



The Effect of Training using Training Aids in Some Biomechanical Variables and Accuracy of the Performance of Remote Correction of Basketball Jumping

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Received: 18 Jan 2019

Revised: 21 Feb 2019

Accepted: 25 Mar 2019

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ABSTRACT

The aim of the study was to determine the effect of the training by using the training aids in the development of the motor path to fly the ball to increase the accuracy of the performance of the correction calculated by three points of jumping. The researcher applied the experimental research method to a sample of players of Wasit University basketball team for the period from 20/11/2017 As of 20/1/2018, after the biomechanical analysis of the variables under consideration and the application of the 3-point jump test and the application of the training curriculum and the conduct of remote tests, the results were statistically processed using the Statistical System of Social Sciences (SPSS) (V24), as the researcher concluded that it is possible to find that the training means of assistance used by the researcher contributed to the improvement Albyumkanikih the values of variables through the availability of the correct path for the arc fly ball, which made it easier for the player to take the right angles to the skill of correction calculated by three points from jumping.

Keywords: training aids - biomechanical variables – basketball.

INTRODUCTION

Instruments and tools are an important training tool used in the field of sports which is used as an educational tool in learning the skills of sports and at the same time to provide live experiences and high impact, "The use of these tools and devices leads to raising the process of education and training and shorten the time affected by performance. The kinetic analysis is also one of the most important tools on which the trainer is based. He is able to observe the performance of the player with close eyes and helps to increase the instructor's awareness. The kinetic analysis is a tool To deal with all tasks related to the performance of skills, where the analysis depends on the basis and rules on





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the entry to the depth of human performance and reveal secrets ", and thus the analysis is" sorting and tabulating the many data with its main components, and then logically processed Balznh with a suitable standard and specific to shift from its formula Quantities of the deaf to another useful meanings to solve the problem addressed by the researcher "

In the opinion of the researchers, most failures leading to failure to achieve field injury calculated by three points due to the existence of mechanical errors during the performance of the skill either in the vertical jump and take the appropriate height in addition to the corners of the arm during the correction and take the right angle of the bow of the ball during the correction process , Hence the importance of dynamic analysis of each skill for the purpose of full knowledge in all its aspects in a way that achieves the benefit by identifying the exact details and aspects of the movement of the mechanical body and knowledge of the causes and finding solutions to resolve Problems related to the training of this skill and to achieve the best performance through the use of corrective training methods for technical performance on the basis of some biomechanical indicators and their impact on the accuracy of the performance of the skill of scoring calculated three points in the basketball.

The research aims to : Identifying the values of some biomechanical variables for accuracy of correction calculated by three points of jumping basketball. To identify the impact of training using training aids in some biomechanical variables and accuracy of the performance of the correction calculated by three points of jumping basketball. The researchers assume There are statistically significant differences between the tribal and remote tests of the values of the biomechanical variables of the accuracy of the correction calculated by three points of jumping basketball. There are statistically significant differences between the tribal and remote tests of the accuracy of jumping by three points in the basketball for young people and for the tests of the dimension.

MATERIALS AND METHODS

Research Methodology

The researchers followed the descriptive research approach in the associative method of regression type. The associative method is defined as "the kind of research that can detect whether there is a relationship between two or more variables, and then know the strength and direction of this relationship"

Search community and sample

The limits of the research are the players of the Wasit University basketball team participating in the Iraqi Universities Championship (2016-2017) of (15) players. The sample was chosen from (10) players in a random way represented 66.66% (-0.661, 0.701, -0.521), respectively, within the parameters of (3+), which means that they are naturally distributed within the average kawse curve.

Means of gathering information, tools and devices used in research

The researchers used the following tools: Video Camera Type (National-M3 -Video device type (National) -Tape measure -Medical balance -Scale length (1) meter. -. A regular basketball court -Basket balls number (15) - Change the path of the ball correction The height of the tool is about 3.66-3.75 m in relation to the proximity and after the player is eliminated from the panel of the ring. Performance description: - The player is the process of correction (from the stability - jump) from the front of the barrier, so that the player of the correction from the top of the circular ring installed in the barrier to make the arc of the flight path of the ball suitable for the purpose of the player away from the player defender and thus achieve the appropriate angles to the corner Starting - entering the ball) to the goal during the correction. Angle control device for ball entering the throat -It is a tool that is made of flexible iron covered with plastic on the board. It is 90 cm in front and 70 cm wide and 10 cm above the throat :Performance





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description As this tool contributes to the player to throw the ball high to allow it to pass from the top of the tool and thus change the angle of entry of the ball by making the arc of the path of the ball increased by increasing the angle of the ball also in proportion to the rise of the player corrected during the correction process. Control tool of the player during shooting. It is made of flexible iron covered with plastic, which is a column with a variable height relative to the length of the player (from the top is in the form of the letter T) (will be coated with sponge material either from the bottom and be fixed with a base placed on the ground). Performance description : The player will perform the correction of the stability of the front of the device to touch the wrist of the player corrected the piece of sponge, which is in the form of (T) after the left of the ball hand-corrected, to ensure that the ball out of the highest point of the correction.

Measuring tools and tests in the study

According to the data of the current study was adopted as provided by modern technology. First: kinetic analysis. By using a digital video camera (120 ppm) to record the three-point remote correction test and to analyze the movement of the motion later to extract the biomechanical variables. The camera was positioned vertically on the center of gravity of the player who is aiming at 1.20 m and a distance of 6.50 The right of the player who stands near the left corner for the goal of the basket and outside the arc of the remote correction three points. Second: Correction tests calculated by three points in the basketball: Purpose of the test: Measuring the ability to pick up by jumping - three points (Account Grade: - Calculates the time since the player received the ball until the end of the eighth attempt after the ball left the hand of the laboratory player. Time division to (60) tha (The player will score a score for each successful jump -. The player (zero) of the score for each case of a failed jump correction -. Collecting degrees (accuracy) of successful attempts - Total grade (final): division of the output of precision over time

Field research procedures

Exploration Experience

For the purpose of identifying the obstacles to work that may face the process of the field experiment, the researchers conducted a pilot experiment on five players from the University of Wasit and using a video camera on Tuesday, 16/11/2017 and at four o'clock and at the Martyr Maitham Habib Hall Sports in Kut. The aim of the exploratory experiment was to make sure that things were mechanical: the efficiency of the camera used in the experiment. Ensure the validity of tools and device - Recognize the distance of the height of the camera from the ground and the distance of the camera away from the center of performance skipping skill. .The extent of the sample's understanding and response to the tests. The extent to which the Task Force applies its obligations to ensure the legalization of exercises used and the validity of the research sample.

Main experience

Tribal tests: from 18/11/2017 until 19/11/2017, The main experience: from 20/11/2017 until 20/1/2018, Remote tests: from 21/1/2018 to 23/1/2018

Suggested exercises

A number of exercises have been prepared to match the biomechanical variables that were created for the sample, on the basis of which the locomotor path of the ball of the ball was developed for the correction calculated by three points of jumping basketball. The exercises were applied in the main section of the training module for the experimental group. Ranged from 24_30 minutes.

© The total number of training units (24) training units.

© Number of training units per week (3) training modules.



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The weekly training days are Sunday, Tuesday, Thursday, The exercises were conducted after conducting the appropriate tests and imaging and analysis of the biomechanical variables to find the best variables through the successful correction according to the test used. In light of this, the proposed training and training aids were developed for the purpose of developing successful correction.

Statistical means

The Social Statistical Pouch (SPSS) (V24), (statistical package for social sciences), was used to address the results of arithmetic mean, standard deviation, intermediate, torsion and T (T).

RESULTS AND DISSCUSSION

Table (1) Shows the values of the computational environment of the biomechanical search variables and the correction calculated by three points in the tribal and remote basketball and the value (t) calculated, the tabular and the significance level. From the review of the results of the tribal and remote tests of the experimental group in the tests of the biomechanical variables and the skill of accuracy of three-point jump correction. The obvious development of the experimental group, which was subjected to the training modules using the auxiliary training methods which contributed to the improvement of all search variables. Which included training using the training aids that give the ability to the player who leads the correction on the possibility of taking the right bow of the ball path, which has positively affected the development of the performance of art The training modules are complemented by the presence of training aids which contribute greatly to shortening the effort and time to reach the desired goal. The experimental group has increased its performance through the use of the exercises which were developed by the researchers in the main section of the training unit, which confirmed the training precision resulting in reduced time and an increase in the number of duplicates and accuracy of performance.

In the opinion of the researchers that the impact of various exercises overlapping with some of the training aids used to develop the variables under consideration led to this improvement, the more the training modules are based on scientific basis commensurate with the nature of the sample and the use of appropriate training to make the training process more effective in skill training, Schmidt noted that "organizing training in a varied or variable manner using stimuli or means is more effective in learning from training or organizing training consistently without changing from one repetition to the next." These results are consistent with the study of Louay Ghanim Al- "Where and D The researchers proposed that the means have a significant impact on access to perfection skill stage also lead to a rise in the average performance of the transmission accuracy after receiving training on the proposed program". The evolution in the nature of the biomechanical variables represented by the linear velocity of the arm, the elbow and the hip, as well as the angle angles (direction, attack, launch, entry), were a natural result of the nature of the training tools and tools used.

While taking the best angles to do the jump to reach the highest point and then the process of correction in addition to the sense of the start of the ball from the hand and take the right angle as much as possible to achieve the main goal of the correction is the entry of the ball Of the throat and the achievement of the three points, which makes the interaction between the forms of these exercises and form the real performance as much as possible, as obtaining the highest amount of strength must be consistent with the matching of the doses of this force with the movement required and to the performance time that should be appropriate for the use of this force speed considerations , and that means and training tools increase the sense of movement "sensory knowledge is important for the development of skill" , and this is confirmed by (Hale) "The sense of movement works to strengthen the pathways of the player by bringing mental images of skill In an orderly manner where it works to engage the verb In the muscles of the body in the performance of the skill , and it plays an important role in the process of compatibility of activity of complex movements that require differentiation between different parts, " which is clear to us the effectiveness of training





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tools and methods prepared by the researchers, which contributed to the improvement of both The angle of starting the ball during the correction and take the right bow and thus reach the right angle to enter the ball to the throat, which effectively contributed to the development of three-point jump, although the angle (45) degree is the ideal corner in the arrival of the ball to the ring as short as possible and less Effortless but flat (50) and above do not allow the ball to be hit by the opponent even if they are tall because the high flight of the ball will be high and will affect the angle of the ball entering the ring and the proportion of correction, and this "The arc of the ball of the ball and the back of the ball reduces the fall of the ball under the influence of gravity and works to create the angle of entry of the ball in the ring appropriately" , which requires the player to exert greater muscle strength, which of Which requires greater force to push from the legs and elbow arm, and flex the wrist during movement Hess 1980 states that "bending the wrist during the final movement of the ball helps determine both the speed and the angle of the ball" .

The final stage of the correction is the follow-up when all the joints of the body In the move until the ball out of the arm of the player is corrected, that is, there is a force moved to the arm is the part that is pushing the ball or the last part is contact with the ball and on the basis of strength and speed determines the path of the ball, "The force gained from the trunk goes to the arm and then the ball to gain speed Appropriate ", (Hay, James G) refers to " the high starting point of the ball is determined by , and that the height of the start of the ball has a close relationship with the angle of the start of the ball as some players are shooting during the jump before reaching the highest point in the air and some of them do the correction during the landing, and some of the correction when he arrived The researcher goes with the opinion that the best position for the correction is when the player reaches the maximum height possible in the air because that situation away from the player who performs the scoring of the player first Defender and allows this situation the opportunity for the player who is doing the correction to meet the target clearly And Easy.

CONCLUSIONS

The training methods used by the researcher contributed to the improvement of the values of the biomechanical variables of the skill of correction calculated by three points of the jump of the experimental group. The training methods contributed positively to improving the accuracy of the correction calculated by three points of jumping. There is a positive effect of the training aids in the development of the correction process by providing the right path of the ball of the ball, making it easy for the player to take the appropriate angles for correction. The various training using the training tools and methods was a key factor in developing the incentive to jump in the manner required to perform the jump.

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Table (1) Shows the values of the computational environment of the biomechanical search variables and the correction calculated by three points in the tribal and remote basketball and the value (t) calculated, the tabular and the significance level

Significance	Level of error	The value (t) calculated	Evaluate the computational settings of the tests						Variables
			E	P-	Dimensionality		Tribal		
					P	s	P	s	
D	0.000	7.52	0.23	0.59	0.15	4.20	0.18	3.61	Linear velocity is the moment of throwing
D	0.000	6.24	0.27	0.57	0.29	3.38	0.16	2.81	The linear velocity of the hip is the moment of payment
D	0.000	7.20	0.14	0.38	0.14	3.39	0.07	3.01	Linear speed of the joint joint
D	0.000	8.10	0.48	3.89	1.76	51.89	1.30	55.78	Angle of attack
D	0.000	10.39	0.25	2.67	0.66	6.44	1.65	9.11	Direction angle
D	0.000	16.44	0.70	11.66	1.80	58.33	1.73	46.67	The starting angle of the ball
D	0.000	9.38	2.94	9.22	3.96	56.22	3	47	Angle of entry ball
D	0.000	9.18	0.47	1.46	0.52	3.29	0.08	1.83	3 point correction

The value (t) of the table (2.26) level of significance (0.05) and the degree of freedom (9)





Isolation of Leishmania Species Causative Agent of Cutaneous Leishmaniasis from Patients in Endemic Areas of Iraq: Identification and Genotyping using Molecular Methods

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Received: 17 Jan 2019

Revised: 19 Feb 2019

Accepted: 23 Mar 2019

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ABSTRACT

Background: Cutaneous leishmaniasis (CL) remains a serious public health concern in some parts of Iraq. Molecular characterization is now the most reliable method to differentiate between them and to determine their phylogenetic relationships. The aims of this study to evaluate the reappraisal of the diagnosis and epidemiology of CL in some parts of Iraq, by different parasitological, cultural, and this is the first study conducted to characterize *Leishmania* species causing CL among Iraqi people using the sequence analysis of ITS1. **Methods:** A total of 86 cases of suspected cutaneous leishmaniasis were checked for *Leishmania* amastigote using Giemsa-stained smear; however the Novy Macneal Nicolle (NNN) culture led to the growth of promastigotes in all samples, then the samples were examined using PCR and RFLP-PCR methods. Also, phylogenetic tree analysis based on the Kinetoplast DNA partial sequence that used for *Leishmania* sp. confirmative detection. **Results:** The highest infection (100 %) appeared by using PCR while the lowest infection (65.1%) appeared by culture on NNN media. The present study was revealed that the highest infection (60 %) caused by *L. major* than *L. tropica* (40 %) and



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there were two type of *L.major* and *L.tropica*; type A (40 %) and type B (60 %). Our results showed that 53(61.6%) of CL patients were had single lesion and 33(38.4%) had multiple lesions, and the ulcerative wet type lesions were present in 65.1 %, while the nodule dry type lesions were present in 34.9%. Conclusion: *Leishmania* isolates collected from different parts of Iraq showed that *L.major* and *L.tropica* are the agents of CL. Moreover, the present study revealed that the genetic properties of a species can play an important role in the clinical manifestations, pathogenesis, epidemiology, and classification of the parasites. Control measures and health strategies should have high priorities to help treat the existing cases and prevent the expansion of the disease to new areas.

Keywords: Cutaneous leishmaniasis, PCR,Culture, DNA Sequencing

INTRODUCTION

Leishmaniasis comprises a group of disease caused by the genus *Leishmania* widely distributed in tropical and subtropical regions throughout the world. These diseases transmitted by various species of Phlebotomus sandflies in Old World. *Leishmania* genus can be divided into several species complexes [1]. It occurs in three clinical forms including cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL) [2]. Many *Leishmania* spp. cause skin ulcers and nodules. The protozoan *Leishmania* is an obligatory intracellular parasite which exists in two distinctive forms. In man and other hosts it occurs as a non-flagellated amastigote form, while in culture and gut of sandflies the flagellar or the promastigote form is seen. They are neither found in the peripheral blood nor in any visceral organ. The amastigote are small, round to oval, bodies which measure about 2-5µm and found only in the macrophages of infected vertebrate hosts, The flagellar or the promastigote forms are seen in the culture media and in the gut of the sandfly [3].

In all areas of Iraq there had also been cases of cutaneous leishmaniasis. The course of the disease is much more gentle than that of kala-azar (VL). In 2001 there were 625 cases of cutaneous leishmaniasis, 955 cases in 2000 and as many as 8779 cases in the peak year 1992 (45 cases for every 100 thousand citizens). Cases of cutaneous leishmaniasis caused by *L. tropica* mostly occur in the suburbs of big cities (Baghdad, Mosul) among large conglomerations of people where the sanitary conditions are unsatisfactory. Incidences caused by *L. major* are much more common; they appear primarily in rural areas, especially in the northern and southern provinces of the country [4,5]. The diagnosis of CL in Iraq is based on clinical signs of the disease, microscopic observation of parasites in stained skin biopsies and culture [6]. The differentiation at the genus level based on the amplification of the kinetoplast minicircle DNA (kDNA, about 10000 copies per cell) or the variable sequences of the small subunit ribosomal DNA genes (SSU rDNA, 40-200 copies per cell) [7].

They allow identification of *Leishmania* parasites only at the generic and/ or subgeneric level. Both targets have also been used for the development of real-time PCR assays in order to determine parasite burden in clinical samples. The ability to distinguish between *Leishmania* species is crucial for a correct diagnosis of the disease as well as for making decisions regarding treatment and control measures. This is especially useful in areas where several *Leishmania* species co-exist [8]. The PCR-RFLP of the internal transcribed spacer (ITS) of ribosomal DNA is one of the most widely used approaches for the detection and identification of *Leishmania* species in the Old World [9]. Furthermore, sequence analysis of the internal transcribed spacer 1 and 2 (ITS1 and ITS2) of ribosomal region has been used in several studies for intra-species inference of phylogenetic relationships among *Leishmania* populations [10]. New high-throughput sequencing technologies have opened the door for population genome analyses and genome-wide association studies. Genome of the *L. major* species was the first to be fully sequenced [11] followed by *L. infantum* and *L. braziliensis* [12]. Treatment of CL is not easy especially when the causative agent is *L. tropica*. Although pentavalent antimonite derivatives are still considered as the main treatment available for CL, their



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efficacies are not promising and resistance has been reported. Maglumineantimoniate (MA) (Glucantime® Rhodia Laboratories, Rhone-Poulenc, France) is considered as the only standard treatment available for CL in Iran. However, MA treatment is administered via parenteral route which needs multiple painful injections, is accompanied by several side effects and questionable efficacy [13]. The aims of this study to evaluate the reappraisal of the diagnosis and epidemiology of CL in some parts of Iraq, by different parasitological, cultural, and this is the first study conducted to characterize *Leishmania* species causing CL among Iraqi people using the sequence analysis of ITS1.

MATERIALS AND METHODS

Collection of samples

A total of 86 cases of suspected cutaneous leishmaniasis were included in this study during a period from October 2013 to the February 2014. Skin lesion samples were collected from patients in Iraqi's hospitals and placed in sterile test tubes, then transported to laboratory and stored in -20°C until genomic DNA extraction step.

Parasitological Examination

Small amount of aspirated fluid was taken and smeared on a clean glass microscope slide then left it to dry, then fixed using 100% absolute methanol for 30 seconds and left it to dry again and stained with Giemsa stain for 20 minutes, then rinse with tap water and dry the slide, and then examined it under oil immersion lens of the light microscope (Olympus CH2, Japan). Amastigote was diagnosed as round or spherical shape with distinctive kinetoplast. In this case was declared positive. When no amastigote was seen after 15 minute of inspection, the smears was declared negative [14].

Culture

The lesions and the adjacent normal-looking skin around them were cleaned, sterilized with 70% ethanol, and allowed to dry. Similar to the preparation of the slide smears, a small amount of the scraped tissue was inoculated on the liquid phase of Novy-McNeal-Nicolle (NNN) medium (10% of rabbit blood). The culture was incubated at 25°C and examined for parasite growth by the inverted microscope and also light microscope every 4 days until promastigotes were seen or up to one month before being discarded as negative. The cultures were made at least in duplicates for each case [15].

Genomic DNA Extraction

Genomic DNA was extracted from frozen skin lesion samples by using (Genomic DNA Mini Kit, Geneaid. USA). The extraction was done according to company instructions by using frozen Blood extraction Protocol method with Proteinase K. After that, the extracted DNA was checked by Nanodrop spectrophotometer, and then stored at -20 °C at freeze until used in PCR amplification.

PCR amplification

PCR assay was carried out by using specific primer for small subunit (SSU) ribosomal RNA (rRNA) and 5.8S rRNA regions that are related to ribosomal ITS1, the primers forward primer (CTGGATCATTTTCCGATG) and reverse primer (TGATACCACT TATCGCACTT) were used to amplify approximately (350 bp PCR product) in *L. major* and *L. tropica*. The primers were provided by (Bioneer company, Korea). Then PCR master mix was prepared by using (AccuPower® PCR PreMix kit. Bioneer. Korea).





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The PCR premix tube contains freeze-dried pellet of [Taq DNA polymerase 1U, dNTPs 250 μ M, Tris-HCl (pH 9.0) 10mM, KCl 30mM, MgCl₂ 1.5mM, stabilizer, and tracking dye] and the PCR master mix reaction was prepared according to kit instructions in 20 μ l total volume by added 5 μ l of purified genomic DNA and 1.5 μ l of 10 p mole of forward primer and 1.5 μ l of 10pmole of reverse primer, then complete the PCR premix tube by deionizer PCR water into 20 μ l and briefly mixed by Exispin vortex centrifuge (Bioneer. Korea). The reaction was performed in a thermocycler (Techne TC-3000. USA) by set up the following thermocycler conditions; initial denaturation temperature of 95 °C for 5 min; followed by 30 cycles at denaturation 95 °C for 30 s, annealing 50 °C for 30 s, and extension 72 °C for 30 s and then final extension at 72 °C for 7 min. The PCR products were examined by electrophoresis in a 1.5% agarose gel, stained with ethidium bromide, and visualized under UV illumination.

RFLP-PCR

RFLP-PCR were used in genotyping of *L. major* type (LmA&LmB) as well as *L. tropica* type (LtA&LtB) by using HaeIII (Promega, USA) that digested the PCR products into 140-bp and 220-bp fragments corresponding with *L. major*, and 60-bp and 200-bp fragments corresponding with *L. tropica* in buffer at 37°C for 1 hours. Restriction fragments were separated in 2% agarose gels in 1 \times TBE buffer and visualized by staining with ethidium bromide on a UV Transilluminator.

Sequence analysis

Phylogenetic tree analysis based on the Kinetoplast DNA partial sequence that used for *Leishmania* sp. confirmative detection. The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version) [16,17].

Statistical Analysis

The suitable statistical method was used in order to analyze and assess the results by using T-test in Minitab version [18]. The comparison of significant (P-value) in any test were: S= Significant difference (P<0.05), HS= Highly Significant difference (P<0.01), and NS= Non Significant difference (P>0.05).

RESULTS

Table 1. The prevalence of positive cases of CL by using different diagnostic methods. The highest infection (100 %) appeared by using PCR while the lowest infection (65.1%) appeared by culture on NNN media.

Table 2. The distribution of CL cases in relation to the clinical features. The wet type lesions represent (65.1%) and the dry lesions were (34.9%). Also, the single lesions recorded (61.6%) than multiple lesions (38.4%).

Table 3. The diagnosis of two species of *Leishmania* by using PCR method. The present study was revealed that the highest infection (60 %) caused by *L. major* than *L. tropica* (40 %).

Table 4. The diagnosis of two species of *Leishmania* by using RFLP-PCR method. The present study was appeared that there were two type of *L. major* and *L. tropica*; type A (40 %) and type B (60 %).

Figure 1. Promastigote forms of *Leishmania* spp.

Figure 2. PCR amplification results

Figure 3. RFLP-PCR analysis results

Figure 4. Multiple sequence alignment analysis of the partial Kinetoplast DNA sequence for local *Leishmania* species isolates and NCBI-Genbank *Leishmania* spp. isolates by using MEGA 6.0, multiple alignment analysis tools. The multiple alignment analysis similarity (*) and differences in Kinetoplast DNA nucleotide sequences.



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DISCUSSION

Correct diagnosis of *Leishmania* species is essential to determine the clinical prognosis and a species-specific therapeutic approach [19]. Specification of different species of genus *Leishmania* depends on several factors such as the geographical distribution of an isolate, the clinical finding of the disease and the epidemiology of the vector and the animal reservoir [20]. A round shape *Leishmania* parasite without flagellum was grown in culture media with biphasic NNN media as demonstrated by light microscopy examination. The change of promastigotes to amastigotes did take place completely in culture. These finding again emphasize that optimal condition for propagation of axenic amastigotes vary and have to be determined for each *Leishmania* species isolates. DNA isolated from promastigote forms obtained from in vitro culture of *Leishmania* allowed for optimization of PCR reaction [21].

The diagnosis of CL classically relies on microscopic examination and in vitro cultivation. These classical methods require the presence of a relatively high number of viable or morphologically intact parasites; this may pose a problem particularly in the chronic phase of CL where parasite levels in skin lesions are very low. In contrast, the molecular approach is both sensitive and specific [22]. In this study we set up a well documented, genus-specific PCR to detect *Leishmania* species in clinical cutaneous samples and compared this method with classical methods. Ulcerative wet type lesions were present in 71.9 %, while the nodule dry type lesions were present in 28.1 %. These observations are in agreement with those reported from Iraq [6], Iran [23], Colombia [24], Pakistan [25], and Afghanistan [26]. The present study indicated that the incidence rate of multiple lesions in CL patients was 32.8 % . This result could be due to long periods of exposure to Plebotomine sandflies and the high population density of sandflies in this area.

PCR methods using either genomic or kinetoplast DNA(kDNA) are now frequently cast in this role. When the amplicon is digested with restriction enzymes, it is possible to identify almost all pathogenic *Leishmania* species by RFLP, allowing direct, rapid characterization and identification of the infecting parasite [27]. Several DNA targets were used for DNA amplification, such as the SSU rRNA gene , the ITS regions , the microsatellite DNA or extrachromosomal DNA, such as the repetitive kinetoplast DNA (kDNA) minicircles. Related to the sequence variation in the first part of the spacer, the ITS1-DNA target allows the identification of almost all medically relevant *Leishmania* parasites with the use of only one restriction enzyme (HaeIII) for amplicon digestion [28]. As a result of digestion with HaeIII, ITS1-PCR products yielded 140-bp and 220-bp fragments corresponding with *L. major*, and 60-bp and 200-bp fragments corresponding with *L. tropica*. In this study, we applied ITS1-RFLP as a tool for identification of *Leishmania* species. For a further characterization of DNA polymorphisms within *L. major* and *L. tropica* isolates from different areas of Iraq, we used sequencing of the amplified ITS1 region of representative strains of each RFLP pattern. Through PCR-RFLP, a genetic polymorphism was determined for *L. major* as LmA and LmB and for *L. tropica* as LtA and LtB for a number of samples. This may be related to either strain heterozygosity or mixed strains, as isolates were not cloned. Also, the Giemsa-stained slides were examined by both microscopy and ITS1-PCR. Most of the slides that were high scored amastigote numbers as microscopy- positive were also positive by PCR-RFLP. Although the costs for PCR-RFLP diagnosis are higher and its concordance is lower than microscopic examination, but this method can identify *Leishmania* species without the need for cultivating them [17].

Identification of the causative *Leishmania* agent of CL is an important issue since choosing treatment strategy and prognosis of the disease depend on the causative species. Characteristics of the lesion and epidemiological information are not enough to define *Leishmania* species especially in endemic areas with mixed ACL and ZCL infections [29,30]. Cutaneous leishmaniasis is the most severe form of leishmaniasis that is caused by the species of *L. major* and *L. tropica*. Although differentiation between these two species is important because of the differences in their epidemiology and pathology, this had been a difficult task. Typing this parasite using isoenzyme analysis is difficult and requires a large amount of the parasite or may sometimes be unreliable . The advent of molecular tools





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provided methods which are robust in discriminating these two species and to study their phylogenetic relationships [31,32].

CONCLUSIONS

Leishmania isolates collected from different parts of Iraq showed that *L.major* and *L.tropica* are the agents of CL . Moreover, the present study revealed that the genetic properties of a species can play an important role in the clinical manifestations, pathogenesis, epidemiology, and classification of the parasites.there were two type of *L.major* and *L.tropica*; type A (40 %) and type B (60 %).

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Table 1. Distribution of CL cases by different diagnostic methods

Result	Giemsa-smear (%)	Culture on NNN (%)	PCR (%)
Positive	67/86 (77.9%)	56/86 (65.1%)	20/20 (100%)
Negative	19/86 (22.1%)	30/86 (34.9%)	0
Total	86/86 (100%)	86/86 (100%)	20/20 (100%)

Table 2. Distribution of CL cases in Relation to the Clinical Features

No. of Lesions	Types of Lesions		Total
	Dry (%)	Wet (%)	
Single	18/86 (20.9%)	35/86 (40.7%)	53/86 (61.6%)
Multiple	12/86 (14%)	21/86 (24.4%)	33/86 (38.4%)
Total	30/86 (34.9%)	56/86 (65.1%)	86/86 (100%)





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Table 3. PCR results

<i>Leishmania sp.</i>	PCR result (%)
<i>L.major</i>	12 positive (60%)
<i>L.tropica</i>	8 positive (40%)

Table 4. RFLP-PCR results

<i>Leishmania sp.</i>	RFLP-PCR result	
	Type A	Type B
<i>L.major</i>	4	8
<i>L.tropica</i>	4	4



Figure 1.Promastigote forms of *Leishmaniaspp.*

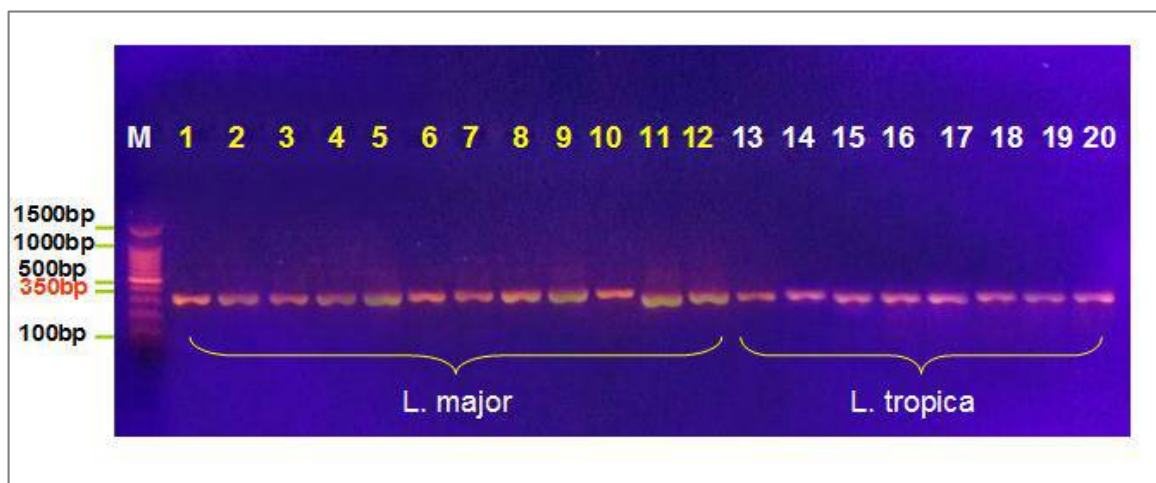


Figure 2. PCR amplification results





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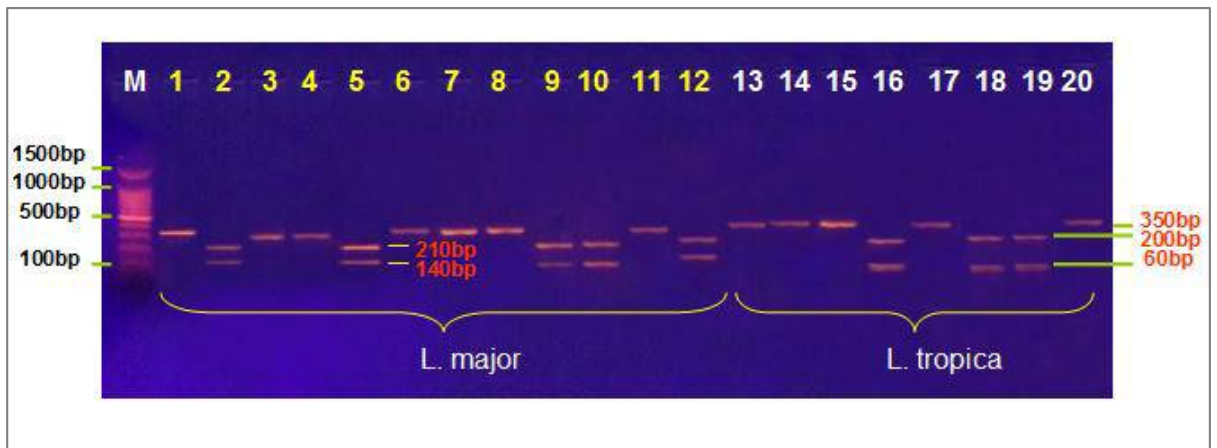


Figure 3. RFLP-PCR analysis results





Eco Friendly Technologies for Finishing: Cutting Waste and Gaining Benefits

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Received: 16 Jan 2019

Revised: 20 Feb 2019

Accepted: 25 Mar 2019

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ABSTRACT

The textile industry is considered as the most ecologically harmful industry in the world; therefore Environment awareness in textile industry has increased significantly with the increased awareness towards ecology. Due to the toxicity of the dyes and chemicals used in textiles and inappropriate discharge of such waste has led to serious environmental and health issues. This has given an impetus to the rising demand for producing textile products through environment friendly and sustainable processes. From an environmental point of view, the clothes we wear can cause a great deal of damage since textiles are used next to the skin and is called second skin. A new concept of fabric finishing with eco friendly finishing techniques is emerging now days with an objective to replenish damage caused by the traditional chemical processing of textile materials. In order to reduce and save earth from the pollutions caused by industries, there also comes in to existence stipulated norms and standards for the finished goods and the way of operation. Finishing of fabrics in an eco-friendly manner is getting very advanced these days. Science has come up with many technologies for the eco processing of textiles, which includes, enzymatic finishing of textiles, plasma technology, biotechnology, ultrasonic, super critical carbon dioxide, nanotechnology, laser treatment and micro encapsulation method. Here, an attempt has been made to explore the possibility of finishing textiles with a suitable textile processing method that delivers not only eco friendly finished products but also adding to the functionality and durability of the garment. Thereby, developing textiles with smart functioning using new ecological products to provide extra comfort and increased performance in an ecofriendly manner.





Keywords: eco friendly finishing, second skin, plasma technology, super critical carbon dioxide.

INTRODUCTION

It is well known that every customer product has less or more impact on the environment, which the consumer does not know. Any product, which is made in a way that significantly reduces the harm it would otherwise cause to the environment, could be considered as eco-friendly product. The textile chemical processing plays an important role in controlling the pollution load for environment. Due to the increasing requirements on the dyeing and finishing of textile fibres and fabrics, the society demand for textiles that have been processed by eco-friendly sound methods. Problems related to toxicity and other health hazards have resulted in the replacement of chemical processing by more eco-friendly physical methods and use of eco-friendly processing. Slowly, consumers in India are taking lead in prompting manufacturers to adopt clean technologies to produce eco-friendly products. Therefore, the researchers and scientists are working to solve issues by converting the chemical treatments into biological or environment friendly treatments. A finishing process that is most suitable and with in the norms of eco label standards is called Eco Finishing. Finishing of fabrics in an eco-friendly manner is getting very advanced these days and Science has come up with many technologies for the eco processing of textiles. Discussed below are some textiles finishing techniques that gives a significant contribution in the protection of environment.

Ultrasonic assisted wet processing

The ultrasound is one of the green approach towards green technology[1]. Sound generated above the human hearing range (20 Hz to 20 kHz) is called ultrasound. Ultrasonic represents a special branch of general acoustics, the science of mechanical oscillations of solids, liquids and gaseous media.[3]

Basic principle

In liquids, longitudinal vibrations of molecules generate compression and refractions, i.e. areas of high pressure and low local pressure and gives rise to cavities or bubbles, which expand and finally during the compression phase, collapse violently generating shock waves. The phenomena of bubble formation and collapse (known as cavitations) are generally responsible for most of ultrasonic effects observed in solid/liquid or liquid/liquid systems.[3]. The ultrasonic waves can be generated by variety of ways. Mostly it is produced by piezo-electric and magnetostrictive transducers.

Mechanisms of Ultrasound Energy

1. Increasing swelling of fiber in water.
2. Reducing glass transition (T_g) temperature of the **fiber**.
3. Reduce the size of the dye particles. It helps to enhance the transport of the dye to the fiber.[2]

Applications of Ultrasound Energy

1. The scouring of wool to reduce the fiber damage and enhance rate of processing.[1]
2. Mechanical processes (weaving, finishing and making up for cutting and welding woven, nonwoven and knitted fabrics) and wet processes (sizing, scouring bleaching, dyeing, etc).[4]
3. It is more beneficial to the application of water insoluble dyes to the hydrophobic fibers.
4. Dyeing of fibres such as polyester which are structurally compact with a high level of crystallinity and without recognized dye sites.
5. Increasing fibre swelling in water during dyeing.
6. Ultrasonic washing, desizing, scouring etc improves reaction rates, product yields.[2]



**Pooja Choudhary and Ekta Sharma****Benefits of Ultrasound Energy**

1. Energy savings by dyeing at lower temperatures and reduced processing time.
2. Environmental improvements by reduced consumption of auxiliary chemicals.
3. Increased color yields.
4. Enzymatic treatments supplemented with ultrasonic energy resulted in shorter processing times, less consumption of expensive enzymes, less fiber damage, and better uniformity treatment to the fabric. [2]

Microwave heating

Microwaves are electromagnetic waves whose frequency ranges from 1000MHz to 10,00,000 MHz. Dielectric heating involves the heating of electrically insulating materials by dielectric loss. A changing electric field across the material causes energy to be dissipated as the molecules attempt to line up with the continuously changing electric field. The heating mechanism is through ionic conduction. Depending on the acceleration of the ions through the dye solution, it results in collision of dye molecules with the molecules of the fiber. Microwaves transfer energy into materials by dipolar polarization and ionic conduction mechanisms to cause localized and rapid superheating of the reaction materials.

Application Microwave Energy in Textile sector

MW energy is observed to be used in heating, drying, condensation process, pre treating, dyeing and pressing, finishing process, surface modifications of textile materials etc [11]

Advantages

- The use of MW energy is more efficient than conventional method.
- Shortens the duration of the application, while protecting the product quality and increasing the efficiency of applied method by virtue of the uniform heating
- The use of industrial microwave systems avoids combustible gaseous by-products, eliminating the need for environmental permits and improving working conditions.
- It pulls down the production cost. Lack of high temperature heating surfaces reduces product fouling in cylindrical microwave heaters. This increases production run times and reduces both cleaning times and chemical costs.[10]

Biotechnology

Biotechnology can be broadly defined as "using organisms or their products for commercial purposes." Biotechnology uses enzymes in different process in order to achieve different goals. Enzymes are proteins formed by long linear chains of amino acids linked by peptidic bonds. They are biological catalysts capable of accelerating the chemical reactions without being used up. These are proteins folded into complex shapes that allow smaller molecules to fit into them.[8]

Applications in textile processing

- a. Enzymatic desizing (eg Amylase)
- b. Enzymatic bio scouring- an eco friendly process (depending upon impurities)
- c. Enzymatic bio polishing
- d. Biofading



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- e. Enzymatic degumming of silk
- f. Decolourization of textile waste water
- g. Enzymatic printing (Latest development)
- h. wool carbonizing

Advantages

- Replacement of harsh and energy demanding chemical treatments by enzymes in textile processing
- Environmentally friendly routes to textile auxiliaries such as dyestuffs
- Novel uses for enzymes in textile finishing
- New diagnostic tools for detection of adulteration and Quality Control of textiles
- Waste management [9]

Plasma treatment

Plasma treatment is a revolutionary technology for surface finishing and pretreatments. Plasma is matter that exists in the form of ions and electrons. Basically, it is a gas that's been electrified charged with freely moving electrons in both the negative and positive state. Plasma treatment uses a controlled vacuum plasma to alter the surface of a material in order to improve bonding, printing, painting, coating, or wettability. The process is performed in a plasma chamber under vacuum pressure. The working process is dry, environmentally friendly and inexpensive; a fabric is normally treated at a speed of 13-20 m/min. It is possible therefore to obtain anti-static, antimicrobial, stainproof or flame retardant fabrics, with clearly higher performance, with better resistance to washing and wear and tear, and with finishing that is at times more simple and less expensive. [13]

Applications

- Desizing of cotton fabrics.
- Hydrophobic enhancement of water and oil-repellent textiles
- Anti-felting/shrink-resistance of woollen fabrics.
- Hydrophilic enhancement for improving wetting and dyeing.
- Hydrophilic enhancement for improving adhesive bonding
- Removing the surface hairiness in yarn.
- Scouring of cotton, viscose, polyester and nylon fabrics.
- Anti-bacterial fabrics by deposition of silver particles in the presence of plasma.
- Room-temperature sterilization of medical textiles. [12]

Advantages

- Compact and require less space
- Less chemicals used
- Less operations and manufacturing cost
- Support for green technology and environment [12]

Microencapsulation Method

Micro-encapsulation is a process in which small capsules of many useful properties are made by using tiny particles or droplets surrounded by a coating. The material inside the microcapsule is called as the core material whereas the wall is called a shell, coating, or membrane. Usually, microcapsules have diameters between a few micrometres and a



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few millimetres. This technique is now widely used in Textile finishing also. [6]. The release mechanisms of the core contents vary depending on the selection of wall materials and more importantly, its specific end uses. [5]

Mechanism of microencapsulation

Microencapsulation can have two distinct objectives as shown in figure 6

1. On one side, to block the substance within the microcapsule, necessarily rigid, when the diffusion of product is not desired.
2. The progressive liberation of the active ingredient within the microcapsule which, in this case should have more fragile wall.

Use of Microencapsules in Textiles

- Fragrance finishes
- Phase-change materials
- Fire retardants
- Polychromic and thermo-chromic microcapsules (colour-changing technology)
- Antimicrobials
- Counterfeiting skin softeners
- Insect repellent

Advantages

This process is more advantageous to conventional process in terms of economy, energy saving, eco-friendliness and controlled release of substances. [7]

Foam finishing

Foam is a dispersion of a gas in a liquid. Here the liquid is generally water, and the gas usually is air. A wet processing which uses air in the form of dispersion foam is called foam finishing. The foam is metered onto the fabric to ensure that there is no excess liquor which must be removed and recycled. Foam dyeing and finishing of textiles has demonstrated as an economically very attractive technology to conserve energy. Foam finishing has a wide application on cotton, synthetic fibres and their blends. Special equipments can be designed for foam sizing, bleaching, mercerizing dyeing and finishing.

Advantages

- Offers potential savings in materials and energy
- Faster production.
- The process converted water phase into micro foam.
- Foam processing overcomes all problems associated with conventional finishing processes.
- Reduces the problem of uniform distribution over the fabric.[14]

Clariant's Foam Eco Care

Clariant's Textile Chemicals Business Unit showcased its innovative products, processes and effects at Technotex 2013 in Pragati Maidan, New Delhi. Clariant has demonstrated its technologies that address environmental concerns.





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One such example is in the finishing area. Foam Eco Care is a wrinkle-free technology for cotton fabrics, which allows reduced water wastes. Anti-wrinkle finishes are traditionally applied with a dry-curing process, which by definition requires no water. The problem with the dry-curing process is that the anti-wrinkle effect is not entirely satisfying: on a scale of 1 to 5, it reaches an average 3. The industry therefore came up with a so-called moist-curing process which allows a very high anti-wrinkle effect – such as Clariant's Eco Care - scoring at up to 3.5 - 4. The moist-curing process has one major downside: it weakens the fabric and lowers its resistance to abrasion, which translates for instance into damaged shirt collars or cuffs. Another downside of the moist-curing process is that, by definition, it requires water for the pad application. Together with Cotton Incorporated, Clariant came up with an all-inclusive solution: Foam Eco Care, a foamed-based curing at low temperature. Foam Eco Care provides the much coveted high anti-wrinkle finish effect (scoring at 3.5 - 4), offers improved abrasion resistance and fabric strength and, last but not least, allows reduced water usage. This is due to lower wet pick-up and to the fact that no after-wash is needed as Foam Eco Care contains low formaldehyde (substantially less than the 75ppm permitted limit). No additional washing and drying process are necessary. Energy savings consequently follow.[15]

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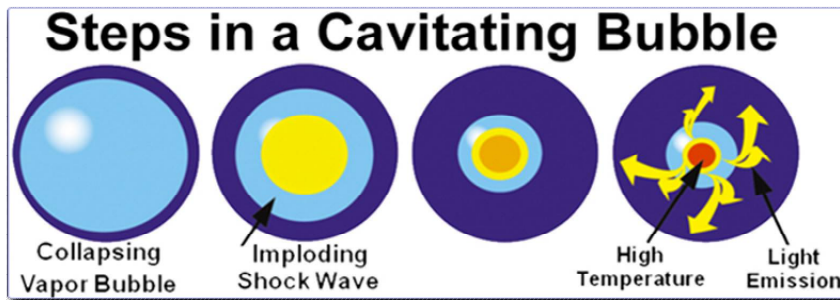


Fig.1. Steps in a cavitating Bubble

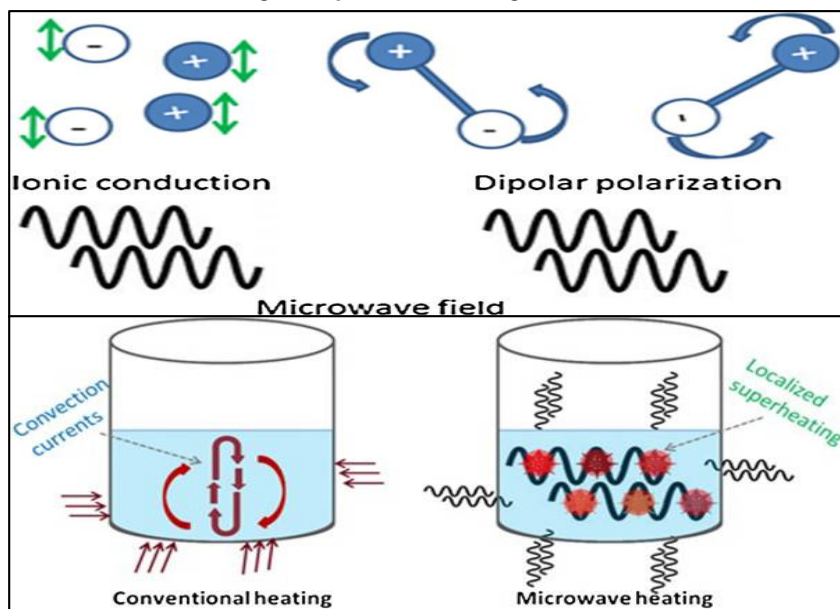


Fig.2. Localized superheating

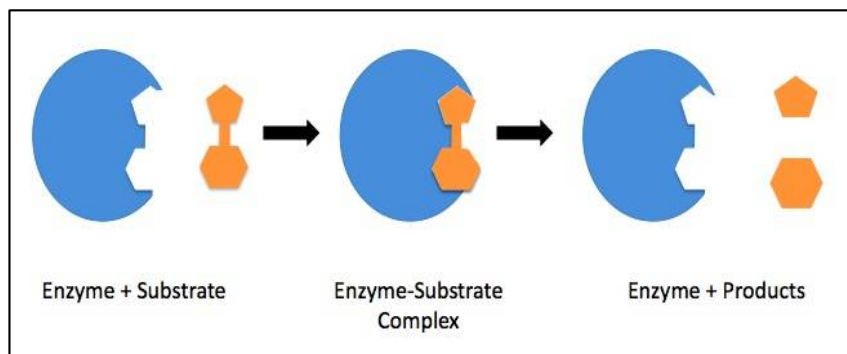


Fig.3. Enzymes work on lock and key mechanism





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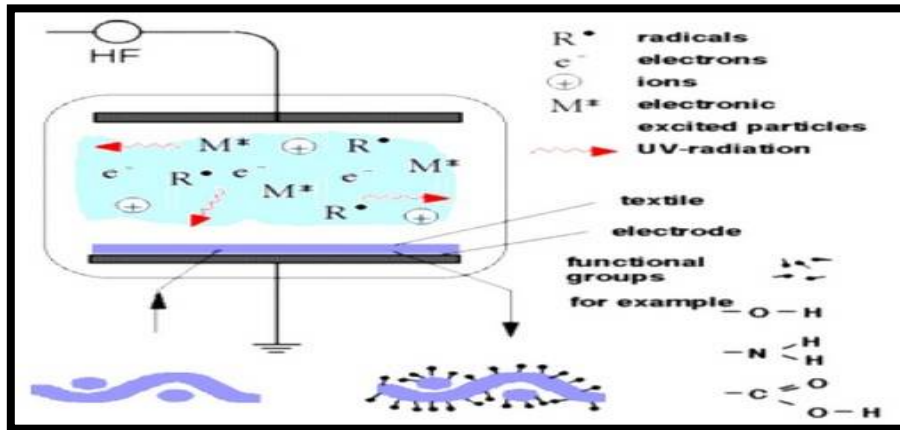


Fig. 4. Plasma surface modification

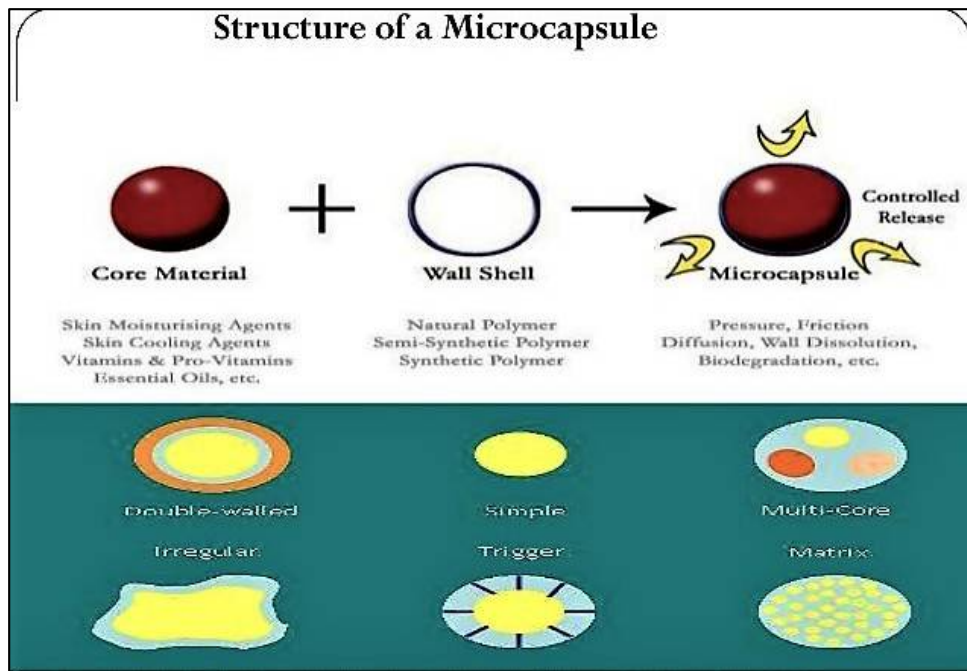


Fig. 5. General structure of a microcapsule



Fig.6. Mechanism of microencapsulation





Pooja Choudhary and Ekta Sharma



Fig.7.Result of insufficient abrasion resistance

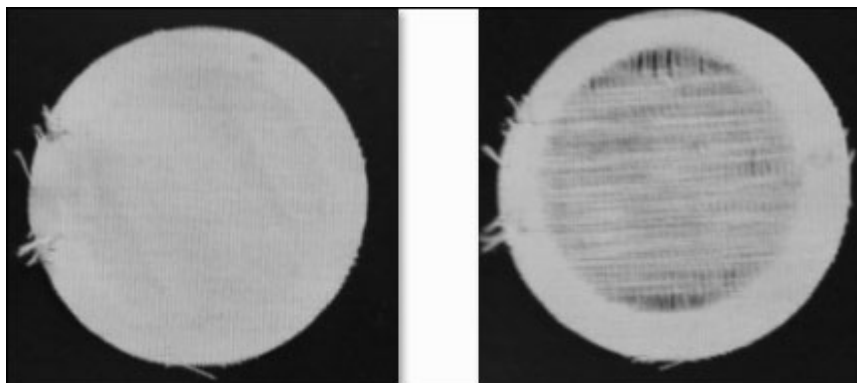


Fig.8. Abrasion resistance with Foam Eco Care (left) vs. conventional method (right)





Analyzing the Financial Deficit with in US Cities: a Case Study of Rock Hill

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Received: 09 Dec 2018

Revised: 12 Jan 2019

Accepted: 15 Feb 2019

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ABSTRACT

Over the last years, Rock Hill has tried to recover from a major crisis of large financial deficit and management problems. This case study aims at understanding the characteristics of the Rock Hill city and its financial situation. To do so, it looks to be imperative to analyze various dimensions such as past budgets, the demographic composition, and the potential brand of the city. Basically, the city of Rock Hill's budget appears to mirror the national budget because they both have problems in terms of the growing budget deficit.

Keywords: Financial Deficit, Rock Hill, America.

INTRODUCTION

The current budget process

Based on an interview with Philip Scherry, an Aldermen of Rock Hill who has six years of experience in preparing budgets, there has not been an issue between the Aldermen and the Mayor since 2006. At that time, the city has been audited and the previous Mayor was accused of improperly handling funds. In fact, the previous Mayor employed pension funds to benefit himself. As a consequence, he was tried, found guilty, and sent to jail. However, the new administration has been very careful about handling the city funds. Before looking at the history of the budget, the current budget process is presented. The budget process is structured as follows. The Personnel Department decides on whether city personnel deserve raises or not. If so, they assign the same percentages to all compartments. For instance, if the amount has been augmented by 5%, it is raised by the same amount for everyone including fire fighters, police officers, and the staff of the City. The financial director is able to decide the amount of raises for all personnel. Then, the budget is examined by each Department Head. Each one lists what its department needs. Each department's phone bill is dealt with one contract. Expenses are constant from year-to-year, so the overall billing is non-discretionary. In the third step, each Department Head looks at capital expenses, normally during two or three





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budget meetings. They send their budget requests to the City Hall Financial Director which makes a budget sheet and sends it to the City Administrator. So the heads submit the budget and the Financial Director makes sure the budget numbers are accurate. In addition, the third step examines the major expenses that the city may incur. For example, two of the capital expenses were a fire truck and street repairs. The city should strengthen security (police and fire) and the ability for people to safely travel in the city streets. Obviously, the Financial Director determines how much money can be safely budgeted. In this sense, each budget is unique. For example, the projected revenue was about \$5,500,000 in 2011, while the city only received only \$4,500,000 as taxes. Therefore, the city cut about a million dollars worth of expenses, especially from street repairs. Some expenses are committed, while others can be deferred to the following year. There are some urgent expenses that should be paid, such as bank payments. Delinquency of debt means no future financing. Finally, and by ordinance, the budget is approved in a public meeting. The budget contains restricted funds, which means that specific taxes are mandated for specific uses, such as monies for parks only or street repairs only. The budget must be of course monitored. This is done on a monthly and quarterly basis by the Financial Director.

The financial situation of the Rock Hill city

The evolution of revenues and expenditures

Historical overview

A historical analysis of past budgets shows that Rock Hill was affected by the national financial situation. As Figure 1 indicates, between 2004 and 2005, there has been a rise of revenues. However, from 2005 to 2007, the revenues continually decreased. Starting from 2007, revenues have evolved in a quasi-stable manner. Turning to budget expenditures, the figure suggests that the highest values have been recorded in 2005 and 2006. There has been particularly a peak of expenditures in 2005 reaching \$10,971,016. However, starting from 2007, expenditures were almost stable and reach its lowest level in 2011 (\$3,192,830). The increase of expenditures in 2005 and 2006 are due by the fact that the city of Rock Hill bought a land to build a new city hall, but it could not afford it and had to sell it to the ARCO Company (Gillerman).

A year-by-year analysis

For the fiscal years of 2004-2005, the total revenues of Rock Hill City were \$4,502,647. This amount came from two kinds of sources. The first consists of program revenues, which include charges for services (11.2%) and federal and state grants (7%), while the second kind of revenues is general revenues which include taxes (72.6%), licenses (4.7%), investment income (0.5%), the sale of capital assets (2.2%) and miscellaneous (1.9%). It is thus clear that taxes represent the main source of revenues in the Rock Hill city during the fiscal year 2004-2005. The total expenditures during the same year were \$4,597,690, spent as follows: \$1,124,631 for administration (24.4%), \$1,237,179 for the police department (27%), \$170,883 for facilities maintenance (3.7%), \$859,402 for the fire department (18.7%), \$642,262 for public works (14%), \$157,600 for parks and recreation (3.4%), \$68,321 for the housing department (1.5%), \$160,568 for the municipal court (3.5%), and \$176,844 or 3.8% of expenditures as interest on long-term debt. During the fiscal year 2005-2006, the total revenues of Rock Hill City increased by 32.4% to reach \$5,960,395. These amounts came from service charges (\$621,408), operating grants and contributions (\$279,311), taxes (\$3,431,607), licenses (\$205,477), investment income (\$10,964), gain on the sale of capital assets (\$1,327,677) and miscellaneous (\$83,951). Regarding expenditures, they also increased by 138.6% to reach \$10,971,016. The main expenses include administration with \$1,023,263 (9.4%), the police department with \$1,169,508 (10.7%), the fire department with \$838,408 (7.6%), the public work with \$841,467 (7.7%), parks and recreation with \$150,530 (1.4%), the housing department with \$80,499 (0.7%), the municipal court with \$152,712 (1.4%), the economic development with \$6,435,596 (58.6%) and finally the interest on long-term debt with \$279,033 (2.5%). In same year the City of Rock Hill did not have facilities maintenance expenses, but there was a new additional expense that came from the economic





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development fund of \$6,435,596. This number specifies a deficit of \$5,010,621. In the fiscal year of 2006-2007, the total revenues of the City of Rock Hill were about \$4,732,118. This came from two sources. The first was program revenues (19.9%), which include charges for services, \$533,536 (11.3%), and operating grants and contributions of \$408,578 (8.6%). The second kind of revenues is the general revenue mainly consisting of taxes (72%), licenses (4.6%), investment income (0.6%), and miscellaneous (3%). During this year, there were no gains from the sale of capital assets. Compared to the previous year, total expenses of the Rock Hill City increased to reach \$7,021,073. Expenditures included those of the administration (10.2%), the police department (13.9%), the fire department (10.2%), public works (7.4%), parks and recreation (1.8%), the housing department (0.8%), the municipal court (2.2%), economic development (51.7%), and the interest on long-term debt (1.8%). In same year, there were additional expenses of \$3,405. A share of the economic development was allocated for a municipal building which serves as the city hall, the police and fire departments. To pay these expenses, the city planned to set aside specific funds. However, these funds have been never materialized.

During the fiscal year 2007-2008, total revenues of the City of Rock Hill were about \$3,494,301. This amount came from different sources, namely property taxes (\$258,573), utility taxes (\$1,027,268), inter-governmental costs (\$216,539), sales tax (\$953,004), licenses and permits (\$225,955), fire and court costs (\$504,355), and miscellaneous revenue (\$308,607). In this year the total expenses of Rock Hill City was \$3,916,462. These expenses included administration expenses (\$886,768), the municipal court (\$154,126), fire department (\$826,426), housing (\$78,184), parks and recreation (\$265,062), the police department (\$829,411), and public works (\$876,485). During this year, the budgetary items changed from the previous year probably due to a change in the administration. For example, there have been new Mayor, financial director, and city administrator (Gillerman and Scherry).

During the fiscal year 2008-2009, the total revenues of the City of Rock Hill were \$4,508,264. This amount comes from property taxes, \$260,271, utility taxes, \$813,280, inter-governmental funds, \$202,438, sales taxes, \$852,389, licenses and permits, \$221,004, fire and court revenue, \$544,907, and miscellaneous revenue \$1,613,975. The total expenses of the Rock Hill City were \$3,879,937. These expenses included administration expenses, \$1,325,349, court expenses, \$157,659, fire department, \$784,604, housing, \$83,911, parks and recreation, \$159,542, police department, \$902,908, and public works \$465,964. In the fiscal year 2009-2010, the total revenues of the City of Rock Hill decreased to \$3,028,402. Among others, this amount comes from property taxes (8.5%), utility taxes (28.9%), inter-governmental funds (6.5%), sales taxes (28.4%), licenses and permits (7%), fire and court costs (14.7%), and miscellaneous expenses (6.3%). In this year, the total expenses of Rock Hill City were \$3,245,862. These expenses include administration expenses (21%), court expenses (4.9%), fire department, (26.3%), housing (2.6%), parks and recreation (3.4%), police department (27.9%), and public works (13.9%).

The evolution of the budget deficit

Since the audit made between 2005 and 2007 and the change in the administration, the City of Rock Hill has reduced a deficit of about \$5,010,621. The deficit continues to affect city improvement projects. As shown in Figure 2, the higher deficit came from the economic development fund of \$6,435,596. As mentioned earlier, funds served to buy a land and build a new city hall. However, policymakers were unable to afford to build a new city hall and had to sell the land to ARCO Company. During the fiscal year 2007-2008, the deficit was less than in 2006-2007, but represented \$422,161. The only fiscal year during which the city had a surplus was 2008-2009 (\$628,327). In 2009-2010 and 2010-2011, it had a deficit of \$217,460 and \$217,267 respectively. According to the Gillerman Report, the city was looking for ways to raise revenues in order to reduce the budget deficit, including the sale of new bonds and the establishment of new businesses. During the fiscal year 2010-2011, total revenues of the City of Rock Hill were \$3,227,603. This amount comes from property taxes (\$269,495), utility taxes (\$790,508), inter-governmental funds (\$203,923), sales taxes (\$832,950), licenses and permits (\$195,140), fire and court revenue, (\$417,590), miscellaneous (\$122,240), and transfer from another funds (\$395,757). The total expenses of Rock Hill City in this year were \$3,444,870. These expenses included administration expenses (\$977,362), municipal court (\$120,061), fire department





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(\$798,442), housing (\$116,990), parks and recreation (\$122,095), police department (\$863,554), and public works (\$446,366). According to the Annual Budget of Rock Hill of the fiscal year 2011-2012, total revenues of the City of Rock Hill reached \$3,192,830. This amount comes from property taxes (\$265,422), utility taxes (\$815,284), inter-governmental funds (\$208,796), sales taxes (\$843,776), licenses and permits (\$195,927), fire and court costs (\$418,383), miscellaneous (\$42,191), and transfer from another funds (\$403,051). The total expenses of Rock Hill City were \$3,192,830. These expenses include administration expenses (\$638,591), municipal court (\$135,843), fire department (\$803,665), housing (\$107,567), parks and recreation (\$139,652), police department (\$879,402) and public works (\$488,110).

The demographic profile of the Rock Hill Community

Rock Hill is surrounded by Glendale, Webster Groves, Ladue, Richmond Heights, and Maplewood. In general, except Webster Groves, Ladue, and Glendale, the other cities have similar demographic characteristics. The City of Rock Hill has a population of 4,620 residents, of which 70.6% are white, 23% are African American, and 2.8% are Hispanic. Compared to surrounding cities, Rock Hill has more residents who are African American. For example, African Americans accounted for 23% of the total population while they represent only 0.8% in Ladue, 0.88% in Glendale and 6.8% in Webster Groves and 13% in Richmond Heights. The residents of Rock Hill City have a median age of 36.9 years. This median age is similar to surrounding cities, except for Ladue. The median age is 40 years in Webster Groves, 36 years in Richmond Heights, 34.7 years in Maplewood and 40 years in Glendale. The average household income, as Table 1 indicates, of Rock Hill residents is \$65,438, although the income for a typical family reaches \$72,870. Ladue and Glendale are much higher in terms of incomes. Residents of Ladue have an average household income of \$142,000, while those of Glendale have an average income of \$75,000. On the other hand, the Rock Hill has a higher average income higher than Webster Grove, Richmond Heights and Maplewood.

In comparison to Richmond Heights and Maplewood, Rock Hill has fewer families living under the poverty line. Rock Hill has 3.5% families living below the poverty line, while those of Richmond Heights and Maplewood were 7.3% and 10.6%, respectively. On the contrary, the poverty rate in Glendale and Ladue is smaller than the Rock Hill city and were about 0.4% and 1.4%, respectively. This is mostly because of higher property values and taxes in these cities. In addition, Ladue has more residents with college diplomas, with 74% receiving their bachelor's degrees. In Rock Hill city, 40% have bachelors. However, 89% of the residents have high school diplomas. In Rock Hill, 85% of houses are occupied by their owners, meaning that only 15% are renting, which is much lower than the state average of 30%. In Rock Hill, the estimated medium value for houses is \$178,508 which is higher than the rest of the state (\$139,700). According to Mr. Philip Scherry, an Aldermen of Rock Hill who has six years and individual meetings with residents, the Rock Hill City is considered middle class in terms of education and housing. However, the majority of houses are older, with 82% built before 1950. Only 10% of houses were built since 2000. Finally, the Rock Hill belongs to the Webster Groves/Schrewsbury Area Chamber. It has small businesses including two banks and no major commercial centers. However, there are numerous restaurants. Webster and Glendale are considered to be bedroom communities (Businesses).

The "Brand" of Rock Hill

In light of the previous analysis, it looks to be essential to define the concept of a city brand. A brand is the residents' impressions of the city and what kind of services the residents expect. Of course, residents should be able to expect police and fire protection, well maintained streets and regular trash collection, which should be regulated by the city. According to Mr. Philip Scherry, an Aldermen of Rock Hill who has six years the Rock Hill city is not specialized in the electricity, water, sewer or natural gas businesses, contrarily to some larger cities that might get into those services. For example, Kirkwood runs their electricity and the city of Saint Louis runs its water industry, while Brentwood runs their trash services. Accordingly, the city of Rock Hill makes a contract with a single trash hauler for the entire city and their contract lasts 5 years and each citizen receives a trash bill. The corresponding budget





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perspective is simple: Rock Hill has the administrator people, the service with experience, the improvement “capital”, and these are the three basic components that make Rock Hill work. However, this is not enough to “brand” a city. Mr. Philip Scherry pointed out that the recent improvement capital was used to fix and improve parks which are a major example of using the budget to enhance the city’s brand. Building parks, schools and other amenities is what will make the Rock Hill city a family-friendly community. This is what Rock Hill wants to be known. Rock Hill has received grants but also spend much money to fix their parks. In 2011, it developed two parks. One of them was financed by receiving a grant, while the other from the budget. The Rock Hill Road Project was funded by a federal grant, but also the city had to match a proportion of it. The city is also very careful about businesses and tries to make them blend in. For example, in 2011, ARCO began the construction on new facilities in Rock Hill, but only if the ARCO landscape and help building a small school. This focus on parks and beautification in the city is important, since they will provide the Rock Hill with a better family environment and a sense of community.

As stated in earlier assignment that the city of Rock Hill is trying to integrate the residents of the community into a discussion about the services and the budget activities, the city is trying to accomplish it. This is one way to get people of the community involved in the budgetary process. The mayor and the city administrator are trying to get feedback from the residents such as problems, services and anything that may improve the life in the city. In addition, they are trying to find out what problems or services the residents care about. One of the complaints was about the demolition of the church in order to build a gas station. Some people believe that the church is a historical building, and it was one of the oldest buildings in the city. So, it is not a good idea to tear it down, according to some citizens. The residents of Rock Hill are trying to improve their city and services. They want to show other people that they are working hard to improve streets and parks because the City has had a negative image owing to the blighted areas. Saint Louisans have negative impressions about the City of Rock Hill and the residents want to change this vision.

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Table 1. Rock Hill city compared to the surrounding cities

City	Populatio	Race	Age	Education	Income	Business and employment
Rock Hill	4620	- White: 76% - Black: 23%	- 45 to 54 years: 16.3% - 25 to 34 years: 15.1% - 35 to 44 years: 14.2% - Median age: 39.5	- High school: 89% - Bachelors: 40%	- Average income: \$65,438 - Capita: \$30,194 - Families below poverty line: 3.5%	- Webster Groves/Schrewsbury area.
Webster grove	23,230	- White: 90.8% - African American: 6.8% - Hispanic: 1.5%	- Under 18 years: 24% - 45 to 64 years: 23% - 65 and older: 17% - Average age: 40 - Household: 31%	-	- Household: \$60,524 - Capita: 31,327 - Families below poverty line: 2.0%	- Bedroom communities

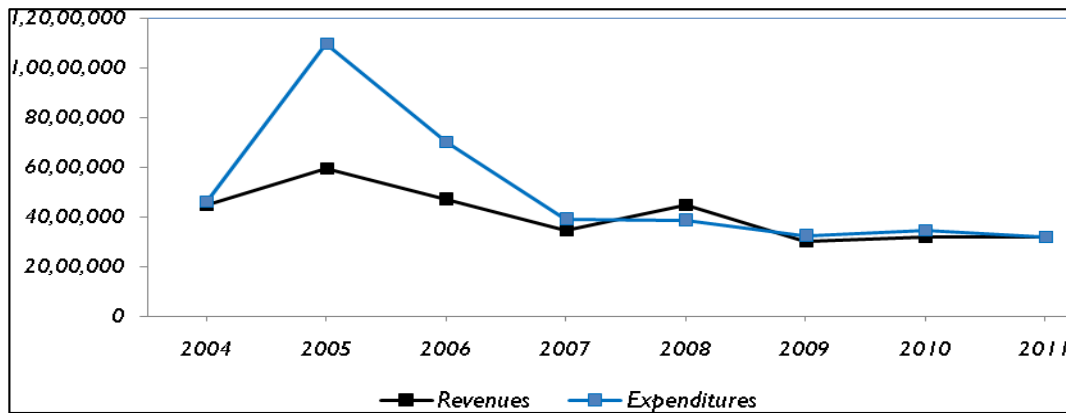




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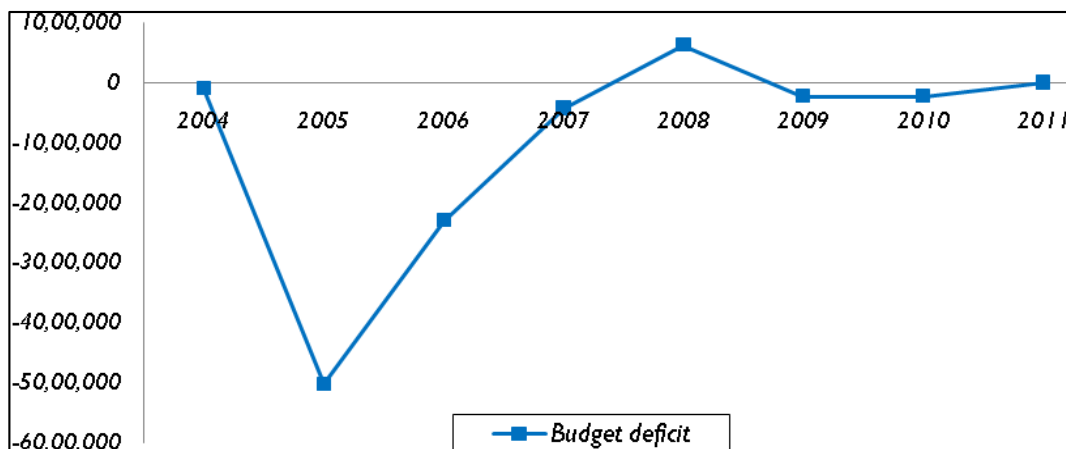
<i>Ladue</i>	8500	- White: 96.8% - African American 0.8%	- Under 18 years: 24% - Average age: 48 years - 31,4% of the family has children	- College degree: 74.5%	- Household: \$142,000 - Capita: \$90,000 - Families below poverty line: 1.4%	- Private companies: 53% - Own business: 17%
<i>Richmond Heights</i>	9600	- White: 81% - African American: 13% - Hispanic: 1.7%	- 64 and older: 14% - Average age: 36 years	-	- Average income: \$50,000 - Families below poverty line: 7.3%	-
<i>Maplewood</i>	8000	- White: 74% - African American: 1.7% - Hispanic: 2,2%	- Average age: 34.7 years	-	- Families below poverty line: 10.6%	-
<i>Glendale</i>	5700	- White: 97% - African American: 0.88% - Hispanic: 1.1%	- 64 and older: 14% - Average age: 40 years	-	- Average income per family: \$75,000 - Families below poverty line: 0.4%	Bedroom communities

Sources: Race in Rock Hill, Rock Hill city, Complete Analysis.



Sources: The author based on financial reports of 2005-2007 and annual budgets of 2007-2012.

Figure 1. Total annual revenues and expenditures of the City of Rock Hill, 2004-2011



Sources: The author based on financial reports of 2005-2007 and annual budgets of 2007-2012.

Figure 2. The Rock Hill annual budget deficit, 2004-2011





Role of *mir-let-7a* Expression in Breast Cancer Diagnosis and Prognosis

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Received: 18 Jan 2019

Revised: 20 Feb 2019

Accepted: 25 Mar 2019

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ABSTRACT

Breast cancer is the most frequent carcinoma in females and the second most common cause of cancer related mortality in women. Early detection of breast cancer is widely reported to be one of the most effective ways leading to better prognosis and lower death rate. For marker discovery, the analysis of miRNA expression signatures in peripheral human blood has been widely used showing to be a promising technique. The *let-7* family is one of the first tumor suppressor miRNAs to be identified. The aim of the present study is detecting the expression levels of *miR-let 7a* gene expression in the peripheral blood of breast cancer patients in comparison with benign and healthy controls as a tool for screening and diagnosis the early stage breast cancers, and estimating the diagnostic and prognostic values of these levels in association with tumor size and lymph node status. The marker was determined in peripheral blood (PB) of 55 patients with Invasive Ductal Carcinoma and samples from 20 healthy donors, and 10 women with newly diagnosed benign breast tumors were served as control group using reverse transcriptase polymerase chain reaction (RT-PCR). *miR-let 7a* gene expression was detected in 30 (54.5%) of peripheral blood of breast cancer patients studied, 1(10%) of the benign tumors but not in any of healthy individuals. It showed statistically significant relations with size of the tumor, and Lymph node involvement. On the other hand, it was statistically non- significant for age of breast cancer patients. The present study results suggest that mammaglobin is a specific molecular marker for detection of breast cancer, discrimination between benign and malignant breast tumors, and it might be of value as a prognostic marker.

Keywords: Cancer breast, *let-7a*, RT-PCR, Prognostic factors.



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INTRODUCTION

Breast cancer is the most frequent tumor after skin malignancies, representing the second most common cancer-related mortality in women. Although TNM staging provides important clinical prognostic information, mammary tumors are known to be biologically heterogeneous with regard to therapeutic responses as well as molecular profiling (1). Early diagnosis of cancer remains a compelling challenge for clinicians; it is the ultimate goal in order to minimize treatment-associated morbidity and mortality and achieve maximal long-term survival (2). Discovery of novel classes of molecular markers in cancer has provided exciting, potentially viable biomarkers that may have utility in early cancer detection (3,4). Ideal biomarkers of tumors should be specific, sensitive, and proportional to tumor load. Early studies clearly demonstrated that circulating miRNAs and tissue miRNAs satisfy these criteria. miRNAs, as mRNA regulators, could serve as novel diagnostic and prognostic candidates, and potential therapeutic targets. Since the miRNA deregulation in breast cancer was first reported in 2005 (5), there have been many studies on the expression of various miRNAs and their roles in breast cancer. mRNA profiling studies have led to the identification of miRNAs that are aberrantly expressed in human breast cancer, with miR-10b, miR-125b and miR-145 being down-regulated and miR-21, and miR-155 being up-regulated. More recent studies have not only identified miRNA downstream targets but also associated particular miRNA expression with prognostic information. miRNA expression studies in breast cancer also revealed the importance and potential use in tumor classification and better prognosis (5). Twenty-nine differentially expressed miRNAs were identified when comparing breast cancer tissue with normal, and a subset of 15 miRNAs could be used to discriminate tumor from normal. In addition, miRNA expression correlated with clinicopathological features such as estrogen receptor (ER) and progesterone receptor (PR) expression (*miR-30*) and tumor stage (*miR-213* and *miR-203*). The differential expression of several *let-7* isoforms was associated with clinicopathological features including PR status (*let-7c*), lymph node metastasis (*let-7f*, *let-7a*), or high proliferation index (*let-7c*, *let-7d*) in tumor samples (6). The *let-7* family is one of the first tumor suppressor miRNAs to be identified. The *let-7* family is composed of 13 members in humans, with both overlapping and distinct functions (7,8). They negatively regulate target gene expression by either translational repression or mRNA cleavage, in a sequence-specific manner (9,10). The role of *let-7* in cancer was first discovered when the *let-7* family was found to negatively regulate *RAS* gene in *Caenorhabditis elegans* by binding to multiple *let-7* complementary sites in its 3' untranslated region. Reduced expression of *let-7* has been associated with shortened postoperative survival in patients with cancer (11), and forced expression of *let-7* family members is able to suppress tumor growth both *in vitro* and *in vivo* (9). The inhibitory function of the *let-7* family in cancer has been corroborated by a number of groups and in various types of tumors (10,12). A systematic review of 43 published studies shows that *let-7* is the miRNA most frequently and significantly associated with clinical outcomes in patients with cancer. The aim of this study was to investigate the utility of a circulating miRNAs (*let-7a*) as potential cancer biomarker, in particular for early-stage disease. This target miRNAs have previously been reported to be dysregulated in various malignancies and have been identified to play key regulatory roles through its functional interactions with critical cancer-associated genes.

MATERIALS AND METHODS

Patients and clinical samples

The blood samples from 55 patients with different stages of newly diagnosed Invasive Ductal Carcinoma were provided by certain Iraqi hospitals (including National center for early detection of tumors and Al-Ilweya teaching hospital) after patients underwent cytopathological (Fine needle aspiration FNA) and histopathological examination. Two control groups were used in this study, 10 samples of patients with benign breast tumors, and 20 samples from healthy donors. The required information about the patients and the histopathologic properties of the tumors were recorded from the patients' files. The samples preservation with TRIzol was done at the Genetic lab of National center for early detection of tumors in Baghdad medical city. Out of 2ml of peripheral blood that drawn into EDTA tubes, 0.5 ml was preserved as whole blood after treating with trizol (sample which was centrifuged at 1,000 xg for 5





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min. at 4°C followed by removing the supernatant and adding phosphate buffer saline (PBS) containing 5% Triton X-100 and vortexed to be homogenized then a 0.75 ml of trizol added to each sample in a ratio of 3 TRIZOL :1Sample volume) then the samples were kept at 80°C. Samples subjected to RNA extraction and molecular study by using microRNA Reverse Transcription and Real Time PCR at Molecular Oncology Unit in Guy's hospital – Kings college/London.

RNA extraction, reverse transcription and real-time RT-PCR assay

The total RNA of breast cancer, benign tumors and healthy control samples was extracted using the TRIzol® LS Reagent(Life Technologies - Ambion CO.) following the protocol provided by the manufacturer. Total RNA was reversely transcribed using TaqMan microRNA Reverse Transcription Kit. The procedure was carried out in a reaction volume of 20 µl following the protocol provided by the manufacturer (Applied Biosystem) cDNA was stored at -80 °C until use.

Analysis of miRNA Gene Expression

In this study *let 7a* miRNAs have been chosen based on its previously documented associations with malignancies (6). Real-time quantitative polymerase chain reaction (RQPCR) quantification of miRNA expression was performed using analyzed using specific primers and probes (Table 1) and TaqMan MicroRNA_ Assays (Applied Biosystems) according to the manufacturer's protocol. Total RNA was reverse transcribed using the Multi- TaqMan microRNA Reverse Transcription Kit (Applied Biosystems). RT_ controls were included in each batch of reactions. PCR reactions were carried out in final volumes of 20 µl using an ABI 7900 HT Fast Real-Time PCR System (Applied Biosystems). Reactions consisted of 1.6 µl cDNA, 10.0 µl TaqMan_ Universal PCR Fast Master Mix, and 1.0 µl TaqMan_ primer-probe mix (Applied Biosystems), and 10.0 µl Nuclease-free water. Reactions were initiated with a 2 minutes at 50°C followed by 10-minute incubation at 95°C, then 40 cycles at 95°C for 15 seconds and 60°C for 60 seconds. *miR-16* was used as an endogenous control to standardize miRNA expression. The gene expression levels and fold change were quantified by measuring the threshold cycle (Ct) which defined as the PCR cycle at which the fluorescent signal of the reporter dye crosses an arbitrarily placed threshold. The Ct is reversely related to the amount of target molecules in the reaction. The classic comparative Ct method was used to calculate the expression level of the gene of interest relative to a calibrator or reference sample using the Ct data below

$$\Delta CT \text{ sample} = Ct_{\text{sample}} - Ct_{\text{endogenous control}}$$

$$\Delta CT \text{ calibrator} = Ct_{\text{healthy control}} - Ct_{\text{endogenous control}}$$

The Ct data were normalized by using endogenous control genes(ABL and miR-16 genes). Normalization factor is the arithmetic mean or geometric mean of Ct values of the selected control genes. The normalized ΔCT data are used to calculate the relative gene expression fold change using a selected calibrator (healthy or reference sample) as below:

$$\Delta\Delta CT = \Delta CT \text{ sample} - \Delta CT \text{ calibrator}$$

$$\text{Fold Change} = 2^{-\Delta\Delta CT}$$

Statistical Analysis

The Statistical Analysis System- SAS (2010)(13) was used to effect of difference factors in study parameters or percentage. The chi-square test at the comparative between percentage & least significant difference –LSD test to the comparative between means in this study.





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RESULTS

The patients' age range was 20-70 years with high frequency of patients in the range of 40-59 years. According to the family history, 50(90.91%) of patients were have negative family history which statistically high significance differences ($X^2=13.473$ **, $p<0.01$) in comparison with patients that have positive family history. According to lymph node status, the percentage of patients number with multiple lymph nodes was higher than those with few or no lymph nodes which showed statistically high significant differences (p value $0.0017^{**}p<0.01$). In regard to the tumor size the highest percentage of patients showed the tumor size 2.0-2.9 cm. which showed statistically high significant differences (p value $0.0014^{**}p<0.01$). Relation between *miR-let 7a* gene expression and clinicopathologic parameters, are listed in Table 2. The present study used a mean value of *miR-let 7a* gene expression (fold change) in healthy control as the cutoff value to separate samples into *miR-let 7a*-positive samples and *miR-let 7a*-negative samples. The expression of *miR-let 7a* was detected at variable levels, normal (fold change \leq cutoff value = 1.562), low (fold change ≤ 4) and high (fold change > 4) in all the 85 study participants with the mean fold change of 4.1892. For *miR-let7a* the cutoff value (4-fold) used to divide breast cancer samples into high expression samples 12(26.67%) and low expression samples 33(73.33 %) (Figure 1). The percentage of *miR-let 7a*-positive breast cancer patients 81.82% ($n=45$) was significantly higher (p value= 0.0027 $p < 0.001$) when compared with *miR-let 7a*-negative patients 18.18%($n=10$) (Figure 2). The results showed that the percentage of patients with high level and with low level of *miR-let 7a* gene expression were significantly higher in comparison with benign tumor patients and healthy controls (p -value 0.0026, 0.0001 $p<0.01$, respectively) that showed normal expression.

The present study also showed high statistically significant differences between high *miR-let 7a*-mRNA expressing sample, low *miR-let 7a*-mRNA expressing samples and normally *miR-let 7a*-mRNA expressing samples 10(18.18%) (p value=0.0027 $p < 0.01$). In addition to assessing the expression levels among the study groups, other clinicopathological features associated with *miR-let 7a* expression were investigated including lymph node status, tumor size, age and family history. The identification of distribution according to the age groups of the present study showed no significant correlation between *miR-let7a* gene expression levels and patients age groups (Figure 3). The present study also showed no significant correlation between *miR-let7a* expression and patients family history (LSD Value = 0.661 NS). In correlation to the lymph node status the results of the present study showed that the percentage of *let 7a* positive patients with multiple lymph node metastasis (85.18%) and with a few lymph node metastasis (82.21%) were significantly different from patients with no lymph node metastasis (p value 0.0149 $p < 0.05$). According to the tumor size the results showed that there was increasing in the *miR-let 7a* gene expression with increasing of tumor size since the highest percentage (100%) of *miR-let 7a* positive patients were with tumor size 4.0 – 4.9 cm which showed statistically high significant differences (p value=0.0019 $p<0.01$).

DISCUSSION

The unique properties of miRNAs, including their remarkable stability, tissue-specific expression profiles, and the ease with which they are quantified, herald these molecules as ideal cancer biomarkers. Our data demonstrated that the that the level of *miR-let 7a* was significantly higher in breast cancer patients than in healthy controls. These results are comparable to those reported by Kerin *et al.*(14) who showed that the level of *miR-let 7a* was significantly higher, on average, in the breast cancer patients than in benign and healthy controls($p<0.01$), corresponding to average fold change of 11.2. Heneghan *et al.*(6) and Wang *et al.*(15) reported that *miR-let 7a* level was observed to be higher in the circulation of patients with breast cancer than in control subjects($p < 0.01$). The present study results are different from that obtained by Lorio *et al.*(5) who showed that *miR-let 7a* was down-regulated in breast cancer patients. Also this results are inconsistent with those reported by Boyerinas *et al.*(10) who showed down-regulation of *miR-let 7a* expression in breast cancer patients when compared with control subjects. In correlation to effect of clinicopathological features (age, family history, lymph node status, and tumor size) on *miR-let 7a* expression, the present study demonstrated that statistically no significant differences in the levels of gene expression with age and



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family history. In correlation to the lymph node status and tumor size, the results of the present study showed that statistically are high significant differences since the highest percentage of *let 7a* positive was observed in patients with multiple lymph node and few lymph node metastasis rather than patients with no lymph node metastasis. As well as there was increasing in the *miR-let 7a* gene expression with increasing of tumor size since the highest percentage (100%) of *miR-let 7a* positive patients were with tumor size 4.0 – 4.9 cm. These results contradicting to those reported by Wang *et al.*, (15) who showed that the expression of *miR-let 7a* was down-regulated in breast cancer samples with lymph node metastasis, suggesting that a reduced *miR-let 7a* expression could be associated with a poor prognosis. Kerin *et al.*, (14) reported that lymph node positive patients were found to have significantly lower levels, on average, of circulating *miR-let 7a* compared to those with node negative disease (p value = 0.002 $p < 0.01$).

Let-7 is widely viewed as a tumor suppressor miRNA. Consistent with this activity, the expression of *let-7* family members is down-regulated in many cancer types when compared to normal tissue and during tumor progression. For some forms of cancer, most or all *let-7* family members appear to be down-regulated (12, 15,16). The down regulation of *miR-let 7a* may be explained by the interaction of *let 7a* with its target mRNA, the *KRAS* oncogene, at the cellular level. An evidence proposes that dysfunctional interaction between *let 7a* and *KRAS*, resulting from a single nucleotide polymorphism in the *let 7a* complementary site in the *KRAS* 3' untranslated region, prevents *let 7a* from binding and exerting its tumor suppressor effect, resulting in over-expression of oncogene (17). A plausible hypothesis is that this particular failure of miRNA and mRNA to bind could lead to lower expression levels of *let 7a* in tumor tissues (18).

The present study was one of the very few studies that showed up-regulation of *miR-let 7a* gene with multiple lymph node metastasis compared with early stage, including Boyerinas *et al.* (10) who reported that *miR-let 7a* levels increased in late stage compared with early stage of cancer. Less frequent, up-regulation of certain *let-7* family members has also been observed, suggesting that *let 7* does not play a tumor suppressor function under all circumstances and / or in all tissues. The up-regulation of *let-7b* and *let-7i* was associated with high grade transformation in Lymphoma (19,20), indicating that increased expression of *let-7* family members could be used as a prognostic marker to identify patients at risk of high grade transformation, or for higher grade cancer. The most detailed mechanistic analysis of an up-regulated *let-7* family member was performed for *let-7a*. Hypomethylation of the *let-7a* locus was found to cause higher expression of *let-7a* in epithelial ovarian cancer (21) and lung cancer (22).

This study first focused on the rule of *miR-let 7a* expression in discrimination between malignant and benign tumor rather than healthy controls. It was found that *miR-let 7a* was significantly over-expressed in breast cancer patients compared to benign tumor patients and healthy controls, which suggest the diagnostic value of this microRNA in breast cancer. The present study also focused on the potential relationship between the expression levels of significantly over-expressed *miR-let 7a* and various breast cancer clinicopathological features, the study observed significant correlation between *miR-let 7a* expression and advanced clinical stage (multiple lymph node status and large tumor size), which indicates that *miR-let 7a* may be a good candidate as a molecular prognostic marker in breast cancer.

CONCLUSION

The present study results showed that *miR-let 7a* expression can be consider as a promising diagnostic marker for breast cancer since it's have the ability to discriminate between malignant, benign breast tumors and healthy controls. It's also can be considered as potential prognostic marker since its overexpression associated with multiple lymph node status which reflect its role in breast cancer metastasis and its prognostic value which in turn can be target for breast cancer therapy and monitoring tumors metastasis.





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Ethics approval and consent to participate

The current study was approved by the Institution of Genetic Engineering and Biotechnology Committee and under agreement of Iraqi MOH.

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Table 1. Primers and Probes sequences

Primers and Probes used with RT-qPCR		
Primer	Sequence	Melting temperature
MGB 1-F	5'-TGCCATAGATGAATTGAAGGAATG-3'	47.2 C°
MGB 1-R	5'-TGTCATATATTAATTGCATAAACACCTCA-3'	47.9 C°
MGB 1-P	5'-TCTTAACCAAACGGATGAACTCTGAGCAATG-3'	55.5 C°
ABL-F	5'-TGGAGATAACACTCTAAGCATAACTAAAGGT-3'	C°
ABL-R	5'-GATGTAGTTGCTTGGGACCCA-3'	C°
ABL-P	5'-CCATTTTTGGTTGGGCTTCACACCATT-3'	C°

Table 2: Relation between miR-let 7a gene expression and clinicopathologic parameters.

Variable	Total	miR-let 7a Expression				P-value
		Expression		No		
		No.	%	No.	%	
Breast cancer						
Benign tumors						
Healthy controls						
Age groups						
20-29	2					
30-39	11					
40-49	15					
50-50	15					
60-70	12					
Tumor size (cm)						
1.0-1.9	14	12	85.71	2	14.29	0.0001
2.0-2.9	19	12	63.16	7	36.84	0.0024
3.0-3.9	18	17	94.45	1	5.55	0.0001
4.0-4.9	4	4	100	0	0	0.0001
Lymph node status						
Negative	9	6	66.67	3	33.33	0.0027
Few	19	16	82.21	3	15.79	0.0025
Multiple	27	23	85.18	4	14.82	0.0025
Total	55	45	81.82	10	18.18	0.0015





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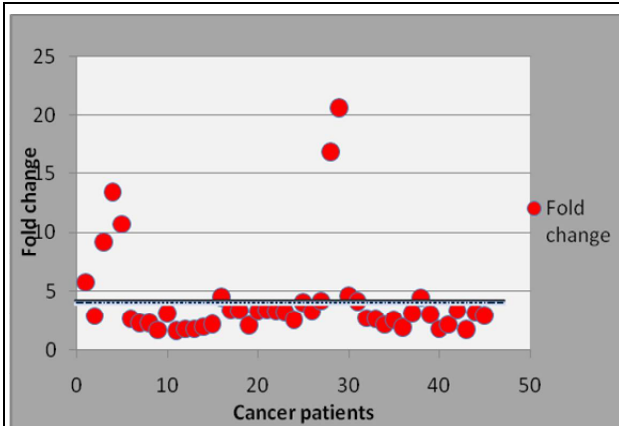


Figure 1. Differences in *miR-let 7a* gene expression levels according to fold change in breast cancer patients (n=45)

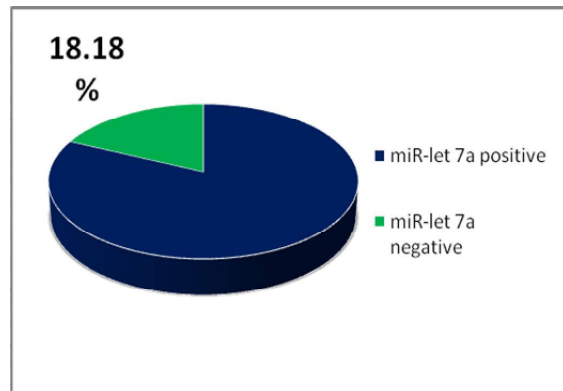


Figure 2. Distribution of breast cancer patients according to *miR-let 7a* gene expression

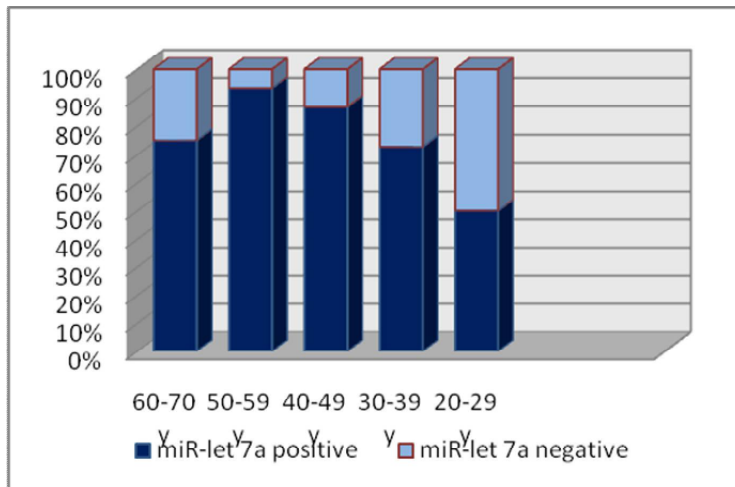


Figure 3. Differences in the percentage of *miR-let 7a* gene expressing samples with patients age groups





Typhoid Relapse, Trends and Management Practices in Pakistan

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Received: 19 Jan 2019

Revised: 22 Feb 2019

Accepted: 25 Mar 2019

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ABSTRACT

Background: The occurrence of Typhoid fever has significantly increased in the past few decades and it is now considered as the major cause of mortality in Pakistan. The treatment and diagnosis of this endemic condition is still challenging due to amplified antibiotic resistance and relapse cases. **Objective:** This study aims to evaluate practices of health care providers in relation to the follow-up routine of the patients, factors promoting relapse and resistance and complications observed during treatment. **Methodology:** This cross-sectional survey was conducted in all four provinces of Pakistan i.e. Sindh, Punjab, Baluchistan & Khyber Pakhtunkhwa during September 2018 - November 2018. Total 949 health-care providers participated in this survey. The study was designed to collect information related to the follow-up patterns, relapse, resistance and complications of the typhoid patients. Statistical analysis was done on statistical package for social science (SPSS version 22.0). **Results:** A high rate of relapse cases were reported in comparison to resistance i.e. 69% vs 31%. Out of the total, around 90% clinicians recommended a follow-up visit after treatment completion. 35.6% of the patients visited health care providers after 3 weeks of discontinuing the therapy. Gastrointestinal, Neuropsychiatric, Respiratory and Cardiovascular complications were observed in health care setting during long-term typhoid treatment. **Conclusion:** The reason behind the increase in number of relapse cases might be due to patient careless attitude towards follow-up. Patient's awareness regarding the relapse and management of typhoid can be the key solution.

Keywords: Typhoid Fever, Relapse, Resistance, Follow-Up Visits, S. Typhi.





INTRODUCTION

The complications in the treatment of enteric fever have increased with the passage of time not only because of rising expenditure but also due to the resistant *S. Typhi* species against the antibiotics used for treatment purpose. In addition to this, typhoid fever is also associated with numerous nonspecific symptoms and complications that cause life-threatening effects which preserves the poverty cycle and enhances the financial burdens[1]. Each year around 22 million new typhoid cases are reported and the mortality rate among the typhoid patients is rapidly increasing especially among low resource areas [2]. The major management strategy in this regard is the appropriate use of antibiotics, while misuse or overuse of antibiotics leads to antibiotic resistance [3]. According to the Indian Association of Pediatrics (IAP) task force, proper timely management of typhoid fever is necessary to reduce the morbidity and mortality [4]. It is recommended that proper diagnosis, appropriate disease monitoring, treatment of associated complications and accurate antibiotics use leads to favorable outcomes[4]. Relapse of typhoid fever results mainly due to reinfection or recrudescence[5]. The relapse usually occurs 1 to 3 weeks after the recovery from the first typhoid episode and is milder than the first one[6]. The relapse is normally termed as recrudescence if the strains of initial and second attack are identical. While the reinfection occurs when the strains of the first and second attack are different [7&8]. The typhoid patients are recommended for follow-up 3 months after treatment completion to identify relapse or further complication associated with the previous infection [9].

Around 5-10% of all typhoid cases undergo relapse after initial recovery[9]. Usually, it occurs within one week after therapeutic discontinuation but in rare cases, it has also been reported 70 days later[9&10]. The typhoid relapse cases can be managed with the same choice of drugs as that for the first attack as these patients already have isolates with antimicrobial susceptibility. While the reinfection can be distinguished by the differences in the susceptibility pattern[7&8]. Despite of the fact that typhoid relapse is milder but it must be treated with the same line of action as the first incident. Usually, recovery can be seen within one to two days of appropriate antibiotic use. The sufferers after effective recovery become long term silent carriers and may have recurrent typhoid relapses later in their life[11]. However, prolonged use of antibiotics are preferred for the cure of these carriers but the recommendation is exclusive for the patients with gallstones[12]. As this endemic disease is common in Asian countries and spreads rapidly. Early identification of disease development and complications, close follow-up is required for appropriate therapeutic approach and controlled typhoid associated mortality. Through this study, our aim was to evaluate the clinical practice of health care providers in relation to the follow-up routine of the patients, factors promoting relapse and complications observed during treatment.

MATERIALS AND METHODS

This cross-sectional survey was conducted in all four provinces of Pakistan i.e. Sindh, Punjab, Balochistan & KPK during September 2018 -November 2018. Study was designed under declaration of Helsinki and independent ethical committee approval was also obtained from Pakistan Medical Association Committee on ethics (Reference Number: OB/213/PMC/16). Total 949 health-care providers participated in this survey. A paper based form was designed for systematic data collection. Data collectors designated by the investigator, fully informed the health-care providers about terms and conditions, objectives, constraints and duration. Data was collected by means of a structured questionnaire including seven closed-ended questions. The study was designed to collect information related to the follow-up patterns, relapse, resistance and complications of the typhoid patients. The collected data was then coded and entered into a database. Double entry of data was done by 3rd party data punch operator on Epidata v.3.1. Statistical analysis was done on statistical package for social science (SPSS version 22.0).





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RESULTS

Initially, the survey was distributed among 1800 health care providers out of which responses were received from 949. Table 1 indicates that 66.8% of the health care providers recommend follow-up visit 1 month after the treatment completion while 15.8% agreed with the duration of 2 months and 17.4% said that the follow-up duration can extend up to 3 months after completion of typhoid treatment. It was evident through the study results that typhoid relapse was more common as compared to resistance. According to the data provided by the health care providers 36.9% patients visited 3 weeks after therapy discontinuation, 33.7% visited with typhoid relapse after 2 weeks, while 29.4% were those who visited the clinics within a week with adverse effects due to treatment discontinuation. Gastrointestinal, Neuropsychiatric, Respiratory and Cardiovascular complications were observed in health care setting during long-term typhoid treatment (Table 2).

DISCUSSION

The management of Enteric fever is still a challenge even after a hundred years of the *Salmonella Typhi* isolation by Gaffkey in 1884 [13]. The absence of appropriate diagnostic technique results in decreased cure rate and increases fatality. Therapeutic strategies must target the antibiotic sensitivity of *S. Typhi*. Proper sanitation, prompt diagnosis, effective vaccination and appropriate timely treatment are necessary for disease control in endemic areas [13]. The resistant *S. Typhi* strains must be tested with advanced molecular methods in laboratories under expert supervision [14]. Whereas in countries with no such diagnostic facilities regional collaboration with neighboring country is recommended. Help can also be acquired through a World Health Organization (WHO) Collaborating Center for this purpose [14]. It is evident from previous literature that the bacterium of enteric fever sheds off for up to 3 months from the feces of untreated typhoid patient. Hence, it is recommended that 3 stool cultures must be performed after 1 month of treatment completion in order to identify carrier state [9]. On the basis of our findings, close follow-up visit was highly recommended for disease monitoring by most of our enrolled clinicians (Table 1). Mostly the clinicians recommended the first follow-up visit, 1 month after the completion of therapy while 19% opted for 3 months duration for the first follow-up visit (Table 1). John and his colleagues in a similar study recommended that infected patients must re-visit the clinicians for 3 months after treatment in order to avoid relapse [15]. Moreover, they concluded that the follow-up must be taken within a week to avoid all sorts of uncertainty. If there are symptoms of recurrence, it is mandatory to recheck it diagnostically. Patients with positive results must undergo retreatment to clear out infectious agents [9&16].

The relapse rate reported by the health care providers in our study is consistent with previous literature i.e. 69% relapse cases and only 31% resistance cases were reported (Table 1). According to a study, 27 million typhoid cases are reported per year with a relapse rate greater than 5% to 20%. Despite of increased relapse rate the factors leading to relapse are still unknown [17&18]. Around 35.6% clinicians reported that typhoid patients re-visited their clinics with adverse effects within 3 weeks after discontinuing prescribed antibiotics (Table 1). Also supported by a study published in Annals of Saudi medicine, typhoid relapse is usually associated with medication non-adherence and the recurrence is observed within 2-3 weeks after therapy discontinuation [19]. Typhoid patients are exposed to numerous complications due to the fact that *S. typhi* holds capacity to cause damage to every system in the human body. Intestinal perforation (1-3% cases) associated with high mortality rate [20-22]. Moreover, complication associated with cardiovascular system include myocarditis leading to heart failure, hepatitis leading to liver failure, pneumonia causing respiratory failure and intravascular coagulation, arthritis, and orchitis are also observed in rare cases [20]. Similar trend was observed in our study, the health care providers observed various complications associated with different systems of the body. Where Intestinal haemorrhage among the gastrointestinal complications, Disorientation among neuropsychiatric, Cough among respiratory and Weak pulse among cardiovascular complications over-shadowed the other less common complications observed during the treatment duration (Table 2).





CONCLUSION

The reason behind the increase in number of relapse cases might be due to patient careless attitude towards follow-up. Furthermore factors for medication compliance should be identified in future studies. Patient's awareness regarding the relapse and management of typhoid can be the key solution. As this infectious disease is widespread in Pakistan, proper vaccination, sanitation and hygienic lifestyle must be maintained to avoid this endemic condition. Government health Sector must initiate sanitation/health campaigns and programs must be organized to educate the local population for maintaining personal hygiene as well.

ACKNOWLEDGEMENTS

We would like to express our special gratitude to the entire team involved in this study. We are thankful for their support throughout the study.

Funding

This study was supported by Advance Educational Institute & Research Center.

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Table 1. Shows the observation, recommendation provided by health care provider.

		n	%
Follow up recommendation after treatment of typhoid	<i>no</i>	91	9.6
	<i>yes</i>	858	90.4
Follow-up duration advised to typhoid patients after completion of treatment	<i>1 month</i>	634	66.8
	<i>2 month</i>	150	15.8
	<i>3 month</i>	165	17.4
Type of typhoid cases reported in clinical practice	<i>Relapse</i>	676	71.2
	<i>Resistance</i>	273	28.8
Observed relation between relapse of the disease and medication compliance	<i>Always</i>	194	20.4
	<i>Most of the time</i>	391	41.2
	<i>Occasionally</i>	298	31.4
	<i>Never</i>	66	7
Duration of visit after discontinuation of therapy in a relapse patient	<i>1 week</i>	279	29.4
	<i>2 week</i>	320	33.7
	<i>3 weeks or more</i>	350	36.9

Table 2. Complications observed in health care setting during long-term typhoid treatment

		n	%
Gastrointestinal complications	<i>Intestinal haemorrhage</i>	706	74.4
	<i>Perforation</i>	243	25.6
Neuropsychiatric complications	<i>Disorientation</i>	854	90
	<i>Delirium</i>	56	5.9
	<i>Convulsions</i>	39	4.1
Respiratory complications	<i>Cough</i>	881	92.8
	<i>Pneumo typhoid</i>	44	4.6
	<i>Ulceration of posterior pharynx</i>	24	2.5
Cardiovascular complications	<i>ECG changes (Nonspecific)</i>	57	6
	<i>Tachycardia</i>	106	11.2
	<i>Weak pulse</i>	699	73.7
	<i>Hypotension</i>	87	9.2





Modified Decomposition Method for Analytic Treatment of Non-linear Volterra- Fredholm-Hammerstein Integral Equations of the Second Kind

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Received: 15 Dec 2018

Revised: 17 Jan 2019

Accepted: 20 Feb 2019

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ABSTRACT

In this article, non-linear Volterra-Fredholm-Hammerstein integral equations of the second kind (NVFHIEK2) considered. The modified Adomian decomposition method is been used to solve the NVFHIEK2. Some illustrative examples are prepared to show the pertinent features of the method. The results reveal that the MAD method is very effective and simple and gives the exact solution.

Keywords: Modified decomposition method, non-linear Volterra- Fredholm-Hammerstein integral equations.

INTRODUCTION

Recently a great deal of interest has been focused on the applications of the Adomian method to solve a wide variety of stochastic and deterministic problems [1]. The solution is the sum of an infinite series which converges rapidly to the accurate solutions. In this paper, we extend the modified decomposition method to solve non-linear Volterra-Fredholm-Hammerstein integral equations of the second kind. A non-linear Volterra-Fredholm-Hammerstein integral equation [3] can be written as the following:

$$u(x,t) = f(x,t) + \int^x k_1(x,t) R(u(x,t))dt + \int^1 k_2(x,t)N(u(x,t))dt, \quad (1)$$

where $u(x, t)$ is an unknown function that will be determined $k_1(x,t)$, $k_2(x,t)$ is the kernel of the integral equation, $f(x, t)$ is an analytic function, and $R(u)$ and $N(u)$ are the linear (non-linear) function of u . The concepts of integral equation have attracted much interest in recent years for analytical and numerical treatments. Due to the increased





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interest in non-linear integral equations, abroad classes of analytical and numerical solution methods have been used to handle these problems [4]. However, this analytical solution method is not easy to use and require tedious work and knowledge. To overcome the tedious work involved in the existing strategies and to minimize the proliferation of terms in the Adomian scheme, the modified Adomian decomposition method is studying. In this paper, we aim to obtain analytical solutions for non-linear Volterra-Fredholm-Hammerstein integral equation of the second kind. Although the modified forms introduce a slight change in the formulation of the Adomian recursive relation, it provides a qualitative improvement over standard Adomian method. In addition, the modified technique may give the exact solution for non-linear equation without any need for the Adomian polynomials. Although this slight variation is quite simple, it demonstrates the reliability and the power of the modification.

The Modified decomposition method

The standard Adomian method defines the solution $u(x)$ by the series

$$u(x,t) = \sum_{i=0}^{\infty} u_i(x,t) . \quad (2)$$

Substituting the series decomposition (2) into both sides of equation (1) and assuming that the function f can be expressed as the sum of two parts, f_0 and f_1 . Therefore we set

$$f = f_0 + f_1 . \quad (3)$$

In view of this assumption, we propose a slight variation is that only the part f_0 is assigned to the zeroth component u_0 and the remaining part f_1 is combined with the other terms (integral part) to define u_1 , based on the suggestion, we formulate the following modified decomposition method:

$$\sum_{i=0}^{\infty} u_i(x,t) = f_0(x,t) + f_1(x,t) + \int_0^x k_1(x,t) R \left(\sum_{i=0}^{\infty} u_i(x,t) \right) dt + \int_0^1 k_2(x,t) N \left(\sum_{i=0}^{\infty} u_i(x,t) \right) dt . \quad (4)$$

The modified decomposition method introduces the use of the recurrence relation

$$\begin{aligned} u_0(x,t) &= f_0(x,t) , \\ u_1(x,t) &= f_1(x,t) + \int_0^x k_1(x,t) R(u_0(x,t)) dt + \int_0^1 k_2(x,t) N(u_0(x,t)) dt , \\ u_{k+2}(x,t) &= \int_0^x k_1(x,t) R(u_{k+1}(x,t)) dt + \int_0^1 k_2(x,t) N(u_{k+1}(x,t)) dt , \quad k \geq 0 . \end{aligned} \quad (5)$$

As stated before, we may need only two iterations to derive the exact solution. If more than two iterations are needed, $N(u(x,t))$ should be represented by Adomian polynomials which can easily be generated for all types of non-linearity. The choice of $f_0(x,t)$





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Terms has a strong influence on facilitating the recurrence relation, and as a consequence, accelerates the convergence of the solution. Also the exact solution must be a term of $f(x, t)$ or to be a part of series of $f(x, t)$. This means that the success of this method depends mainly on the proper choice of f_0 and f_1 . We have been unable to establish any criteria to judge what forms of f_0 and f_1 can be used to yield the acceleration demanded. At present f_0 and f_1 are selected by trials. Several illustrative examples are used to show the pertinent features of the modified method.

The Noise Terms phenomenon

The noise terms phenomenon gives a useful tool in that, if it appears, it gives a fast convergence of the solution by using two iterations only. It is significant to note that the noise terms may appear only for inhomogeneous problems. It is important to note that these terms may appear for inhomogeneous problems, whereas homogeneous problems do not general noise terms [5]. A necessary condition for obtaining exact solution in minimum iteration (only two iterations) is that the exact solution must be a part of noise terms (or a part of series of noise terms). To give a clear overview of the content of this work, several illustrative examples of *NVFHIEK2* have been selected to demonstrate the efficiency of the method and to confirm the necessary condition for obtaining exact solution.

Illustrative Examples

The method of this paper is useful for finding the solutions of non-linear *VHIEK2* in terms of modified decomposition method. We illustrate this by solving the following five examples

Example 1: [3]

Consider a non-linear *VFHIEK2*'s:

$$u(x) = x - 1 - \exp(x - 1) + \exp(-1) + \int_0^x ((x - t) \exp(u(t)))dt + \int_0^1 (xt \exp(u(t)))dt .$$

We decompose $f(x)$ as follows:

$$f_0 = x - 1,$$

$$f_1 = \exp(-1) - \exp(x - 1) .$$

The modified decomposition technique admits the use of the recursive relation given in the form

$$u_0 = x - 1 ,$$

$$u_1 = \exp(-1) - \exp(x - 1) + \int_0^x (x - t) \exp(u_0(t))dt + \int_0^1 xt \exp(u_0(t))dt .$$

This gives





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$$u_1 = \exp(-1) - \exp(x-1) + \int_0^x (x-t) \exp(u_0(t)) dt + \int_0^1 xt \exp(u_0(t)) dt .$$

This gives

$$u_0 = x - 1 ,$$

$$u_1 = \exp(-1) - \exp(x-1) + \int_0^x (x-t) \exp(t-1) dt + \int_0^1 xt \exp(t-1) dt = 0$$

It follows immediately that $u_k = 0, k \geq 2$, and the exact solution

$$u(x) = x - 1$$

readily obtain

Example 2:

Solve a non-linear VFHIEK2's:

$$u(x) = 3x - \frac{1}{4}(x^2 + 1) + \frac{1}{2}x^2 \ln(x) - x \ln(x) + \int_0^x (1-t) \ln(u(t)) dt + \int_0^1 (x-t) \ln(u(t)) dt$$

We decompose $f(x)$ as follows:

$$f_0 = x ,$$

$$f_1 = 2x - \frac{1}{4}(x^2 + 1) + \frac{1}{2}x^2 \ln(x) - x \ln(x) .$$

The modified decomposition technique admits the use of the recursive relation given in the form

$$u_0 = x ,$$

$$u_1(x) = 2x - \frac{1}{4}(x^2 + 1) + \frac{1}{2}x^2 \ln(x) - x \ln(x) + \int_0^x (1-t) \ln(u_0(t)) dt + \int_0^1 (x-t) \ln(u_0(t)) dt .$$

This gives

$$u_0 = x ,$$

$$u_1(x) = 3x - \frac{1}{4}(x^2 + 1) + \frac{1}{2}x^2 \ln(x) - x \ln(x) + \int_0^x (1-t) \ln(t) dt + \int_0^1 (x-t) \ln(t) dt = 0.$$





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It follows immediately that $u_k = 0, k \geq 2$, and the exact solution

$$u(x) = x,$$

readily obtain.

Example 3

Solve a non-linear VFHIEK2's:

$$u(x) = 2x - 2 - x \sin(x - 1) - x \cos(1) + \cos(1) - \cos(x - 1) - \sin(1) + \int_0^x t \cos(u(t))dt + \int_0^1 (x - t) \sin(u(t))dt$$

We decompose $f(x)$ as follows:

$$f_0 = x - 1,$$

$$f_1 = x - 1 - x \sin(x - 1) - x \cos(1) + \cos(1) - \cos(x - 1) - \sin(1).$$

The modified decomposition technique admits the use of the recursive relation given in the form

$$u_0 = x - 1$$

$$u_1(x) = x - 1 - x \sin(x - 1) - x \cos(1) + \cos(1) - \cos(x - 1) - \sin(1) + \int_0^x t \cos(u_0(t))dt + \int_0^1 (x - t) \sin(u_0(t))dt$$

This gives

$$u_0 = x - 1,$$

$$u_1(x) = x - 1 - x \sin(x - 1) - x \cos(1) + \cos(1) - \cos(x - 1) - \sin(1) + \int_0^x t \cos(t - 1)dt + \int_0^1 (x - t) \sin(t - 1)dt = 0.$$

It follows immediately that $u_k = 0, k \geq 2$ and the exact solution $u(x) = x - 1$,

Readily obtain.

CONCLUSION

This paper presents the use of the reliable modified Adomian decomposition method for solving non-linear Volterra-Fredholm-Hammerstein integral equations of the second kind. The modified Adomian decomposition method is implemented in a straight forward manner and provided significant improvement by requiring only





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two iterations to obtain the exact solution in most cases. Accelerating the convergence of the modified Adomian method requires that the exact solution must be a part of $f(x, t)$ or series of $f(x, t)$.

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A Study on Effects of Compounds from *Alpinia ocinarum* Hance on Antiviral Activity

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Received: 15 Dec 2018

Revised: 17 Jan 2019

Accepted: 20 Feb 2019

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ABSTRACT

Dengue viruses classified into 4 serotypes (DV1, 2, 3 and 4) are etiological agents of dengue fever (DF) and dengue hemorrhagic fever (DHF) that are arthropod-borne diseases and cause a serious global health problem. There are no specific approved drugs or vaccines for the treatment or prevention of infectious DV and there are very few compounds known to inhibit the replication of this virus. The purpose of this investigation was to extract and identify the bioactive phytochemicals from smaller galanga (*Alpinia officinarum* Hance). The chloroform extract of this plant and Structural elucidation of the isolated compounds was carried out on the basis of spectral analyses showed that these compounds were galangin, kaempferide and kaempferide-3-O- β -D-glucoside, which had not been previously reported in this species. Galangin or 3,5,7-trihydroxyflavone and its derivatives are a flavonoid compounds which have various pharmacological properties including, bactericidal, anticancer, antioxidant and anti-inflammatory. This study aimed to investigate antiviral activity of galangin and its derivatives, extracted from *Alpinia officinarum* Hance on enveloped DNA and RNA viruses including herpes simplex virus type 1 (HSV-1) and dengue virus type 2 (DV-2). Antiviral activities at two steps of the viral infection including pre- and post-entry to the host cells were investigated using HSV-1 strain KOS and DV-2 strain 16681. Sub-cytotoxic concentration of galangin was analyzed by MTT assay. Antiviral activity was determined by plaque reduction assay, virus yield reduction assay, polymerase chain reaction (PCR), western blot and reverse transcriptase PCR. The results showed that sub-cytotoxic concentrations of galangin tested in Vero and A549 cells were 6.67 and 8.00 μ g/ml, respectively. Galangin had no anti-HSV-1 and anti-DV-2



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activities at post-entry step. However, at pre-entry step, galangin exhibited anti-HSV-1 infection by reduction of plaque, whereas no inhibitory effect on DV-2 infection. To confirm the anti-HSV-1 activity, The HSV-1 DNA and proteins were detected by PCR and western blot analysis. The results showed that the HSV-1 DNA and proteins were not detected at 72 hours after infection. The results demonstrated that galangin has anti-HSV-1 infection in the step before viral entry to the host cells and it would be a promising new candidate for HSV-1 therapeutics.

Keywords: Dengue virus, dengue hemorrhagic fever, *Alpinia officinarum* Hance, anti-viral activity

INTRODUCTION

In recent years, many natural compounds have been studied for their ability to prevent and treatment different of several diseases. Galangin or 3,5,7-trihydroxyflavone and its derivatives, a naturally occurring flavonoid, is a polyphenolic compound extracted from *Alpinia officinarum* Hance (1-4). It has been attributed to a wide variety of mechanisms including anti-cancer by such as apoptosis of hepatocellular carcinoma (5). The bactericidal activity of galangin against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* has been reported (6, 7). In addition, galangin and its derivatives have been found to have anti-viral activity and anti-inflammatory (8-10). Acute, recurrent and chronic herpes simplex viruses (HSV) infections occur worldwide. HSV-1 and HSV-2 are members of the *Herpesviridae* family. They cause recurrent orolabial and genital infections. HSV-1 is a widely prevalent DNA virus that causes a range of human diseases. Persistent mucocutaneous lesions of HSV can also lead to disseminated visceral infections, such as esophagitis, hepatitis, or pneumonia, as well as meningoencephalitis, in immunocompromised hosts⁽¹¹⁻¹⁴⁾. Acyclovir is a drug of choice for herpes treatment and newer anti-viral agents are valacyclovir and famciclovir. However, these drugs are expensive for Thai peoples and have to be imported. Dengue viruses classified into 4 serotypes (DV1, 2, 3 and 4) are etiological agents of dengue fever (DF) and dengue hemorrhagic fever (DHF) that are arthropod-borne diseases and cause a serious global health problem. There are no specific approved drugs or vaccines for the treatment or prevention of infectious DV and there are very few compounds known to inhibit the replication of this virus. Dengue virus (DV) is an RNA virus in the *Flaviviridae* family and is an important public health problem, especially in Thailand. It causes a variety of clinical syndromes ranging from a self-limiting febrile illness to a severe hemorrhagic fever and a shock syndrome⁽¹⁵⁾. At present, there are no effective anti-viral drugs for treatment DV infection.

There are many Thai medicinal herbs of which anti-viral action is considered to be largely nonspecific. Some compounds have been extracted but no further study of antiviral activity. This study aimed to investigate antiviral activity of the galangin and its derivatives, extracted from *Alpinia officinarum* Hance on the DNA virus, HSV-1 and the RNA virus, DV-2.

MATERIALS AND METHODS

Preparation of compounds

The fresh rhizomes of smaller galangal were digged from Saengduan Konekratoke's paddy field located in Chokchai District, Nakhon Ratchasima Province in July and December 2007, June and November 2008. The plant specimen has been deposited at the National Herbarium after it was identified by Dr. Paul J. Grote, School of Biology, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima Province. The rhizomes of smaller galanga was separated from the stems, washed thoroughly, and dried in an oven at 50 °C for three days. The dried samples were then ground to powder the powder was sequentially extracted with hexane, chloroform and methanol respectively. Galangin and its derivatives isolated from these crude extracts as described previously



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(2) and dissolved in dimethyl sulfoxide (DMSO; Amresco®; Solon, Ohio, USA) to make a stock solution at a concentration of 5 mg/ml. DMSO did not exceed 0.5% in each experiment and this percentage did not interfere with cell growth.

Cell lines

Vero cell line, from African monkey kidney, was cultured in Opti-Eagle's minimal essential medium (Opti-MEM; Gibco-BRL, Gaithersburg, MD, USA) supplemented with 2% fetal bovine serum (FBS; Gibco-BRL, Gaithersburg, MD, USA) at 37°C in a humidified, 5% CO₂ atmosphere incubator. C6/36, a mosquito cell line from *A. albopictus*, was cultured in Leibovitz's L-15 medium (Gibco-BRL, Gaithersburg, MD, USA) supplemented with 1% tryptose phosphate broth, and 10% FBS at 28°C without CO₂. LLC-MK₂ cell line, from Rhesus monkey kidney, was cultured in Opti-MEM supplemented with 2% FBS at 37°C in a humidified, 5% CO₂ atmosphere incubator. A549 cell line, type II human lung alveolar epithelial cell carcinoma, was cultured in Roswell Park Memorial Institute medium 1640 (RPMI-1640) (Gibco-BRL, Gaithersburg, MD, USA) with 10% FBS at 37°C in a humidified, 5% CO₂ atmosphere incubator. HSV-1 strain KOS was propagated and titrated by plaque assay in Vero cells. DV2 strain 16681 was propagated in C6/36 cells and titrated by fluorescent focus assay in LLC-MK₂ cells⁽¹⁶⁾. The titer of virus used in each experiment was 0.01 multiplicity of infection (MOI).

Virus preparation

The stocks of DV2 strain 16681 were prepared in C6/36 cell line and titrated by immune fluorescence technique and confirmed by reverse transcriptase polymerase chain reaction (RT-PCR)

Cytotoxicity study of the compounds from *Alpinia officinarum* Hance

Vero and A549 cells were grown and treated with different concentrations of galangin in 96-well tissue culture plates (Costar®; Corning Inc., Cambridge, New York, USA). After incubation at 37°C for 3 days, the number of viable cells in each well was determined by adding 3-(4,5-Dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT, (SIGMA®; Sigma-Aldrich, Saint Louis, Missouri, USA)) solution to a final concentration of 0.5 mg/ml. The cells were incubated at 37°C for 4 hrs. The formazan crystals produced in the cell was dissolved with 100 µl of DMSO and optical densities (OD) were quantified at a wavelength of 540 nm using an ELISA reader (Tecan Sunrise™). Cell viability was evaluated as the percentage of the mean value of OD resulting from the three cell controls, which was set 100%. The 50% cytotoxic concentrations (CC₅₀) of galangin were calculated from the mean dose-response of three independent assays.

Anti-viral activity of galangin at pre-entry step⁽¹⁷⁾

HSV-1 and DV-2 were pretreated with or without galangin (0 - 6.67 µg/ml) for 1 hr at 37°C and subsequently 50 µl of the mixture were adsorbed on Vero and A549 cells, respectively. Dextran sulfate (1 mg/ml) was used as a positive control. In order to exclude any influence of DMSO, all experiments included media containing the same concentration of DMSO as the compound-treated viruses. The cells were incubated at 37°C for 1 hr for viral adsorption. After washing, the cells were cultured in media at 37°C for 3 days.

Anti-viral activity of galangin at post-entry step

HSV-1 and DV-2 were adsorbed on Vero cells and A549, respectively at 37°C for 1 hr, then the unadsorbed viruses were removed. The cells were incubated in media with galangin (0 - 6.67 µg/ml) at 37°C for 3 days. Acyclovir (ACV)



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and ribavirin were used as positive controls of HSV-1 and DV-2, respectively. In order to exclude any influence of DMSO, all experiments included media containing the same concentration of DMSO as the compound-treated cells.

Assessment of anti-HSV-1 activity by plaque reduction assay

In the experiments of pre- and post-entry step, carboxymethyl cellulose (CMC, SIGMA®; Sigma-Aldrich, Saint Louis, Missouri, USA) was added in the media in the concentration of 0.8% w/v before the cells were cultured at 37°C for 3 days. Then the cells were fixed with 10% formalin for 30 min and stained with crystal violet for 30 min. After that, the cells were washed with water and the plaques were counted.

Assessment of anti-HSV-1 activity by virus yield reduction assay

After the experiment of pre- or post-entry step, the supernatant of each experiment was collected and made a serial 2-fold dilution with MEM. A 50 µl of each dilution was adsorbed on Vero cells at 37°C for 1 hr. After washing, the cells were incubated for 3 days at 37°C. The cells were fixed with 10% formalin for 30 min and stained with crystal violet for 30 min. After that, the cells were washed with water and the plaques were counted.

Assessment of anti-HSV-1 activity by polymerase chain reaction (PCR)

After the experiment of pre- or post-entry step, DNA was extracted from the treated cells using the PUREGENE™ DNA purification system (PUREGENE®; Gentra Systems Inc., Minnesota, USA). The presence of HSV-1 DNA was determined by PCR assay as previously described.⁽¹⁸⁾ The 194-bp amplified PCR product was determined using 1.5% agarose gel electrophoresis.

Assessment of anti-HSV-1 activity by western blot analysis

After the experiment of pre- or post-entry step, the treated cells were lysed with a lysis buffer and protein was extracted. The extracted protein was determined for HSV-1 glycoprotein B (gB) by western blot using primary polyclonal antibodies against gB (kindly provided by Dr. Gary H. Cohen and Dr. Roselyn J. Eisenberg) and the secondary antibody, horseradish peroxidase (HRP)-conjugated goat anti-rabbit immunoglobulin G (Zymed®; Invitrogen Co., California, USA) according to the protocol of the previous study.⁽¹⁸⁾

Assessment of anti-DV-2 activity by reverse transcriptase polymerase chain reaction (RT-PCR)

After A549 cells had been infected with DV-2 for 3 days, RNA was extracted from the cells using TRIzol® Reagent (Invitrogen™, Carlsbad CA, USA) according to the manufacturer's protocols. DV-2 RNA was examined by one step RT-PCR using SuperScript™ III one-step RT-PCR system with platinum® Taq DNA polymerase (Invitrogen™; Foster, California, USA). Viral gene specific forward and reverse primers were 5'-TCA ATA TGC TGA AAC GCG CGA GAA ACC G-3' and 5'-TTG CAC CAA CAG TCA ATG TCT TCA GGT TC-3', respectively.⁽¹⁹⁾ Each 25 µL reaction mixture contained 5 µL of RNA sample, 1x RT-PCR buffer, and 0.2 µM for each primer, 1.6 mM MgSO₄, 200 µM dNTP and SuperScript™ III RT/platinum® Taq mix enzyme. After cDNA had been synthesized at 50°C for 30 min, DNA was denatured at 94°C for 2 min then PCR was performed for 40 cycles. Each cycle consisted of 95°C for 30 sec, 55°C for 1 min and 72°C for 1 min and followed with a final extension at 72°C for 10 min. The 511 bp PCR product was detected on 1.5% agarose gel electrophoresis.



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RESULTS AND DISCUSSION

Compounds 1, 2 and 3 were obtained and the UV, IR, ¹H NMR and ¹³C NMR spectrum showed that compounds 1, 2 and 3 are 3,5,7- trihydroxyflavone (galangin), 3,5,7-trihydroxy-4 -methoxyflavone (kaempferide) and kaempferide-3-O- -d-glucoside, respectively (Fig. 1). All spectronic data agree with those reported in the literature.(25 -28) Keampferide and kaempferide-3-O- -d-glucoside had not been previously reported in this species.

Cytotoxicity of galangin

The MTT assay using galangin at concentration of 2-40 µg/ml was performed to determine the cytotoxicity effect of galangin on Vero and A549 cells and observed 50% of cytotoxic concentration (CC₅₀) and the sub-cytotoxic concentration or the maximal concentration which was not toxic to Vero and A549 cells. The results were compared with cell control growing in medium without the galangin and showed cytotoxic effect on Vero and A549 cells in a dose-dependent manner. For Vero cells, the sub-cytotoxic concentration and CC₅₀ were 6.67 and 10.00 µg/ml, respectively. For A549 cells, the sub-cytotoxic concentration and CC₅₀ were 8.00 and 11.11 µg/ml, respectively.

Anti-HSV-1 activity of galangin

To determine the anti-HSV-1 activity of galangin, two steps of the viral infection including pre- and post-entry steps of viral life cycle were investigated by plaque reduction assay using HSV-1 strain KOS. Then the inhibitory effect was confirmed by virus yield reduction, PCR and western blot assays. At pre-entry step, galangin (3.33 and 6.67 µg/ml) and dextran sulfate (1 mg/ml) exhibited 100% inhibition of infection by plaque reduction assay (Fig. 2). The cytopathic effect (CPE) was observed only in cell control which was infected with the same titer of virus but without the addition of galangin. However, at post-entry step, the same concentration of galangin had no significant anti-HSV-1 activity whereas acyclovir completely inhibited viral plaque formation. The inhibitory activity of galangin at pre-viral entry step was further analyzed by virus yield reduction. Viral production from the treated cells at 24, 48 and 72 hr post of infection were investigated by plaque assay. The result showed 100% viral inhibition effect of galangin (Fig 3). This experiment was repeated and the inhibition of viral replication was confirmed by the detection of viral DNA and glycoprotein B (gB) using PCR and western blot, respectively. The viral DNA was not detected at 48 hr post infection whereas it was detected at 72 hr post infection in the experiment of 3.33 µg/ml galangin (Fig 4). Fig 5 shows the same result of 6.67 µg/ml galangin which the viral protein was not detected by western blot

Anti-DV-2 activity of galangin

The inhibition of DV-2 infection by galangin at pre- and post-viral entry steps of viral life cycle were investigated using DV-2 strain 16681. RNA was extracted from the treated cells at 3 days post infection. The viral RNA was detected by one-step RT-PCR using dengue virus specific primers. For the study of inhibition at pre-viral entry step, DV-2 RNA was detected in cells which were infected with DV-2 treated with galangin whereas it was not detected in cells which were infected with DV-2 treated with dextran sulfate. For the study of inhibition at post-viral entry step, DV-2 RNA was detected in DV-2 infected cells cultured in media with galangin whereas it was not detected in DV-2 infected cells cultured in media with ribavirin. These results showed that galangin had no anti-DV-2 activity at both pre- and post-viral entry steps of viral life cycle.

Galangin had a cytotoxic effect on Vero and A549 cells when the cells exposed to the compound at concentration more than 6.67 and 8.00 µg/ml, respectively. The CC₅₀ on A549 cells (11.11 µg/ml) was more than Vero cells (10.00 µg/ml). These differences depend on the characteristics of the cells. A study of galangin purchased from Aldrich, Germany showed that the maximum non-cytotoxic concentration of galangin on primary vervet monkey kidney cells was 47 µg/ml.⁽⁸⁾ The cytotoxic effect was monitored by observation of altered cell morphology. Therefore, the non-



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cytotoxic concentration also depends on the purity of compound and the method used in determination. Herpes simplex virus has a complex sequence of different steps at which antiviral agents might interfere. To investigate the anti-HSV-1 activity in detail, galangin was tested at pre- and post-entry steps of viral life cycle. Dextran sulfate which has been shown to inhibit HSV adsorption⁽²⁰⁾ and to be a potent inhibitor of the infectivities of various HSV-1 and HSV-2 strains⁽²¹⁾ was used as a positive control in inhibition study at pre-entry step. Acyclovir, a potent and selective inhibitor of HSV replication^(22, 23), was used as a positive control in inhibition study at post-entry step.

In the study of anti-HSV-1 activity of galangin at pre-entry step, pretreatment of HSV-1 with dextran sulfate prior to infection caused 100% inhibition of plaques. At a maximum non-cytotoxic concentration and a lower concentration of galangin, plaque was also reduced by 100%. This is similar to a previous study⁽²⁴⁾ which used galangin isolated from the shoots of *Helichrysum aureonitens* against HSV-1. They added 100 TCID₅₀/ml HSV-1 and 12 µg/ml galangin solution simultaneously to Vero cells and incubated at 37°C. Cells were observed daily for the appearance of a cytopathic effect (CPE). The result showed that galangin significantly inhibited HSV-1 infection. Schnitzler P, et al⁽⁸⁾ studied antiviral activity and mode of action of galangin and found that galangin reduced the plaque formation of free HSV-1 by 68.0% when compared with the untreated controls

When the inhibitory effect was confirmed by virus yield reduction assay, the same result was shown. Both concentrations of galangin and dextran sulfate inhibited virus infectivity and there was no virus production from the treated cells. However, the results were different when it was investigated by PCR. HSV-1 DNA was detected at 72 hr post infection when 3.33 µg/ml galangin was used. The result showed that 3.33 µg/ml galangin did not completely inhibit HSV-1 at pre-entry step whereas 6.67 µg/ml galangin completely inhibited HSV-1 at pre-entry step. In the study of anti-HSV-1 activity of galangin at post-entry step, 6.67 µg/ml galangin and 5 µg/ml acyclovir were added in cell culture media after the cells had been infected by HSV-1. The results showed that acyclovir completely inhibited viral plaque formation whereas galangin did not significantly inhibit viral plaque formation. For anti-DV-2 activity, galangin did not inhibit DV-2 at pre- and post-entry steps. These results suggested that anti-HSV-1 activity depends on galangin concentration. The inhibition of HSV-1 appears to occur before entering the cell but not after penetration of the virus into the cell. The action mechanism of this inhibition might be virucidal effect or interference with viral envelope structures which are necessary for adsorption or entry into host cells.

CONCLUSIONS

Galangin has anti-HSV-1 activity at pre-entry step but no inhibitory effect against DV-2 infection. Galangin at a sub-cytotoxic concentration, 6.67 µg/ml, showed complete inhibition of HSV-1 infection at the pre-entry step whereas galangin at 3.33 µg/ml partially inhibited HSV-1 infection. This result indicated that galangin, a flavonoid compound, affects HSV-1 infection in the step before viral entry to the host cells. Therefore, this compound may be used in a treatment of HSV infection in the future.

ACKNOWLEDGEMENTS

This study was supported by a grant from Faculty of Medicine, Khon Kaen University.

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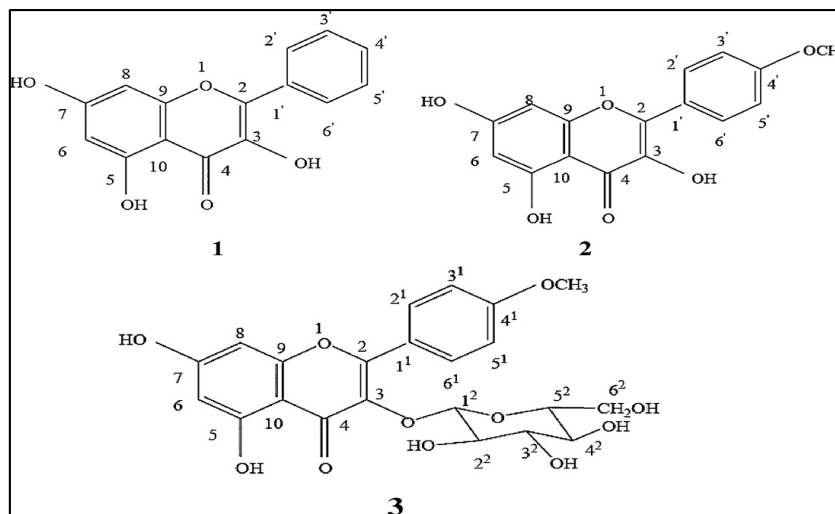


Fig. 1. Structure of compounds 1, 2 and 3

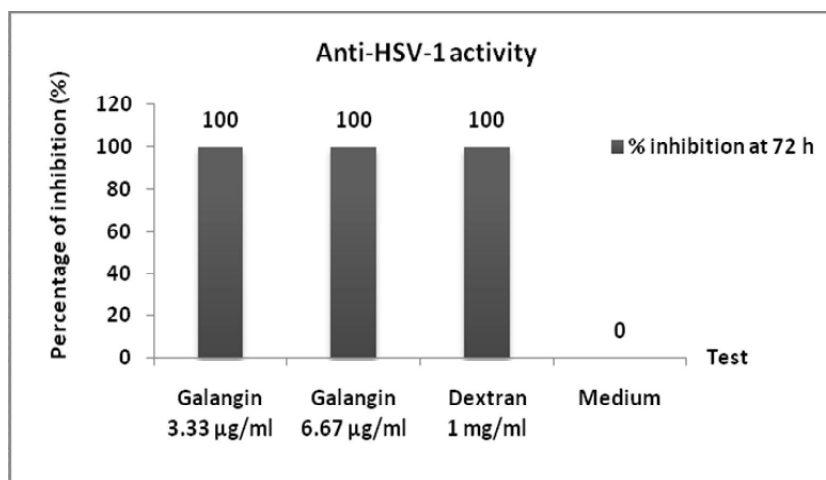


Figure 2. The anti-HSV-1 activity of galangin at pre-viral entry step by plaque reduction assay. HSV-1 was pretreated with or without galangin (3.33 or 6.67 µg/ml) at 37°C for 1 hr and subsequently adsorbed on Vero cells at 37°C for 1 hr. Dextran sulfate (1 mg/ml) was used as a positive control. After washing, the cells were cultured in media with 0.8% CMC and incubated at 37°C for 3 days. The cells were fixed with 10% formalin and stained with crystal violet. The plaques were counted and compared with virus control to analyze % inhibition.





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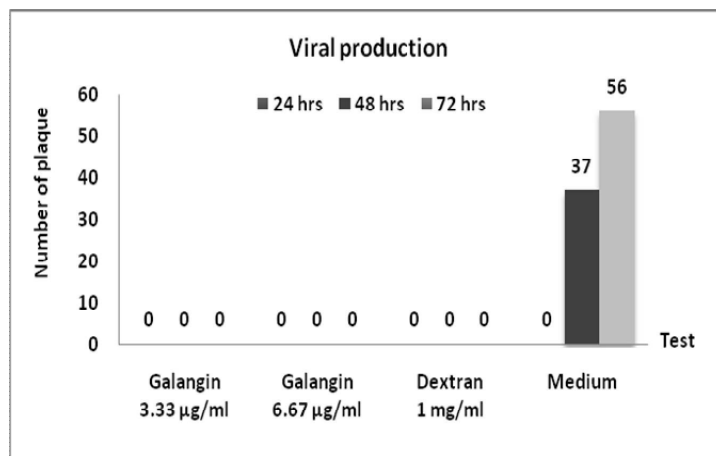


Figure 3. The anti-HSV-1 activity of galangin (3.33 and 6.67 µg/ml) at pre-entry step by virus yield reduction assay. After antiviral activity of galangin at pre-entry step had been performed, the culture medium was collected and adsorbed on Vero cells at 37°C for 3 days. Vero cells were fixed with 10% formalin and stained with crystal violet. The plaques were counted.

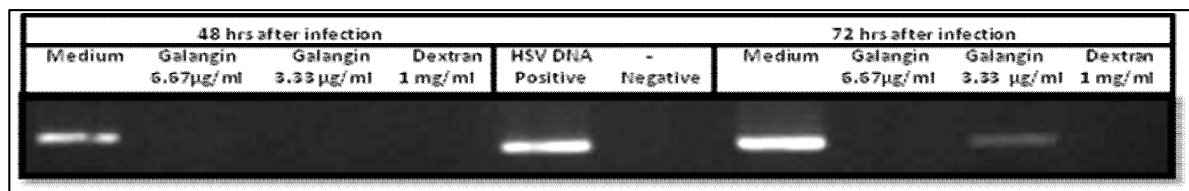


Figure 4. The anti-HSV-1 activity of galangin (3.33 and 6.67 µg/ml) at pre-entry step by PCR assay. DNA was extracted at 48 and 72 hr post infection and used to amplify HSV-1 DNA polymerase gene by PCR. The 194-bp amplified PCR product was determined using 1.5% agarose gel electrophoresis.

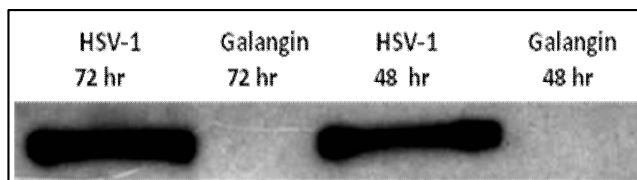


Figure 5. The anti-HSV-1 activity of galangin (6.67 µg/ml) at pre-entry step by western blot. Proteins were extracted from the treated cells at 48 and 72 hr post infection. The HSV-1 protein, gB (90 kDa) was determined by western blot analysis using specific antibodies.





Pre & Post Stress Evaluation in Coronary Angiography and Angioplasty Patients in Tertiary Care Hospital of Karachi - Pakistan

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Received: 18 Jan 2019

Revised: 21 Feb 2019

Accepted: 25 Mar 2019

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ABSTRACT

Background: Coronary angiogram is considered as the finest interventions that can be utilized for coronary artery disease (CAD) evaluation. It identifies the exact location and figure out disease severity. Despite of the fact that this intervention is a blessing in the medical world but also a menace. Coronary angiography patients experience numerous stress induced physiological alterations. **Objective:**The purpose of the study was to evaluate perceived stress experienced by the patients of coronary angiography and angioplasty, resulting in different physiological changes. **Methodology:**In this pre-post interventional study a total of 100 patients undergoing coronary interventions were enrolled from Civil Hospital Karachi, National Medical Centre and Karachi Institute of Heart Diseases. All patients undergone a coronary angiography were included in the study while patients with secondary diseases that might affect physical and psychological health such as patients with Acute MI, Heart Failure, Arrhythmia, Respiratory Failure and Diabetes were all excluded. Stress was assessed both before and after the procedure through specially designed questionnaire. Data was obtained and results were statistically assessed using SPSS. **Results:** There were significant alterations in physiological parameters before and after procedure i.e. mean systolic blood pressure (SBP) (132.65 ± 16.79 v/s 140.3 ± 19.87), diastolic blood pressure (DBP) (78 ± 8.41 v/s 81.1 ± 9.94), heart rate (75.75 ± 13.7 v/s 84.41 ± 17.25), temperature (98.36 ± 0.13 v/s 98.39 ± 0.12) and respiratory rate (20.51 ± 1.18 v/s 20.75 ± 1.26). Moreover, significant results were obtained for perceived stress among the study subjects pre and post intervention. **Conclusion:**It can be concluded from study results that higher stress was observed among the subjects after the procedure which was also indicated through their physiological parameters. These alterations can be minimized by taking special precautions and reemphasizing on patients care protocols and advanced techniques are required for improved stress management during the treatment.





Keywords : Coronary Artery Disease, Coronary Angiography, Perceived Stress, Sadaf Stress Scale.

INTRODUCTION

Cardiovascular diseases (CAD) are amongst the most common bases of morbidity and mortality leading to about 17.5 million deaths annually with 80% occurrence in lower and middle income countries [1–4]. In 2014 WHO reported mortality rates due to age adjusted cardiovascular diseases in India were 348 per 100,000 in men while 265 per 100,000 in women, which is about two to three folds higher in USA i.e 170 per 100,000 in men and 108 per 100,000 in women [1]. Though the risk factor associated with cardio-vascular diseases has increased in lower and middle income countries over past decade, still factors accounting for higher incidence related mortalities in those countries are unknown. Some reasons of lower cardiovascular mortality rates in high income countries might be better control of risk factors, enhanced access to verify pharmacological and revascularization therapies [2]. Mostly coronary angiography is used as a first line treatment for patients with coronary arteries diseases as well as Ischemic Heart Disease (IHD) suspected patients [5]. Though it looks simple but it deeply effects patient's physical appearance and performance which are also responsible for alterations in physiological parameters [6] along with changes in stress related sign and symptoms causing several cardiac and non-cardiac complications [7]. This study is aimed to find out an assessment of stress in Coronary angiography patients to link perceived stress in patients before and after the procedure.

Despite of the complications caused, the occurrence and degree of atherosclerotic coronary artery disease (CAD) are accurately identified by coronary angiography [7]. Problems related to this invasive procedure ranges widely from short term abnormality to life threatening conditions that may results in irreversible damages [7]. Luckily because of innovative equipment designs, improved pre-procedural management and increased knowledge of diagnostic operators, the associated risk are minimized significantly [7]. During coronary angiography, a contrast medium was injected in the coronary arterial tree using catheters. Fluoroscopy technique is used to monitor or guiding the catheter for the correct place insertion. Images are then taken that are visualized on monitor. During this procedure patient may experience various stress symptoms [8]. Those stress symptoms might be physically and psychologically harmful. Numerous factors are responsible related to stress level that includes earlier experiences, anxiety, and unbearable pain during procedure, uncomfortable catheterization laboratory environment or fear [8]. When patient is in stress condition, Corticotrophin releasing hormone level rises. In stressful condition, Corticotrophin releasing hormone (CRH) which is the main hormone involved in physiology of stress response, begin to secrete from Hypothalamus as a response to stress, which is then transported to anterior pituitary that activates and enhances adrenocortical tropic hormone secretion. As a result ACTH triggers adrenal cortex to produce higher cortisol levels. Therefore Cortisol is mainly responsible for causing the stress response [9]. The level of the physiological stress response reveals the perceived as well as experienced strain. Evidently, this response increases the amount of work on the circulatory system that might be already compromised due to CAD or any other unusual upset or cardiovascular disease [8]. This study follows patients and evaluate changes that take place as a result of stress during the coronary angiography procedure which includes variations in blood pressure, respiratory rate, heart rate, and temperature.

METHODOLOGY

A pre-post interventional study was conducted at Civil Hospital Karachi, National Medical Centre and Karachi Institute of Heart Diseases. A total of 100 patients coronary angiography patients were selected through purposive sampling technique. All patients with other diseased conditions like Acute MI, Heart Failure, Arrhythmia, Respiratory Failure and Diabetes that might alter physical and psychological health and hinder with study outcomes were excluded. A Pre-designed questionnaire "Sadaf Stress Scale" [10], was utilized for data collection both before and after the procedure. The patient demographic details were recorded after obtaining informed consent from each





patient. Data regarding the physiological parameters (i.e. SBP, DBP, Heart rate, Respiratory rate and temperature) was also taken both pre and post intervention to observe the post interventional alterations. The duration of the procedure ranged from 10-45 minutes. Signs and symptoms of stress were noted prior to the procedure and till six hours of the recovery session. The study was conducted according to the principles of the Declaration of Helsinki (1964) and follows all ethical guidelines. Data was analyzed using Statistical Package for Social Sciences (SPSS) version 22. The variables are represented as Mean \pm S.D (categorical variable) and frequency and percentages (continuous variables). Chi-square test was also applied to evaluate significance, p-value<0.05 was considered significant.

RESULTS

Out of the total 100 patients 76 were males while only 24 were females with a mean age of 57.1 \pm 8.6years and BMI 24.2 \pm 4.6 kg/m². The patients were also categorized on the basis of age, where it was observed that 14 were <50 years of age while 86 were \geq 50 years. We only had 23% smokers out of the total study sample. The type of procedures patient went through were also noted, where 85 were of angiography and 15 of angioplasty. Changes in physiological parameters among the Coronary Angiography Patients before and after the procedure was also observed. As shown in table 2, there were recognizable alteration in the physiological parameters. Both systolic blood pressure (SBP) and diastolic blood pressure (DBP) were altered after procedure (Mean SBP 132.65 \pm 16.79 v/s 140.3 \pm 19.87; Mean DBP 78 \pm 8.41 v/s 81.1 \pm 9.94). Similarly visible changes were observed in respiratory rate and heart rate. According to the study results a significant difference in perceived stress ratio was observed before and after procedure. Only 17% patients reported being stress before procedure while 70% patients reported stress after procedure (p value=0.00).

DISCUSSION

A number of disease are associated with stress and stress itself is the initiator of many, like hypertension and related cardiovascular and cerebrovascular conditions. It is commonly stated that there is strong positive correlation between various diseased conditions and stress [11-13]. In contrast, positive thoughts and good mood are considered as the factors reducing disease risk, limiting the treatment duration and strengthening the immune system [14]. The invasive cardiovascular procedures like coronary angiography effects the patient's quality of life inducing fear, anxiety and stress and in turn increases the complication rate during and after the procedure, might be due to awaited treatment [15-19]. According to American Heart Association (AHA), stress induces set of events that are all interlinked i.e. one effected by another. Persistent stress resulting from invasive interventions results in increased release of adrenaline which in turn effects the physiological parameters such as increasing heart rate, respiratory rate and blood pressure [20]. Also indicated by our study results, physiological changes were observed after the procedure i.e. a significant increase in SBP and DBP was observed among patients after coronary angiography (P<0.05). Results were also significant for respiratory rate and heart rate (Table 2). Heart rate changes might be due to a number of factors other than stress such as post interventional pain. The heart rate variability is the prime response to the nociceptive stimulation as the Autonomic Nervous System (ANS) is sensitive to acute pain [21]. While the causes may include pre-interventional fasting, temperature alterations in the laboratory setting, medications, noise etc [22]. 23% of the study subjects were smokers as shown in table 1, it is universal fact that smoking in itself is the stress inducer and smoking cessation can also be a source of stress for the patients undergoing procedure. According to a study, high perceived stress was observed among the heavy smokers as compared to the other counterparts [23]. Further studies are required to understand the psychology behind smoking associated stress. It is evident that diabetic patients are more prone to stress both physically and psychologically, also concluded by Hagger and his colleagues in a study conducted in 2016, the diabetic patients were more likely to experience distress [24].

According to our results around 70% of the study subjectshad perceived stress after intervention i.e., a significant increase was observed (p<0.05) (Figure 1). It is a known fact that cardiac angiography is associated with a number of stressors that may include intervention duration, unfamiliar place, unfamiliar people, pre and post interventional



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fear and anxiety [8], all of which directly or indirectly leads to stress [25]. Although a sound proof environment was maintained in order to minimize the noise effects moreover, these patients were also kept under special care. There might be other factors leading to perceived stress among these patients but we were unable to monitor them, which counts as the major limitation of this study.

CONCLUSION

It can be concluded from the study results that majority of the patients undergone coronary angiography and angioplasty experienced higher stress that can be due to several reasons and procedure related conditions. Perceived stress during angiography procedure must be assessed, changes in physiological parameters should be, monitored keenly because it aids in treating the patients upon priority basis after assessment. As these associated physiological changes are trigger for post procedural complications that can only controlled by taking special precautions and reemphasizing on patient care protocols and management techniques. Furthermore there is a vital need to deduce and devise a scale to measure stress in patients of Coronary Angiography the present study was an attempt to assess such kind of stress condition in order to overcome unlikely side effects of the procedure.

ACKNOWLEDGEMENTS

We would like to express our special gratitude to the study participants for their support.

Conflicts of Interest

None.

Funding

This work was supported by the Higher Education Commission Pakistan (grant number: 6613/Sindh/NRPU/R&D/HEC/2015).

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Table 1. Demographic characteristics of the study population

Variables	Sub-categories	(n=100)
Mean Age (years)		57.1±8.6
BMI (kg/m ²)		24.2±4.6
Weight (kg)		71.1±12.2
Height (meter)		1.7±0.09
Age Group	<50 Years	14
	≥50 Years	86
Gender	Male	76
	Female	24
Smoking History	Smokers	23
Procedure	Angiography	85
	Angioplasty	15
History	Obesity	18
	Diabetes	47
	Hypertension	81

*Values are given as Mean±SD or percentages





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Table 2: Physiological changes among Coronary Angiography Patients before and after the procedure

	Pre-Evaluation 70%	Post-Evaluation 17%	P-Value
Systolic Blood Pressure	132.65±16.79	140.3±19.87	0.000
Diastolic Blood Pressure	78±8.41	81.1±9.94	0.000
Heart Rate	75.75±13.7	84.41±17.25	0.000
Respiratory Rate	20.51±1.18	20.75±1.26	0.018
Temperature	98.36±0.13	98.39±0.12	0.008

***p-value <0.05 (Significant) *Values are given as Mean±SD**

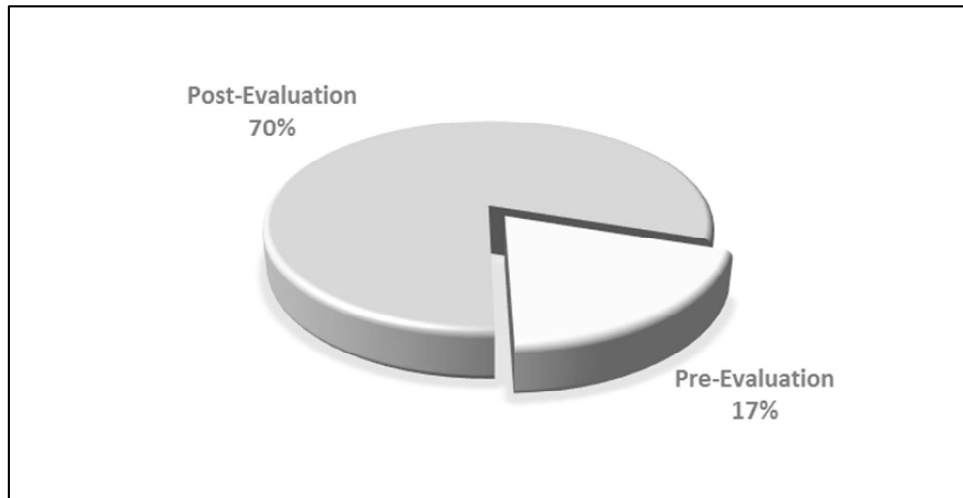


Figure 1: Perceived Stress evaluation before and after the procedure





Object Detection Algorithm Using Deep Learning

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Received: 21 Nov 2018

Revised: 24 Dec 2018

Accepted: 26 Jan 2019

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ABSTRACT

Object detection considered one of the technologies associated with computer vision including a broad variety of utilization in diverse areas that comprise vehicle detection, online images, robot applications, medical image analysis, security and the interaction between human and computer. The current methods work sensibly but in specific areas as a result of the progress in science. However, the change in lighting conditions over time or the blocking of part of the object and change of the object viewpoint lead to the failure of traditional methods to detect the objects. Deep learning has progressed so that they have formed algorithms and method in many aspects, outperforming the previous ones. Especially with computer vision issues. In this paper, the properties of convolution neural network have been exploited and shown to propose an object detection approach. The proposed algorithm presents an effective model with a simplified structure that uses less memory and less processing power while maintaining good accuracy and speed. This algorithm allows the computer to tell us directly that this image includes a specific object and its position in the image. The proposed depends on extracting the features of the object by using CNN and then working on these features or characteristics to predict the class and location of the object between the tested species by using another convolutional neural network which contains two outputs. The first output contains ten outputs for predicting the classes and the other contains four outputs to predict dimensions Location of the object. The customized detection approach was tested on the pascal object detection benchmark and it was effectively able to detect objects of varying scale, lighting conditions, and orientation. This model has achieved 48.61% mAP. The results have been discussed in details and re-examined in future work especially in the field of deep learning and computer vision.

Keywords: Object detection, computer vision, deep learning, convolution neural networks, artificial intelligence, machine learning, pascal VOC, python.



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INTRODUCTION

There are many tasks which animals and humans are able to do rather easily however the explicit knowledge of how the brains of these intelligent creatures carry out these kinds of tasks is scarce. Doing those tasks involve a knowledge that goes beyond the knowledge of human beings but there is an encouraging information about how neurons work. Furthermore, there are many theories and models on learning strategies for groups of these neurons. These groups are known as neural networks. These networks are known to be learnt by examples [1]. The world has witnessed a vast technological renaissance at the beginning of the 21st century. With the advance in parallel processing and the introduction of Graphic Processing Units (GPUs) with immense computing power artificial neural networks especially deep neural networks have gained a lot of attention.

The enormous use of hand holds devices and the tremendous improvement of these devices as well as the advances in communication technologies have led to a stupendous amount of data recording. This data is easily accessible via the internet. Recently, the majority of this data that is being uploaded to internet is known to be images and videos. The data alongside with the high-performance computing devices have opened a new era for artificial intelligence applications and their uses especially in the field of computer vision [2]. Computer vision has witnessed a great progress with the introduction of deep learning. Furthermore, in certain limited recognition task it even outperforms human capabilities. This progress is mainly the result of new ideas, algorithms and improved networks architectures as well as the opportunity of using these vast data sets in training deep neural networks by using these powerful hardwares [3]. This paper depicts details to all parts of the suggested framework for object detection. It presents the dataset that was used, illustrating experiments, analysing the accuracy and efficiency.

Related works

In [2] conducted a systematic study of the existing research on computer vision applications based on deep learning algorithms and Convolutional neural networks, by studying about 119 papers and classifying them according to the areas of interest, learning model and type of contribution. In [4] provided broadest and different most coverage of the image to help the researcher community in computer vision. a huge scale dataset of more than 15 million high definition images labeled to about 22,000 category called "ImageNet". Images gathered from the web and named by human utilizing Amazon's Mechanical Turk swarmed sourcing instrumented. These images can serve as a useful resource for visual acknowledgment applications such as image classification, localization of objects and object recognition. Achieved promising results in the field of image classification task in that proposed a convolutional neural model [5]. Convolution neural network was trained to identify one and half million high-resolution images from the ImageNet Large Scale Visual Recognition Competition (ILSVRC-2010) challenge categorized into the 1000 groups. Training network takes six days on two GPUs. However, on the test data, they have achieved error rates of 37.5% and 17.0% on the test data which is a much better result than the previous models.

The model size that is mainly restricted to the number of available memory GPUs and the required training time. [6] proposed Spatial Pyramid Pooling (SPP-layer) convolutional layer runs on the entire image only once to get the feature maps. The position of the candidate windows obtained by the selective search respective to the earliest image is mapped to last convolutional layer features maps. Then, the features would be pooled by a multi-level spatial pyramid in a fixed-length feature vector for each window. These representations are provided to the fully-connected network. SPPnet possesses drawbacks. Training is a multi-stage pipeline to extract features, fitting bounding-box regressors. The features are also written to disk which required memory. Fast R-CNN is an end-to-end training object detection model [7]. It trains jointly for classification and bounding-box regression on each labeled the Region of Interest (RoI) pooling layer based on the SPP-layer. Disk accommodation is needed for feature storing neither model layers were renewed all within training. R-CCN is considered as a great success in computer vision and opened the door to object detection approaches based on CNN [8]. The system consisted of three stages. Firstly,



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input images were scanned for possible objects by selective search algorithm to use region proposals generator. Secondly, each category mistake was run through a CNN that extracts features vector. In the last stage the output of each CNN was fed in to a Support Vector Machine (SVM) to classify and a linear regression to find the bounding box of the objects. Region based convolution neural network (R-CNN) achieves 62.4% mAP on VOC2012 datasets. However, it has many drawbacks. The training depends on multiple stages, the training stages are expensive. The file size of extracted feature is extremely high for both Support Vector Machine (SVM). The features are extracted from each proposed area and stored in a disk. This requires large storage space and days of calculation. The third reason is probably the most important slow object detection and it requires almost every minute of the image even by using the graphics processor units.

In [9] have proposed a Region Proposal Network (RPN) to generate proposed areas and used another network to use these proposed areas to detect objects. Boxes most likely containing objects of the training images were proposed by ranking boxes called anchors. Faster R-CNN has a 73.2% mAP on VOC2007. However, Faster R-CNN cannot meet the requirements of the object detection in real time because the calculation features still take a long time. (YOLO) method has been rising up [10]. This approach adopts the idea of regression the detection problem was transformed into regression problem. The input images are divided into several cells and the probability of classification and boxes are predicted from each cell. Predictions were made by the global representation from the image and make pretty utilization of the context. But YOLO also has a problem in that the average precision is only 63.4% mAP on VOC2007 without the region proposal. By using an incorporation of the regression concept of YOLO and also special anchor method of Faster R-CNN designed a way to detect the object inside image by using a single deep neural network [11] originating predictions from feature maps of various scales. At each feature map location, the predicted space of bounding boxes was discretized into a group of default boxes with various aspect ratios and scales.

That doesn't just achieve high discovery accuracy similar Faster R-CNN but also, sure enough, the real-time in speed like YOLO. SSD possess a mAP 72.1% on the VOC2007 test with 58 FPS in GPU, it makes possible to take real-time object detection including high accuracy toward realistic applications. The CNN networks have achieved remarkable success in many areas of computer vision. ILSVRC had applied CNN to image classification and achieved powerfully results between the conventional methods. After this turning point, CNN became a dominant approach in the later computer vision tasks because of its obvious success. In general, R-CNN, SPP-Net, Fast R-CNN and Faster R-CNN were proposed and developed one by one for the object detection that is based on CNN which is a simpler pipeline, a higher precision and less time than traditional detection systems. The R-CNN series of methods are considered a very important branch of object detection at present. Faster R-CNN method is currently the major of object detection, but the speed cannot meet the real-time requirements. SSD and YOLO use single network for speed to predict bounding boxes and classify objects.

Object detection algorithms

Towards making at an indispensable complex task as cataloging and detection real-life objects, a simple and powerful deep CNN object detection algorithm was proposed which is capable of reaching a better performance while demanding least hardware supported and computational costs. The approach consists of two deep networks as shown in figure 1 by using the training set for feature extraction and the other was trained on features for classification and detection objects, respectively. The succeeding of deep learning with several heterogeneous outputs (bounding box and classes) allows the second network to have two head for classification through a softmax layer to infer the transformation between images for class score and by using linear regression to predict the bounding of predicted box. A set of various features makes it hard to develop a robust and generic solution for the problem. A major drawback of this approach lies in modeling a regression problem as a classification one which reduces the representational capabilities of the learned model.





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Dataset

To train the deep neural network for object detection, a vast amount of data onto an actual scenario is required and true ground-truth annotations. So, for the purpose of object detection problem, the PASCAL VOC dataset was used. Images of the dataset were collected from "Flickr "website. Included 11, 530-images with 27,450 annotated regions of interest contain twenty common class objects divided between four-sections (Person, Animal, Vehicle and Indoor). It's proved for classification and detection tasks. The VOC datasets contain some pictures that are taken underneath varied conditions with completely different| object size, orientation, pose, lightning conditions, position, and partial occlusion. Each image has annotations XML file giving a bounding box coordination which are placed within the upper-left corner, and object class label for each object inside the image typically more than the object is given in a picture and bounding boxes might overlap [10].

Preprocessing Data

The task of localization can be considered as the problem of identifying the location of a single object among more than an object. The training set images were pre-processed to create labels with single annotations, because of lower memory footprint and training time, only eight classes were used (person, aeroplane, car, bus, train, motorbike, bird, chair). There should be a save for interested annotations that containing a single object. Before designing the object detection model, itself, pre-process all the dataset to project the images as spatial maps in a (18, 18, 512) dimensional space once and for all. The goal is to avoid repeatedly processing the data from the original images when training the top layers of the detection network. VGG16 CNN model that is used for extracting features of the input image by predicting on a batch of images. The batch of images was created as follow: -

1. Open each image, and the input resolution image is downscale to 300 × 300 due to memory limitations.
2. Stack them as a batch tensor of shape (batch, image size, channels)
3. Preprocess the batch and make a forward pass with the model

Compute representations on all images in the annotations. In other words, the same weight of the model was adopted initialization. The training time for extracting feature took around 5 hours on a single GPU. The final preprocessing step was building ground truth boxes from annotation to use then as true label that will be compared to the output model. As the images was resize to a fixed 300 ×300, the boxes coordinate of the annotations need to be resized accordingly. Using the equation below: -

$$\frac{\text{Each coordinate} \times \text{Image resize}}{\text{Original size}} \dots \dots \dots (4.1)$$

Also, the top-left and bottom-right coordinates were converted to center, height and width by using the equation below.

Compute Center of the Box

Let (x1, y1) topleft coordinate and (x2, y2) bottomright coordinate.

$$cx, cy = \left(\frac{(x2 + x1)}{2}, \frac{(y2 + y1)}{2} \right) \dots \dots \dots (4,2)$$

Compute Height and Width of the Box





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$$\mathbf{width} = \mathbf{x2} - \mathbf{x1} \dots\dots\dots (4.3)$$

$$\mathbf{height} = \mathbf{y2} - \mathbf{y1} \dots\dots\dots (4.4)$$

To give an illustration, the class labels were mapped to corresponding classes names then Build a one-hot encoding of each class. As shown in figure 2

VGG16 Model for Feature Extraction

As mentioned in chapter two deep networks learn representations of the data. Those representations tend to possess a common value so far as possible to be applicable to a host of issues that surpass the specific task that a model was prepared for. This is an advantage as training complex models demand weeks of work on one or extra GPUs. These models can be saved and then reused for a number of further applications, with no or minimal supplementary work. In this approach the VGG16 Net has been used for feature extraction. Only the final fully connected layers were removed. VGG16 Net is one of the most effective model's structures since it fortified the concept that convolutional neural systems should have a profound network of layers for this various leveled illustration of visual information to work.

Oxford's renowned Visual Geometry Group (VGG) was developed a vgg16 network for object recognition and achieved good results on the ImageNet dataset. VGG foster the simplest 3x3 convolutions filter size and 2x2 pooling are used throughout the full network. It was quite famous because doesn't only it works well, but the Oxford teams have made the structure and the weights of the trained network freely available online. The drawback of VGGNet is big and comprises around 160M parameters. Most of the parameters are used in the FC layers. It can detect any one of 1000 different object categories with 7.3% error rate. The model structure is shown in figure 3 [12].

Model architecture implementation

The model has two head for classification and localization. The classification head was softmax layer with eight outputs as number of classes in order to predict the classes probabilities. Categorical cross entropy function was used as loss function for classifying head. Convolution layer with four filters and one by one filter size fit in to bounding box head to predict the coordinates of the predicted bounding box. Mean square error was used as loss function with box head. The model has been trained by using Adam optimizer with 0.0001 learning rate. The model layers and trainable parameters shown in table 1.

Training

Basically, split the dataset into train and test dataset so the training data was 10343 samples and 1149 for validation. Model has been trained by using a categorical cross entropy and Mean square error loss function for bounding box head with Adam optimization algorithm configured with a large momentum and weight decay that is started with a learning rate of 0.001. It was fitted with 64 epochs and a batch size of 32. Model took an average computational time of 33 minutes 3 seconds. These epochs acted like smaller training sessions that ran over all of the data given in training set. During that run, filter values (or weights) were adjusted through backpropagation of a process. It was the part where weights of the layers were correctly tuned with respect to the loss function while carrying out forward and backward passes. The ultimate goal was to achieve a set of parameters which have a certain ability to generalize toward new data. Furthermore, and that ability was reflected by the validation accuracy. The model was tested on the validation dataset to estimate the ability to generalize toward new data, because the validation set contains only data that the model has never seen before and therefore cannot just memorize.





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Evaluating and Testing

Assessing the quality of the model, have to measure whether an object exists in the image (classification) and determining the location of the object (a regression task). Furthermore, in a typical data set there will be many classes and is significantly nonuniform distributed as shown in figure 4. So simple accuracy measurement is not appropriate. It is important to estimate the trade-off among different types of misclassifications. Thus, there is a need to associate a confidence score with each bounding box detected and a discrete score for all classes. In order to evaluate the accuracy of an object detection algorithm on a particular dataset. First, there should be a determination of how well the model predicted the location of the object typically evaluated by intersection over union algorithm which is an evaluating metric monitoring the IoU between ground truth box and predicted box. In order to apply the IoU to evaluate the model need ground truth bounding box from the training set and predicted bounding box from the output model to:

- Compute the intersection area
- Compute the area of both the projected and ground truth boxes
- Compute the intersection over union by taking the intersection area and divided it by sum of predicted and ground truth boxes area minus intersection area. The following equation as shown in figure 5 computes the IoU. An IOU score > 0.5 is consider a good prediction

Model object detections are determined to be true or false depending upon the IoU threshold. Moreover, predicted class and bounding box consider it positive if and only if:

- The maximum of output class of the model is true class
- The IoU between the predicted bounding box and the ground truth box is above a 0.5 threshold.

For the classification task, the results are in the form of a confidence level for each image. The provision of a confidence level allows results to be ranked such as the trade-off between false positives and false negatives can be evaluated, without defining arbitrary costs on each type of classification error. Average Precision (AP) was used to evaluate both classification and detection. For a given task and class as shown in figure 6 the precision/recall curve is computed from a method's ranked output. The recall is defined as the proportion of all positive examples ranked above a given rank. Precision is the proportion of all examples above that rank which are from the positive class and measures how predictions is accurate. The average precision on all dataset are 48.68% as shown in figure 7. The AP measured and shown on the y-axis for each class. Cost of the model on training set and validation set correspondingly over 30 epochs is presented in figure 8. The figure shows that the training loss is saturated at 20.

Cost of class head on training and validation set is shown in the figure 9. The validation cost is decreasing at the early epochs. The range regressor "head" is trained alone without the feature extractor process, and thus the average cost does not show the same characteristics as the cost from classifier and bounding box regressor because they training on different loss function. The accuracy progressed on the training set and validation set for classes head is shown in figure 10. The model training over 63 epochs from the training set and validation set just 30 epochs is plotted for better visual the model behavior.

Arbitrary Results

Figure 11 presents an example of pictures from the test set with the classification rating of class projected on top and the resulting bounding box. The previous mention figure demonstrates that the model is effectively able to come across objects of various scale, lighting circumstances and orientation. The model also figures out the partially occluded objects. Figure 12 shows that the model is successfully detecting classes on a sample of images from the internet



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DISCUSSION

Object detection achieved through predicting bounding boxes gives data about the location of an object of interest in the image. The choice of the model architecture for the detection task was inspired by CS231n lecture 8[31]. The CNN architecture an important consideration while designing a feature extractor. That the feature extractor is the more major part of the detection network pipeline. The feature extractor consists of pretrained classification network trained on the ImageNet dataset. A seeking for an appropriate choice was made by comparing distinct pretrained architectures in terms of their time and accuracy on the validation set. Table 3 shows this. As this network architecture trained on approximately million image that is acceptable at extracting rich hierarchical information. Instead of training from the scratch on the refiner data. Convolution feature map followed by fully-connected layers with individual class scores. can easily extend to build a bounding box regressor. by feeding convolution feature map to a separate set of fully connected layers and transforming the last fully-connected layer to 4 nodes for predicting the bounding box coordinates. then calculate loss of these coordinates with respect to the ground truth bounding box adding it to the loss that already using for classification. the architecture is training the bounding box regressor with backpropagation. Additionally, only fixed number of bounding boxes were predicted. For predicting bounding boxes for n objects, need to have n regression heads and train accordingly.

CONCLUSIONS

Through the short history of programmed object detection, the deep learning plays an effective role overall performance of detection algorithm. The approach that has been designed in this paper is an evidence to this fact. Convolution neural networks have been exposed to eliminate the need for a hand-crafted feature extractor. Convolutional neural network approach draws on the robustness and efficiency automatically learn the object feature based on the given dataset. Also, it is invariant to transformations which is a great asset for certain computer vision applications. On the other hand, it heavily relies on the existence of labeled data. Additionally, of the models investigated CNN are computationally demanding when it comes to training. This thesis presents a simple and scalable object detection algorithm that gives 48.61% mAP relative improvement over the previous results on PASCAL VOC 2012. This performance can be achieved through two insights. Whereas, the first one is to apply high-capacity convolutional networks to objects inside the images. The second is a paradigm for training CNN on the extracted feature to predict classes and bounding box. A promising future direction is to explore how to makes use of the proposed approach as a section of a hardware system to detect and track objects in videos simultaneously and implementing the proposed system on mobile camera.

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Table 1. Model layers and trainable parameters

Layers	Activation shape	Activation Size	Parameters
input	(10,10,512)	165030	-
Conv1	(18,18,4)	1296	2052
Flatten	1296	1296	0
Dropout	1296	1296	0
Head boxes	4	4	5188
Glob avrpool	512	512	0
Dropout	512	512	0
Head class	10	10	5130
Total parameters			12,370

Table 2. Average precision over each object

class	Average precision
aeroplane	69.88%
bird	45.60%
bottle	6.85%
bus	72.71%
car	59.79%
chair	18.10%
motorbike	82.70%
person	41.10%
sofa	34.65%
train	54.37%

Table 3. Accuracy and the time taken to extract features.

Model	Input size	Top-1 err (%)	Time (m)
VGG16	(300, 300)	36.2%	26.45m
VGG16	(244, 244)	44.2%	20.50m
ResNet-50	(300, 300)	38.6%	38.53m
ResNet-50	(244, 244)	40.1%	21.48m





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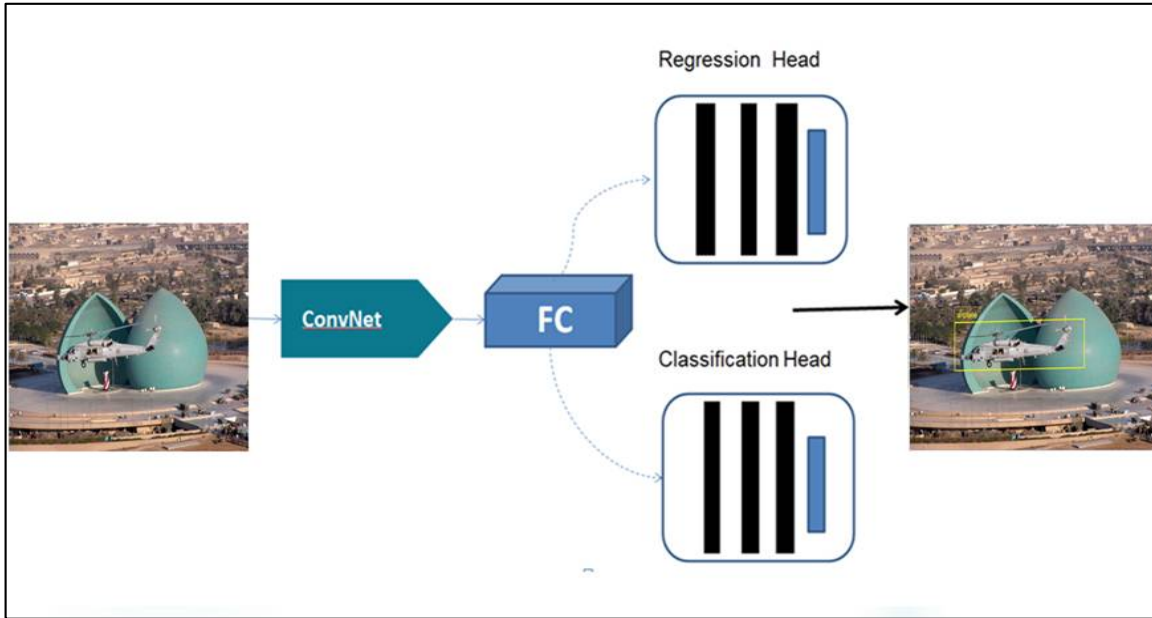


Figure 1. Classification and localization model

```
Classes and boxes shapes: (707, 8) (707, 4)
classes labels:
[[ 0.  0.  1.  0.  0.  0.  0.  0.]]
boxes coordinates:
[[ 150.9  150.4  211.8  299.2]]
```

Figure 2. Ground truth labels

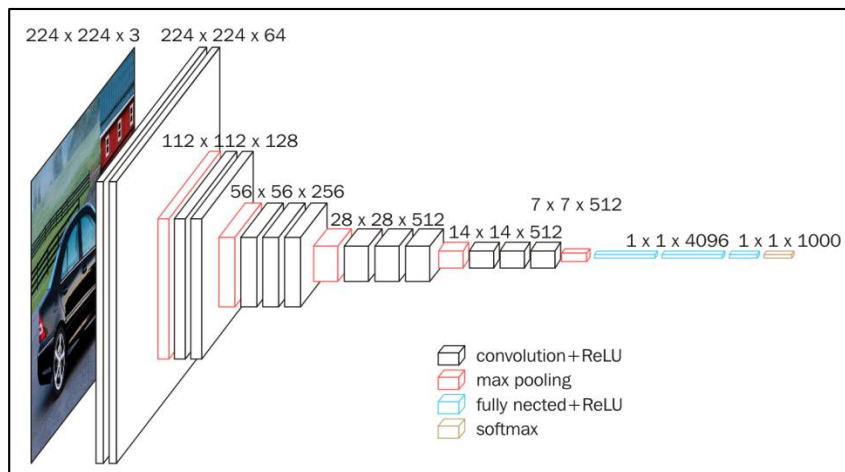


Figure 3. VGG16 architecture





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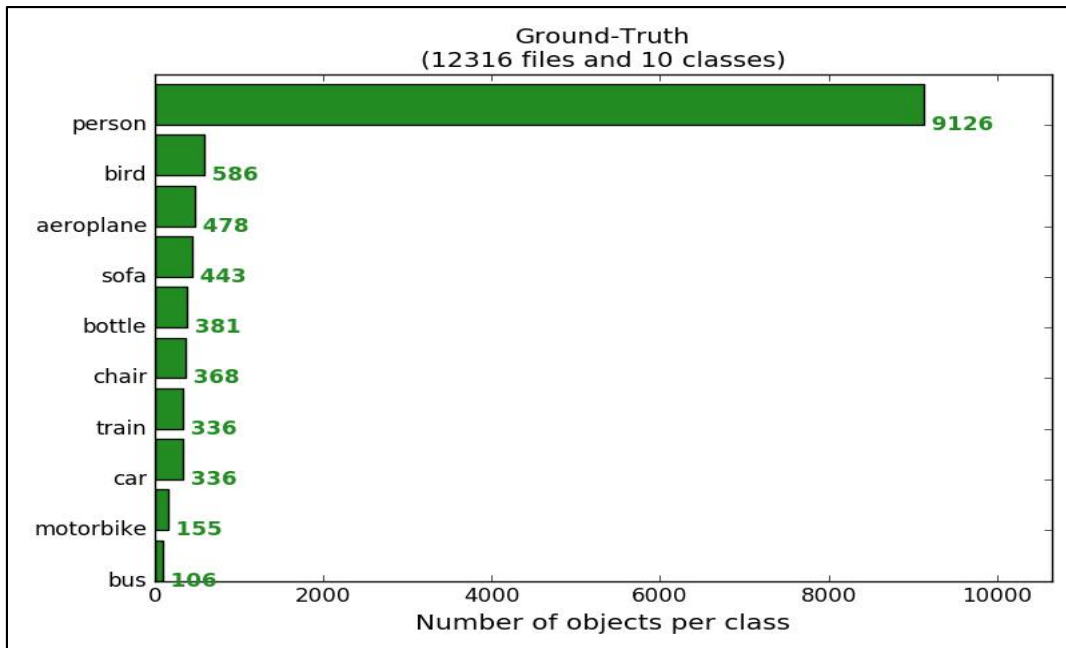


Figure 4. Distributed objects per class

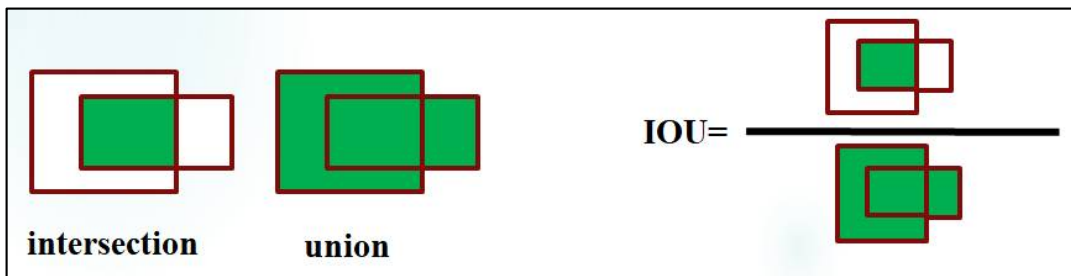
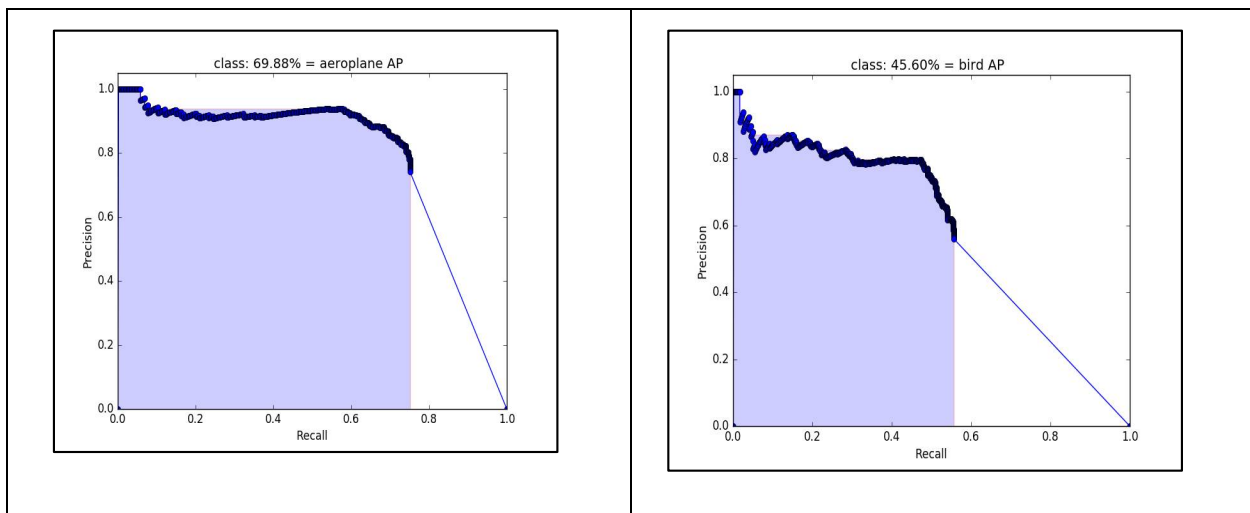
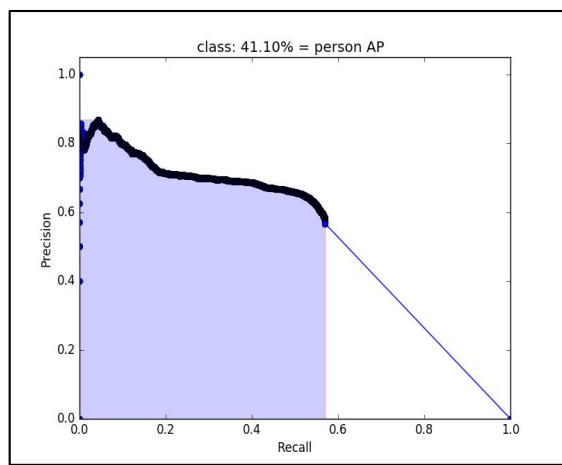
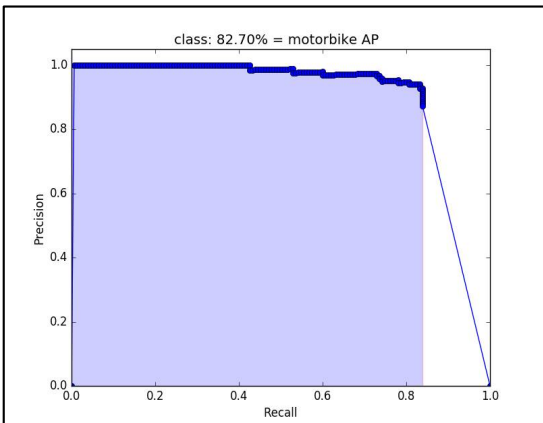
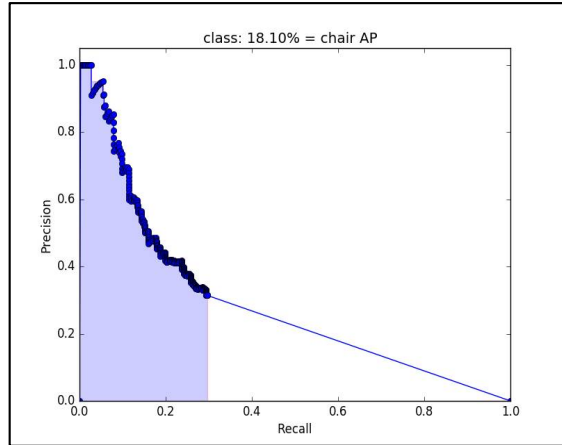
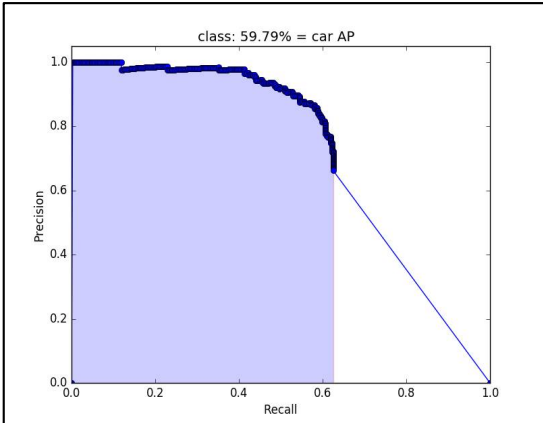
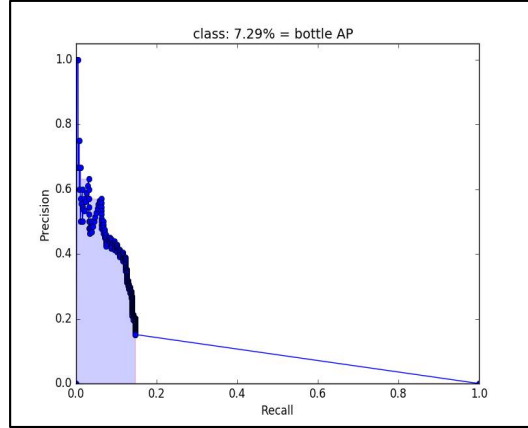
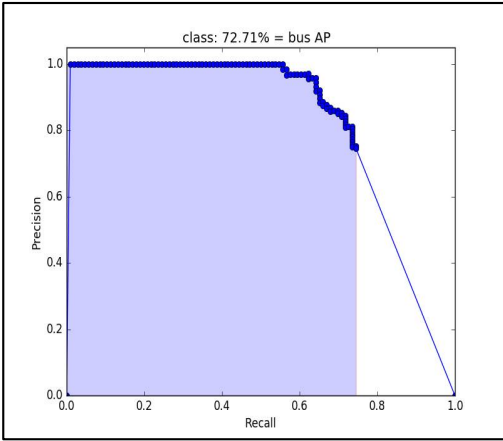


Figure 5. Intersection over union function





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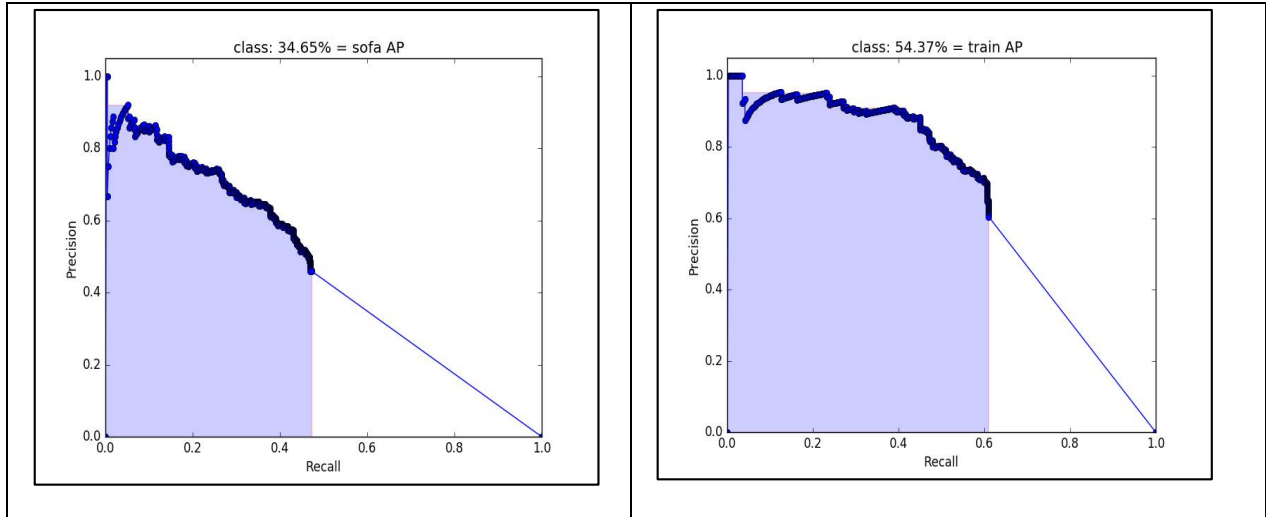


Figure 6. Precision-recall curves for classes on the Pascal VOC object detection benchmark.

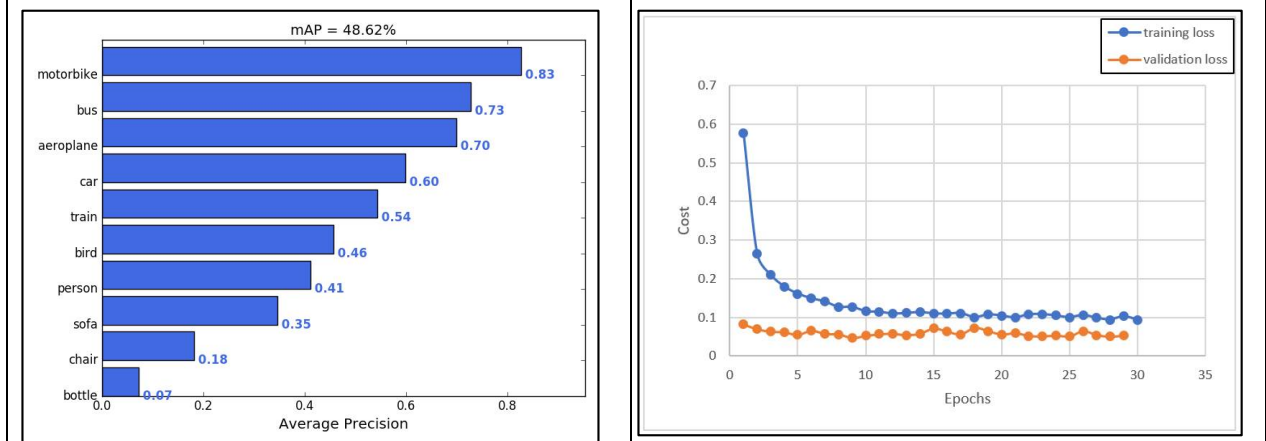


Figure 7. Average precision of all classes

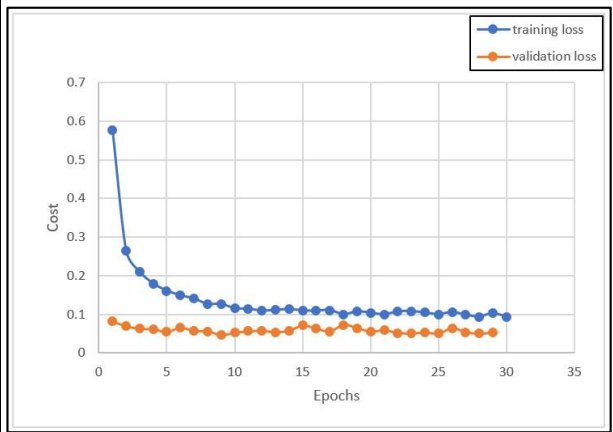


Figure 8. Cost of model against epoch number

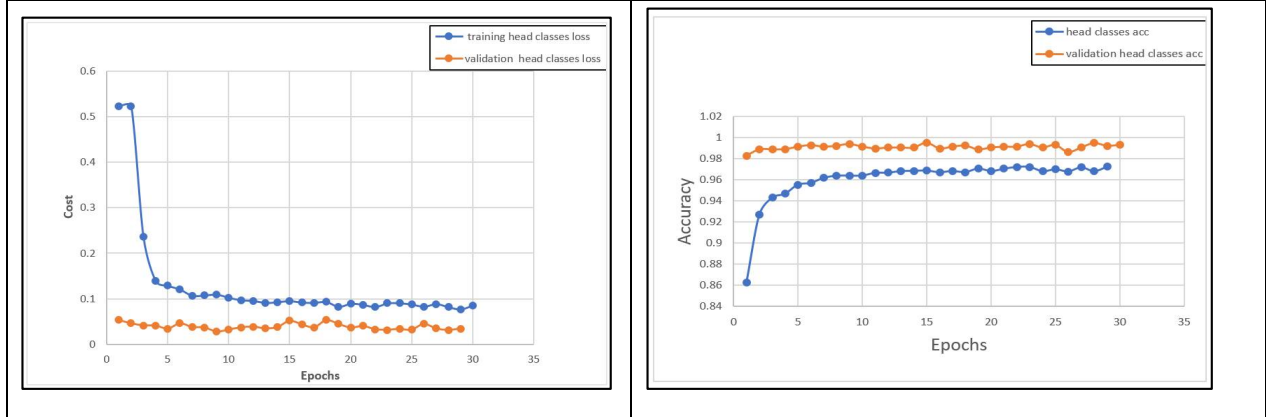


Figure 9. Cost of class head against epoch number

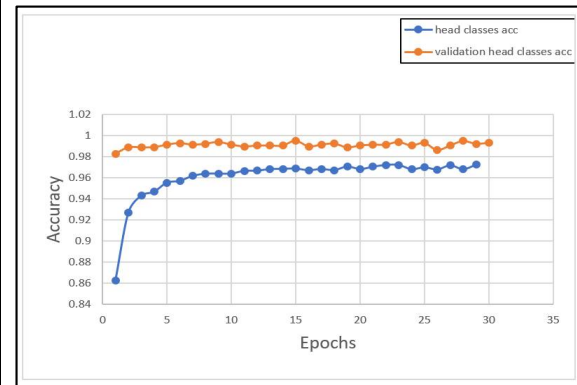


Figure 10. Accuracy from classifier head





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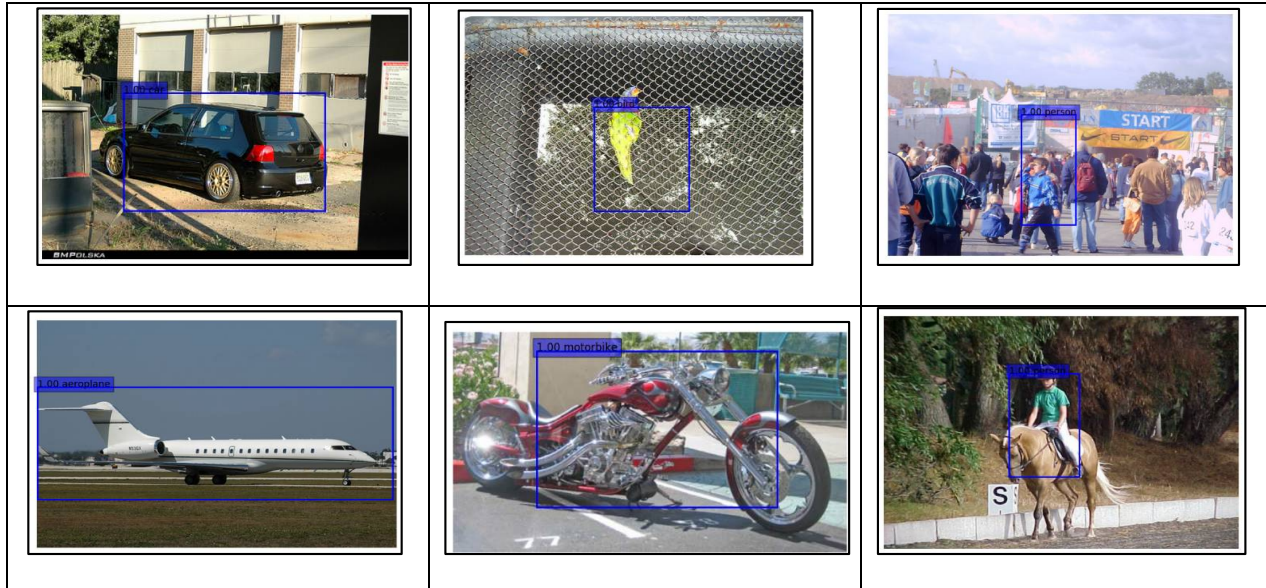


Figure 11: Qualitative results of the detection system for the different classes from the pascal test set

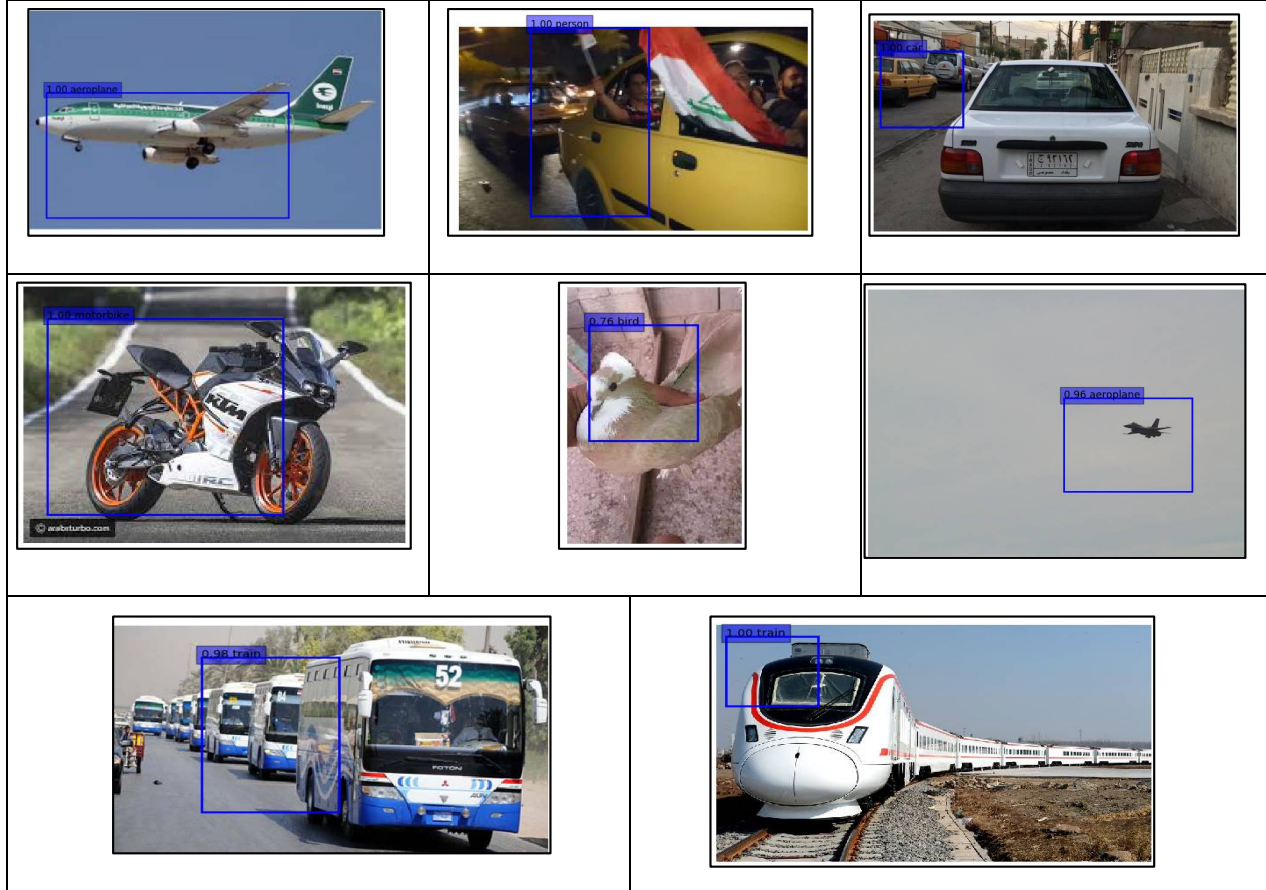


Figure 12. Qualitative results of the model using local images for the different classes





Psychophysiological Consequences of Mobile Phone Addiction; A Study on Mobile Phone Usage and Sleep Quality

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Received: 18 Jan 2019

Revised: 21 Feb 2019

Accepted: 25 Mar 2019

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ABSTRACT

Background: Smartphone has made our life easy but on the other hand over use of smart phone cause ill effects on our health. The most concerned issue nowadays is rising of smartphone abuse among population which alter our sleep quality. **Objective:**The purpose of study is to determine the correlation between smartphone addiction and sleep quality. **Methodology:**The survey is based on Pakistan. Sample possess both males (51%) and females (49%). Subjects of age between 13 and 55 were sampled. Most of them are undergraduates (59%) and students (66%). About 78% belong to upper middle class and only 2% belong to lower class. The primary purpose for mobile phone use in a population is for calls and messages (49.5%) while common among adolescents is social net surfing (25.9%) All the statistical analysis is conducted using Microsoft Excel and SPSS. A self-rated scale is used which is known as Pittsburgh Sleep Quality Index (PSQI) for the access of sleep quality. **Result:**This study shows that smart phone addiction has a parallel effect on sleep quality of an individual (0.000 overall, $p < 0.05$). It is reported that problematic smart phone use may affect sleep initiation, maintenance and sleep efficiency. No relevant gender variations are seen in the frequency of smart phone addiction (-373 overall, $p < 0.05$). There are no important gender variations within the frequency of sleep quality (-097 overall, $p < 0.05$). It shows that there is a direct relationship between age and smart phone addiction and they are depending on each other (0.000 overall, $p < 0.05$). The results of this study show positive outlook towards the age and sleep quality (0.015 overall, $p < 0.05$). **Conclusion:**The development of various programs or seminars are suggested to conduct as to spread awareness regarding impact of smart phone addiction on sleep quality for better future.

Keywords : Sleep Quality, Mobile Phone Addiction, Pittsburgh Sleep Quality Index.



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INTRODUCTION

One of the great developments in technology is Mobile phone. It offers all facilities and functions that has made our life easy and fast. On the contrary, use of cell phone has many negative impacts and may lead to addictive behavior [1]. Smartphones provide combine internet and mobile phone services [2]. It has been reported 1.85 billion people were using smartphone in 2014 across worldwide and it may grow to up to 2.87 billion in 2020 [3]. There is immense amount of popularity of cell phones in youth within a short period [4]. Several survey reports suggest that 50% teens and 27% parents feels that they are addicted to hand phones [5]. In 2012, a poll evaluated that 84% couldn't go a single day without mobile phone devices [6]. Addiction is not only denoted for excessive use of substance but also excessive involvement in activity or technology [7]. Mobile phones overuse and addiction is a very important social problem in many societies. In comparison with adults, teenagers have a greater cellphones addiction rate [8]. Teenagers who use their cell phone imprudently are more prone to restlessness, disrupted sleep, stress and fatigue [9]. The signs of smartphone addiction are unnecessary checking of phone, feeling anxious or agitated without the phone, waking up in the mid night to check the mobile and updates, poor professional performance as a consequence of prolonged phone activities, and distracted with smartphone applications [10].

Sleep is essential need for a healthy body. It is affected by many factors such as lifestyle, environment and stress [11]. Mobile phone addiction has a parallel effect on the sleep quality of an individual. Many studies found linkage between mobile phone addiction and disturbed sleep [12]. Lack of sleep affect brain functioning and information processing [13]. It is reported that problematic mobile phone use may affect sleep construction, by reducing REM sleep, Slow Wave Sleep, and sleep efficiency [14]. Some studies suggested several mechanism related to poor sleep of mobile phone users including, bright light of gadget suppress secretion of Melatonin and delay onset of sleep, use of electronic media devices maybe associated with learning, psychological and physiological problems, gadget in bedroom may affect sleep as messages beep may awake adolescents at night [15]. The prime aim of current study was to determine relationship between mobile phone addiction and sleep quality with respect to gender and age. This study focus on consequences, relationships with factors and demographics of population regarding Mobile phone and sleep. It has been suggested overuse of mobile phone may cause psychological and sleep disturbances [16].

METHODOLOGY

Sampling & Design

The study was conducted using a self-reported questionnaire. Participants were recruited from a wide population and were randomly selected. For this purpose, there were implemented some of the exclusions as well. The Research was based on the data obtained from 600 respondents (approx.) since a sample size too large can create a perception of the significance of a non-existing relationship. Sample possess both male and female gender. Subjects of age between 13 to 55 were sampled and this survey was purely done in Pakistan.

Variables & Tools

The data was collected by self-administered questionnaire including i) Personal data (demographics) ii) Lifestyle iii) Smart phone addiction scale iv) The Pittsburgh Sleep Quality Index (PSQI)

Personal Data

Personal data was gathered from participant's age, sex, educational level, occupation.



**Amas Nadir et al.****Lifestyle**

Question items related to lifestyle includes working hours, social-economic status, bedtime, sleeping hours etc.

Smart Phone Addiction Scale

Smart phone usage of the participant was analyzed by using Smart phone addiction scale [17]. This scale is an effective mean of predicting addiction of smart phone in an individual. Furthermore, by the help of this scale the group which were at high risk for smart phone addiction can be identified.

Pittsburgh Sleep Quality Index

The Pittsburgh Sleep Quality Index (PSQI)[18] is a self-rated scale that assesses sleep quality during the past month. This scale consists of eight items: difficulty with sleep induction; waking up during the night; waking up early in the morning; total sleep time; overall quality of sleep; problems with a sense of well-being; problems with functioning; and sleepiness during the day. Each item is rated on a scale of 0(no problem) to 4(serious problem).

Data Collection Administration

The study has been done on the available data. It was conducted to find out the appropriateness and applicability to the scales of the study. Questionnaires were personally administered by researchers to the selected respondents. These surveys were conducted through personal meetings and telephonic calls.

Statistical Analysis

All the statistical analysis was conducted using Microsoft Excel and SPSS version 10. Frequencies and percentages for categorical variables and mean and Standard Deviations for continuous variables were used in the descriptive analysis. Most of the collected data is interpreted by Microsoft excel since it helps in better evaluation and compilation of a pilot study in a tabulated form and provides a variety of charts to show. Further analysis was done by SPSS version 10 for more accuracy of data. Pearson's Correlation test was applied to find out the association of mobile phone addiction with age, gender and sleep quality, where p value < 0.05 was considered significant.

RESULTS

Out of 649 participants, 328 were males and 321 were females with age group 13-55 years. Among 80% smartphone users mostly are from the upper middle class out of which 428 participants were students and 78% individuals are social. Mostly people worked for 7-8 hours (163 participants) or 5-6 hours (160 participants) so most of them use a mobile phone only 3-4 hours (202 participants) while only fewer 21% (77 participants) use for 7-8 hours. The present data consist of 51% ($n=28$) males and 49% ($n=321$) females. Most of them are undergraduates (59%) and students (66%). The working hours are 5-6 hours and 7-8 hours. About 78% ($n=503$) belong to upper middle class and only 2% ($n=16$) belong to lower class. The people are highly social about 78% of them are social (Table 01). Additionally, 82% of people have a smartphone. Whereas only 3% ($n=22$) have manual phone. About 31% of people use mobile phone 3-4 hours per day. Wi-Fi is commonly available, ($n=353$) 54% have internet access through Wi-Fi (Table 02). Furthermore, our study shows a significant relationship between mobile phone addiction and sleep quality ($p < 0.05$), however, there is no significant relationship between mobile phone addiction, age and Gender ($p > 0.05$). Moreover, age is significantly related to sleep quality ($p < 0.05$) (Table 03).



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DISCUSSION

As smartphone is gaining popularity around the world, their over usage has become epidemic. As reported by Pakistan telecommunication authority, by the end of May 2014, cellular mobile phone users in Pakistan reached 139.20 million [14]. In a study group, mobile phone addiction has a parallel effect on the sleep quality of an individual for any of the age group (0.000 overall, $p < 0.05$). Smartphone over usage results in various psychological and physical health hazards. Many studies found linkage between mobile phone addiction and disturbed sleep [15]. Similar results were reported by Soni, R et al. in a cross sectional study which were conducted on a sample comprises of 587 students [19]. It was reported that problematic mobile phone use affect sleep construction, by reducing REM Sleep, Slow Wave Sleep, and sleep efficiency [15]. The bright light of the gadget suppress secretion of melatonin and delay onset of sleep [20]. It was reported that usage of mobile phone in evening cause disturbance in sleep quality and the rhythmic secretion of melatonin as a consequence it influences the activity of brain especially the activity of the pineal gland, it may also result in disturbed blood flow to the brain and electrical activity of the brain [21]. Moreover, continues use of mobile phone can cause discomfort, such as pain in muscles and headache, which has impact on sleep [22]. Current study revealed no relevant gender variations in the frequency of Smartphone addiction (-373 overall, $p < 0.05$), Similar results were reported in a study conducted by Baifeng and his colleagues [23]. The variations between males and females depend on time of use rather than utilization [24].

Females are mostly concerned with social interactions whereas, males are interested in text messages and business applications [24]. The study shows no important gender variations within the frequency of sleep quality (-0.97, overall, $p < 0.05$) as reported in Kurubaran et al study [25]. In addition, different studies suggest that women are rumored to exhibit poorer sleep quality around the world compared to men [25]. The study shows non-significant relationship between age and gender (.987 overall, $p > 0.05$) as reported by Oliviero et al. In pre - adolescents, sleep quality is affected by web use and time-off, whereas in adolescents, mobile use and range of smart devices are major source of sleep problems [26]. It has been shown through this study that there is a direct relationship between age and mobile phone addiction (0.000 overall, $p < 0.05$), as reported by José De-Sola et al study. Mobile phone addiction greatly affect adults as compared to teenagers so, it verify that as long as someone using the mobile phone, it can be more harmful to them [24]. In general, the data shows that the total time spent on smart phones reduces with age, with the highest times reported for people less than 20 years old, principally adolescents and young adults [24]. Sleep patterns can change across the lifespan in different ways, including lessens in quantity and quality of sleep [27], with up to 50% of older adults report difficulties in initiating and continuing sleep [28]. The results of this study show positive outlook towards the age and sleep quality (0.015 overall, $p < 0.05$), Juan et al also reported similar results. Age is also depending upon the sleep quality and has a direct relationship [29]. Adolescents experience a natural shift in their circadian clock after puberty and usually are unable to fall asleep until 11 p.m. or later [30]. Teenagers addicted to smartphones have totally different sleep quality as compared to adult who are not addicted to it [23].

CONCLUSION

In conclusion, having various physical and psychological consequences of mobile phone abuse it significantly affects sleep quality and efficiency in different ways. Gender has no relevant association with Mobile phone addiction whereas, age is associated with it as adolescents are more addicted to mobile phone compared to adults. It is suggested that more in-depth qualitative and quantitative studies should be conducted and various programs should be organized to spread awareness regarding this issue for better future.

ACKNOWLEDGEMENTS

The authors express gratitude to Department of Physiology, University of Karachi.



**Amas Nadir et al.****Conflict of Interest**

The author declares no conflict of interest.

Funding

None.

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Table 1: Demographic Characteristics of the study population

Variables	Sub-Categories	n(%)
Gender	Male	328 (51)
	Female	321 (49)
Education level	Undergraduate	383 (59)
	Graduate	221 (34)
	Postgraduate	45 (7)
Occupation	Student	428 (66)
	Teaching	52 (8)
	Office job	71 (11)
	Housewife	39 (6)
	Business	19 (3)
	Doctor	6 (1)
	Other	32 (5)
Working hours	3-4 hours	141 (22)
	5-6 hours	160 (25)
	7-8 hours	163 (25)
	9-10 hours	105 (16)
	11-12 hours	80 (12)
Socio-economic status	Upper class	33 (5)
	Upper middle class	503 (78)





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	Lower middle class	94 (15)
	Lower class	16 (2)
Social life	Yes	505 (78)
	No	144 (22)

Table 2. Represents responses regarding mobile phone and internet

Variables	Sub-Categories	n(%)
Type of mobile phone	Smartphone	533 (82)
	Manual Phone	22 (3)
	Both	94 (15)
Usage of Mobile Phone	1-2 Hours	120 (19)
	3-4 Hours	202 (31)
	5-6 Hours	157 (24)
	7-8 Hours	77 (12)
	9-10 Hours	89 (14)
Source of Internet	Wi-Fi	353 (54)
	Data Connection	58 (9)
	Both	238 (37)

Table 3. Association of mobile phone addiction with age, gender and sleep quality

	Mean±SD	Mobile Addiction	Sleep Quality	Age	Gender
Mobile Addiction	3.32 (10.799)	--	--	--	--
Sleep Quality	30.50 (0.763)	.000**	--	--	--
Age	23.91 (8.166)	.000**	.015**	--	--
Gender	0.49 (0.500)	.373	.097	.987	--

** = p<0.05

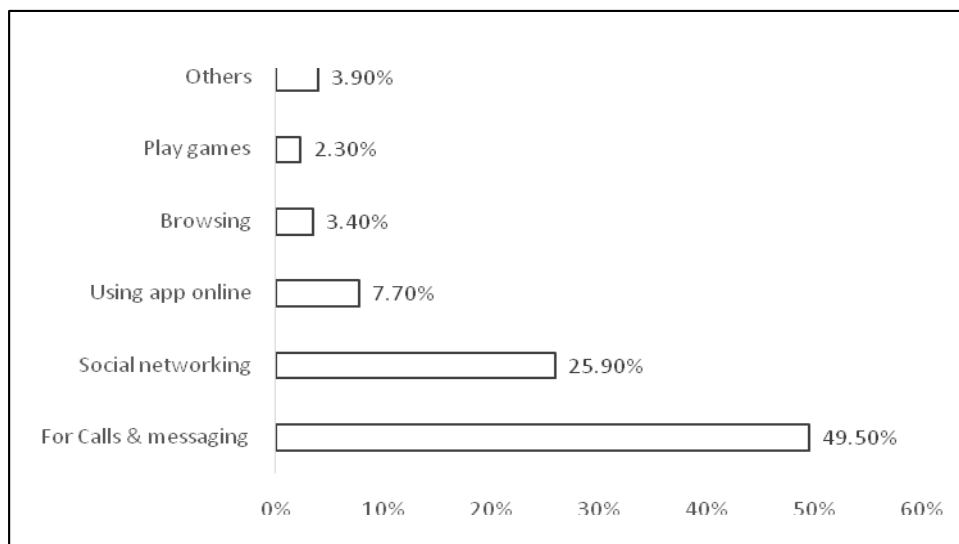


Figure 1: Graph showing primary purpose of using mobile phone among different age groups.





Short-Range Correlation Effect on the Elastic Charge Form Factors and Charge Densities of $^{64, 66, 68}\text{Zn}$ and $^{76, 78, 80, 82}\text{Se}$ Nuclei

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Received: 08 Jan 2019

Revised: 10 Feb 2019

Accepted: 12 Mar 2019

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ABSTRACT

The influence of two-body short-range correlations on elastic charge form factors, charge densities and root mean square charge radii of some fp-shell nuclei (such as, $^{64, 66, 68, 70}\text{Zn}$ and $^{76, 78, 80, 82}\text{Se}$) is examined utilizing the one- and two-body terms in the cluster expansion together with the single particle harmonic oscillator wave functions. The Jastrow-type correlation function is used to implant the effect of the short-range correlations into the elastic charge form factors $F(q)$ and charge densities $\rho(r)$. Both $F(q)$ and $\rho(r)$ depend on the correlation parameter β , which initiates from Jastrow correlation function, and the harmonic oscillator size parameter b . Both β and b are determined by the fit to the experimental elastic charge form factors. The implanting of short-range correlations imitates the experimental charge form factors at all region of momentum transfer considered in the experimental data. It is found that the embedding of short-range correlations is necessary for gaining a notable modification in the calculated elastic charge form factors and charge densities which in sequence leads to describe the data of electron scattering astonishingly. PACS numbers: 21.10.Ft, 21.60.Cs, 25.30.Bf

Keywords: electron scattering from fp- and N50-shell nuclei; elastic charge form factors; charge density distribution; short range correlation effect.

INTRODUCTION

Calculation of elastic charge form factors $F(q)$ and charge densities $\rho(r)$ for atomic nuclei is an interesting and exciting problem [1]. The opportunity of noticing this problem is via the independent-particle model (IPM). The IPM, which is regularly attractive for its simplicity, does not succeed for imitating the observed data at high momentum





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transfer from electron scattering in atomic nuclei [2-11]. Thus, alteration for the single particle potentials (SPP) must be suitably achieved. In fact, the short-range repulsion in the SPP seems proper for light nuclei [12]. For example, via the potential of the harmonic oscillator (HO) (which possess an infinite soft core), the $F(q)$ of ${}^4\text{He}$ may be well imitated, while for the heavier nuclei, for example ${}^{12}\text{C}$ and ${}^{16}\text{O}$, state-dependent potentials look necessary and even then the fitting is poor at higher values of momentum transfer (q) [12]. An additional method, which is somewhat similar, is the inclusion of the short-range correlation (SRC) in the Slater determinant. Many efforts were made in this tendency, connecting commonly light closed shell nuclei in the viewpoint of the Born approximation [4-11, 13-15]. It was initially revealed by Czyz and Lesniak [2] that the diffraction nature in the form factor of ${}^4\text{He}$ may be qualitatively understood by Jastrow-type [16] correlations. Khana [3], utilizing Iwamoto-Yamada cluster expansion with possession only one- and two-body cluster terms, demonstrated that the insertion of SRC offers an adequate explanation for the data on the elastic electron scattering from the ${}^{40}\text{Ca}$ and constructs conjectures for the presentation of the cross section at higher q . Ciofi degli Atti, employing the single pair approximation [4] and the Iwamoto-Yamada cluster increase [5] in s-p shell nuclei, exhibited that elastic electron scattering at higher q seems to offer an adequate sign for the presence of SRC's in atomic nuclei. Bohigas-Stringari [8] and Dal Ri et al. [9] explored the effect of SRC's on the one- and two-body densities via developing a low order approximation (LOA) in the framework of the Jastrow formalism. They showed that the form factors of one-body offer an adequate checkup for the existence of SRC's in atomic nuclei. Stoitsov et al. [11] enlarged the model of Jastrow correlations, mentioned by Ref. [8] within the LOA of Ref. [7], to heavier atomic nuclei like ${}^{16}\text{O}$, ${}^{36}\text{Ar}$ and ${}^{40}\text{Ca}$. The experimental data for these nuclei were very good imitated by this model [11].

Massen et al. [13-15], employing the cluster expansion of Clark and co-workers [17-19], derived a formula for elastic $F(q)$ truncated at the two-body term. This formula (depends on the parameters β and b) was used in the calculation of elastic $F(q)$ of closed shell nuclei (${}^4\text{He}$, ${}^{16}\text{O}$ and ${}^{40}\text{Ca}$) and in an approximate method for the open s-, p-, and sd-shell nuclei. Later, Massen and Moustakidis [20] performed a systematic study for the influence of SRC's on s-, p-, and sd-shell nuclei with entirely avoiding the method achieved in their previous studies [13-15]. An obvious formulas for $F(q)$ and $\rho(r)$ were derived using the factor cluster expansion of Clark and co-workers and Jastrow correlation functions which insert SRC's. These formulas depend on the single particle wave functions and not on the wave functions of the relative motion of two nucleons as was the case of our previous works [21, 22] and other works [4, 10, 13]. In this study, we examine the influence of SRC's on the elastic charge form factors (ECFF's), $F(q)$, the ground state charge density distributions (CDD's), $\rho(r)$, and root mean square (rms) charge radii of some fp-shell nuclei (such as, ${}^{64, 66, 68, 70}\text{Zn}$ and ${}^{76, 78, 80, 82}\text{Se}$). The parameters β and b are determined through assuming them as free parameters to be adjusted so as to imitate the measured elastic charge form factors. It is found that implanting of SRC's is required for gaining a noticeable amendment in the calculated ECFF's and CDD's of considered nuclei which in sequence tends to interpret the data of electron scattering astonishingly.

Theory

The ground state point nucleon density of nuclei is given by [20]

$$\begin{aligned} \rho_p^{gs}(r) &= \frac{\langle \Psi(r_1, r_2, \dots, r_A) | \hat{O}_r | \Psi(r_1, r_2, \dots, r_A) \rangle}{\langle \Psi(r_1, r_2, \dots, r_A) | \Psi(r_1, r_2, \dots, r_A) \rangle} \\ &= N_D \langle \Psi(r_1, r_2, \dots, r_A) | \hat{O}_r | \Psi(r_1, r_2, \dots, r_A) \rangle \\ &= N_D \langle \hat{O}_r \rangle, \end{aligned} \quad (1)$$





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where $\Psi(r_1, r_2, \dots, r_A)$ indicates the many-body wave function of nuclei, A signifies the nuclear mass number, $N_D = \langle \Psi(r_1, r_2, \dots, r_A) | \Psi(r_1, r_2, \dots, r_A) \rangle^{-1}$ designates the normalization factor which is determined such that

$4\pi \int_0^\infty \rho_p^{gs}(r) r^2 dr = 1$, and \hat{O}_r specifies the one body density operator which is given by

$$\hat{O}_r = \sum_{i=1}^A \hat{o}_r(i) = \sum_{i=1}^A \delta(\vec{r} - \vec{r}_i). \tag{2}$$

In terms of the cluster analysis, the expectation value of the operator \hat{O}_r is expressed as

$$\rho_p^{gs}(r) = N_D \langle \hat{O}_r \rangle = N_D \left\{ \langle \hat{O}_r \rangle_1 + \langle \hat{O}_r \rangle_2 + \dots + \langle \hat{O}_r \rangle_A \right\}. \tag{3}$$

The cluster expansion causes to the separation of one-body, two-body, ..., A -body correlation effects on the density. Here, three-body and higher terms is ignored. Thus, in the two-body approximation, the correlated density $\rho_p^{gs}(r)$ (where the effect of SRC's is inserted) is specified by [20]

$$\begin{aligned} \rho_p^{gs}(r) &\approx N_D \left\{ \langle \hat{O}_r \rangle_1 + \langle \hat{O}_r \rangle_2 \right\} \\ &\approx N_D \left\{ \langle \hat{O}_r \rangle_1 - 2O_{22}(r, \beta) + O_{22}(r, 2\beta) \right\}. \end{aligned} \tag{4}$$

It is obvious that the correlated density of eq. (4) depends on the parameter β inserted by the Jastrow-type correlation [16]

$$F = \prod_{i < j}^A f(r_{ij}), \tag{5}$$

where the two-body correlation function $f(r_{ij})$, which is a state-independent function, is expressed as

$$f(r_{ij}) = 1 - \exp[-\beta(\vec{r}_i - \vec{r}_j)^2]. \tag{6}$$

It has the manners: $f(r_{ij}) \rightarrow 1$ for large $\vec{r}_{ij} = |\vec{r}_i - \vec{r}_j|$ and $f(r_{ij}) \rightarrow 0$ for $\vec{r}_{ij} \rightarrow 0$. As a result, the influence of SRC's becomes significant when the parameter β is small and vice versa.

In eq. (4), the one-body term $\langle \hat{O}_r \rangle_1$ is well-known and written as

$$\begin{aligned} \langle \hat{O}_r \rangle_1 &= \sum_{i=1}^A \langle i | \hat{o}_r(1) | i \rangle \\ &= 4 \sum_{nl} \eta_{nl} (2l+1) \frac{1}{4\pi} \phi_{nl}^*(r) \phi_{nl}(r), \end{aligned} \tag{7}$$

where η_{nl} and $\phi_{nl}(r)$ are the occupation probability and the radial part of the single-particle harmonic oscillator wave function of the state nl , respectively. The two-body term $O_{22}(r, z)$ is specified by





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$$O_{22}(r, z) = 2 \sum_{i < j}^A \langle ij | \hat{\rho}_r(1) g(r_1, r_2, z) | ij \rangle_a, \quad (z = \beta, 2\beta) \tag{8}$$

where

$$g(r_1, r_2, z) = \exp(-zr_1^2) \exp(-zr_2^2) \exp(2zr_1r_2 \cos w_{12}), \tag{9}$$

The term $O_{22}(r, z)$ is then originated by expanding the factor $\exp(2zr_1r_2 \cos w_{12})$ in the spherical harmonics [23, 24] and given by [20].

$$O_{22}(r, z) = 4 \sum_{n_i l_i, n_j l_j} \eta_{n_i l_i} \eta_{n_j l_j} (2l_i + 1)(2l_j + 1) \times \left\{ 4A_{n_i l_i, n_j l_j}^{n_i l_i, n_j l_i, 0}(r, z) - \sum_{k=0}^{l_i+l_j} \langle l_i 0 l_j 0 | k 0 \rangle^2 A_{n_i l_i, n_j l_j}^{n_i l_i, n_j l_i, k}(r, z) \right\}, \quad (z = \beta, 2\beta)$$

where

$$A_{n_i l_i, n_j l_j}^{n_i l_i, n_j l_i, k}(r, z) = \frac{1}{4\pi} \phi_{n_i l_i}^*(r) \phi_{n_j l_j}(r) \exp(-zr^2) \times \int_0^\infty \phi_{n_i l_i}^*(r_2) \phi_{n_j l_j}(r_2) \exp(-zr_2^2) i_k(2zrr_2) r_2^2 dr_2 \tag{11}$$

and $\langle l_i 0 l_j 0 | k 0 \rangle$ is the Clebsch-Gordan coefficient.

The CDD's of the ground state $\rho_{ch}^{gs}(r)$ for closed shell nuclei with $N = Z$ may be related to those of point nucleon $\rho_p^{gs}(r)$ by

$$\rho_{ch}^{gs}(r) = \frac{1}{2} \rho_p^{gs}(r), \quad \text{in the unit of (e.fm}^{-3}) \tag{12}$$

It is important to mention that the formulas of eqs. (7) and (10) are created for closed shell nuclei with $N = Z$, where η_{nl} has the value of 0 or 1. For isotopes of closed shell nuclei, the correlated charge densities are still described by the similar formulas (because all isotopic chain nuclei have identical Z) except that we use different values for b and β . For open shell nuclei, we also use the same formulas but with $0 \leq \eta_{nl} \leq 1$.

The mean square charge radii for nuclei is well-defined by

$$\langle r^2 \rangle = \frac{4\pi}{Z} \int_0^\infty \rho_{ch}^{gs}(r) r^4 dr, \tag{13}$$

and the normalization of the CDD's $\rho_{ch}^{gs}(r)$ is given by





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$$Z = 4\pi \int_0^{\infty} \rho_{ch}^{gs}(r) r^2 dr \quad (14)$$

The ECFF's $F(q)$ of electron scattering from spin zero nuclei, may be related with the $\rho_{ch}^{gs}(r)$. In the plane wave Born approximation (PWBA), the waves of incident and scattered electron are described by the plane waves, and $\rho_{ch}^{gs}(r)$ is spherically symmetric and real, thus the elastic $F(q)$ is just the Fourier transform of the $\rho_{ch}^{gs}(r)$, i.e.

$$F(q) = \frac{4\pi}{Z} \int_0^{\infty} \rho_{ch}^{gs}(r) j_0(qr) r^2 dr, \quad (15)$$

Inserting the corrections [25] of the center of mass $F_{cm}(q) = \exp(q^2 b^2 / 4A)$ and finite size $F_{fs}(q) = \exp(-0.43q^2 / 4)$ into eq. (15), we have

$$F(q) = \frac{4\pi}{Z} \int_0^{\infty} \rho_{ch}^{gs}(r) j_0(qr) r^2 dr F_{cm}(q) F_{fs}(q) \quad (16)$$

It is important to refer that, at $q \rightarrow 0$, the target nucleus is supposed as a point particle and with the assistance of eq. (14), the charge form factor is equivalent to unity, i.e., $F(q = 0) = 1$.

RESULTS AND DISCUSSION

The computations of $F(q)$ and $\rho_{ch}^{gs}(r)$ of some even fp-shell nuclei (such as, $^{64, 66, 68, 70}\text{Zn}$ and $^{76, 78, 80, 82}\text{Se}$ isotopes) are achieved on the basis of using the single particle harmonic oscillator wave functions with oscillator size parameter b together with eqs. (4), (12) and (16). These nuclei are supposed to have a core of filled 1s, 1p and 2s-1d shells. Besides, the orbitals 1f and 2p of these nuclei are assumed to have $(Z - 20 - a_{2p})$ and a_{2p} protons, respectively. Two sorts of computations are examined, which refer to the computations made by the harmonic oscillator wave functions without and with the influence of SRC's. The computations of the first sort depend only upon the parameter b while those of the second sort depend upon the parameters b and β . The parameters in sort 1 (sort 2) are determined for each nucleus individually through altering b (both b and β) so as to fit the calculated elastic charge form factors to those of measured ones. The best fitting values for b and β as well as for the values of χ^2 ,

$$\chi^2 = \frac{1}{N} \sum_{i=1}^N \left[\frac{|F_i^{cal.}(q)|^2 - |F_i^{exp.}(q)|^2}{|F_i^{exp.}(q)|^2} \right]^2, \quad (17)$$

are displayed in Table 1. In Table 1, the computed root mean square (rms) charge radii $\langle r_{ch}^2 \rangle^{1/2}$ and the participating of the SRC's,

$$\langle r^2 \rangle_2^{1/2} = \sqrt{\langle r_{ch}^2 \rangle - \langle r_{ch}^2 \rangle_1}, \quad (18)$$





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to the $\langle r_{ch}^2 \rangle^{1/2}$ are also displayed and compared with the corresponding measured ones $\langle r_{ch}^2 \rangle_{exp}^{1/2}$. Table 1 shows that the parameter b , adopted in the calculations of sorts 1 and 2, holds the inequality $b(\text{sort } 1) > b(\text{sort } 2)$, where the implantation of SRC's leads to increase the relative distances among the nucleons which in sequence leads to increase the size of the nucleus. As a result the parameter b , which is related to the nuclear radius (experimentally fixed), must become lesser. In Fig. 1 [Fig. 2], the elastic charge form factors of $^{64, 66, 68, 70}\text{Zn}$ -isotopes [$^{76, 78, 80, 82}\text{Se}$ -isotopes] computed without SRC's (denoted by the dashed line) and with SRC's (denoted by the solid line) are presented and compared with those of experimental data. Here, the proton occupation probabilities for orbitals 1f and 2p are supposed to have $\eta_{1f} = 8/14$ and $\eta_{2p} = 2/6$ in Zn-isotopes [$\eta_{1f} = 12/14$ and $\eta_{2p} = 2/6$ in Se-isotopes]. The computed ECFF's of dashed line in Figs. 1(a)-(d) [Figs. 2(a)-(d)] predict the data very well at the region of low momentum transfer $0 \leq q \leq 0.9 \text{ fm}^{-1}$ [$0 \leq q \leq 0.7 \text{ fm}^{-1}$] and under predict the data at the region of $q > 0.9 \text{ fm}^{-1}$ [$q > 0.7 \text{ fm}^{-1}$], where the experimental data for the second maximum is noticeably under predicted. However, incorporating the effect of SRC's (the solid line) enhances the magnitude of ECFF's at the region of the second maximum which in sequence leads to push them into the line of accordance with the data. It is clear that all diffraction minima and maxima found in the measured data are reproduced in the correct place by the solid line. In addition, both the performance and magnitude of ECFF's displayed by this line exhibit a reasonable accordance with the measured data.

In Fig. 3 [Fig. 4], the dependence of the ground state CDD's on r for $^{64, 66, 68, 70}\text{Zn}$ -isotopes [$^{76, 78, 80, 82}\text{Se}$ -isotopes] calculated without SRC's and with SRC's are presented and compared with those of fitted to the experimental data [26, 27]. The calculated CDD's without and with the effect of SRC's are demonstrated by the dashed and solid lines, correspondingly, whereas those of fitted to the experimental data are demonstrated by the symbols of open circle. Furthermore, the participating of SRC's

$$\rho_2(r) = \rho_{ch}^{gs}(r) - \rho_{1,ch}^{gs}(r), \quad (19)$$

to the $\rho_{ch}^{gs}(r)$ is also demonstrated in these figures. The behavior of $\rho_2(r)$ is described by fluctuations about the r -axis. It is apparent from this figure that the probability of finding a proton near the central region ($0 \leq r \leq 3 \text{ fm}$) of $\rho_{ch}^{gs}(r)$ is larger than the tail region ($r > 3 \text{ fm}^{-1}$). In addition, the implanting of SRC's effect into $\rho_{ch}^{gs}(r)$ leads to reduce significantly the central part of $\rho_{ch}^{gs}(r)$ and increasing slightly the tail part of $\rho_{ch}^{gs}(r)$ as seen by the solid distributions. This means that the inclusion of SRC's tends to increase the probability of transferring the protons from the central region of the nucleus towards its surface which in sequence tends to increase the rms charge radii of nuclei and consequently makes the nuclei to be less rigid than the case when there is no SRC's effect. To keep the nuclear size of nuclei under study within the fixed experimental value, the parameter b must be decreased with considering the effect of SRC's, see Table 1. However, inserting the effect of SRC's into the CDD's of considered nuclei leads to improve the calculated distributions of $\rho_{ch}^{gs}(r)$ (solid lines) and makes them in agreement with the data.

CONCLUSIONS

The effect of SRC's on the form factors and charge densities of $^{64, 66, 68, 70}\text{Zn}$ and $^{76, 78, 80, 82}\text{Se}$ is investigated using the one- and two-body terms in the cluster expansion. The Jastrow-type correlation function is used to implant the effect of





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SRC's into the charge form factors and charge densities. It is concluded that the implanting of SRC's effect in the charge form factors and charge densities is needed for gaining a notable amendment in the computed results and essential for description the data of electron scattering amazingly.

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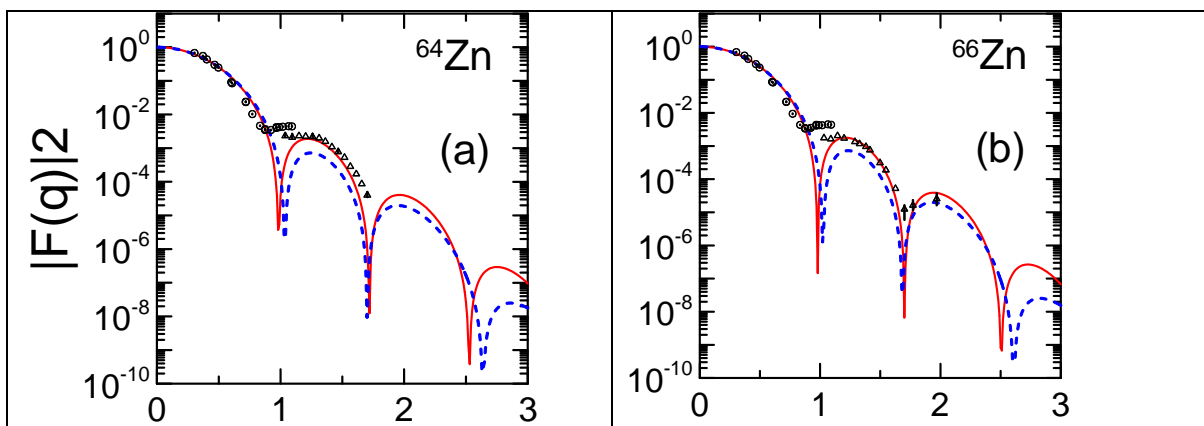




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Table 1: Values for the parameters b , β , and for the contributions of the rms charge radii $\langle r_{ch}^2 \rangle^{1/2}$ of the one- and two-body densities as well as the total rms charge radii for Zn- and Se-isotopes determined by the χ^2 fit to the experimental charge form factor.

Nucleus	η_{1f} η_{2p}	b (fm)	β (fm ²)	χ^2	$\langle r_{ch}^2 \rangle^{1/2}$ (fm)				
					NSR	WSR	Total	Exp.[26,27]	
⁷⁴ Zn	8/14 2/6	2.066	-----	0.618	3.863	-----	3.863	3.895	
		1.941	2.113	0.303	3.631	1.272	3.847		
⁶⁶ Zn		2.088	-----	0.491	3.904	-----	3.904	3.991	
		1.965	2.125	0.264	3.676	1.247	3.881		
⁶⁸ Zn		2.087	-----	0.623	3.902	-----	3.902	3.979	
		1.963	2.118	0.365	3.672	1.253	3.879		
⁷⁰ Zn		2.085	-----	0.836	3.898	-----	3.898	3.983	
		1.961	2.115	0.305	3.668	1.256	3.877		
⁷⁶ Se		12/14 2/6	2.173	-----	0.463	4.127	-----	4.127	4.162
			2.046	1.870	0.335	3.890	1.410	4.137	
⁷⁸ Se	2.164		-----	0.476	4.110	-----	4.110	4.138	
	2.038		1.892	0.342	3.875	1.399	4.119		
⁸⁰ Se	2.110		-----	0.415	4.010	-----	4.010	4.124	
	1.982		1.871	0.272	3.769	1.468	4.045		
⁸² Se	2.135		-----	0.435	4.056	-----	4.056	4.118	
	2.012		1.865	0.322	3.826	1.445	4.089		





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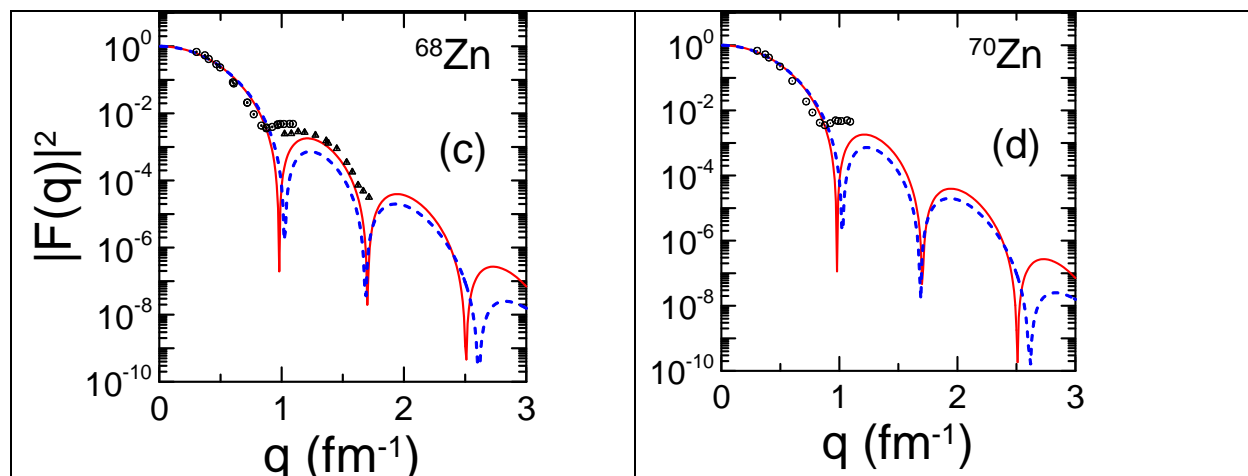


Fig. 1. Elastic charge form factors for Zn-isotopes. The dashed and solid curves are the computed form factors without and with the effect of SRC's, correspondingly. The symbols of circles are the experimental data taken from [28] while the triangles are taken from [29] for $^{64,66}\text{Zn}$, [30] for ^{68}Zn

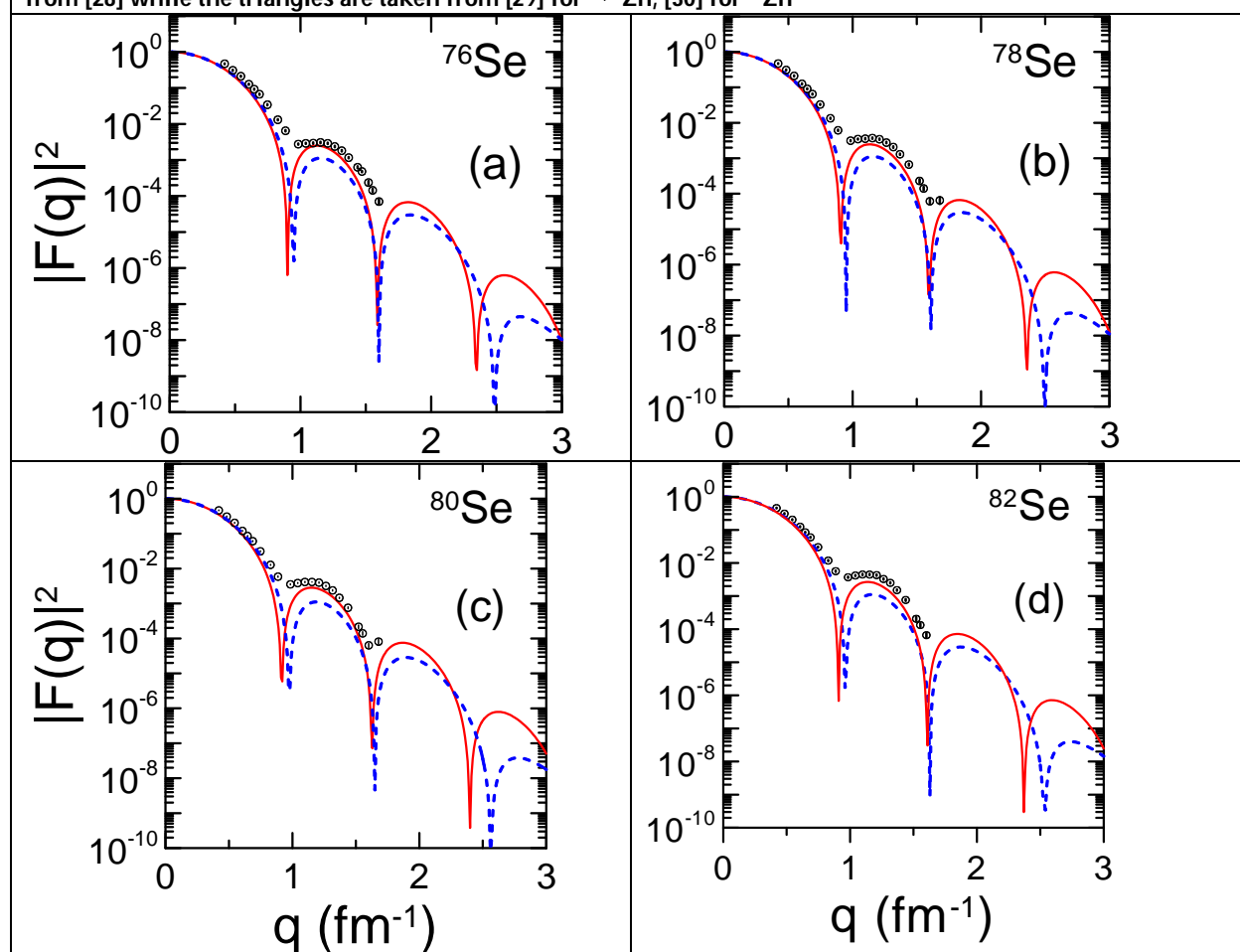


Fig. 2: Same as in Fig. 1 but for Se-isotopes. The experimental data are taken from [31, 32].





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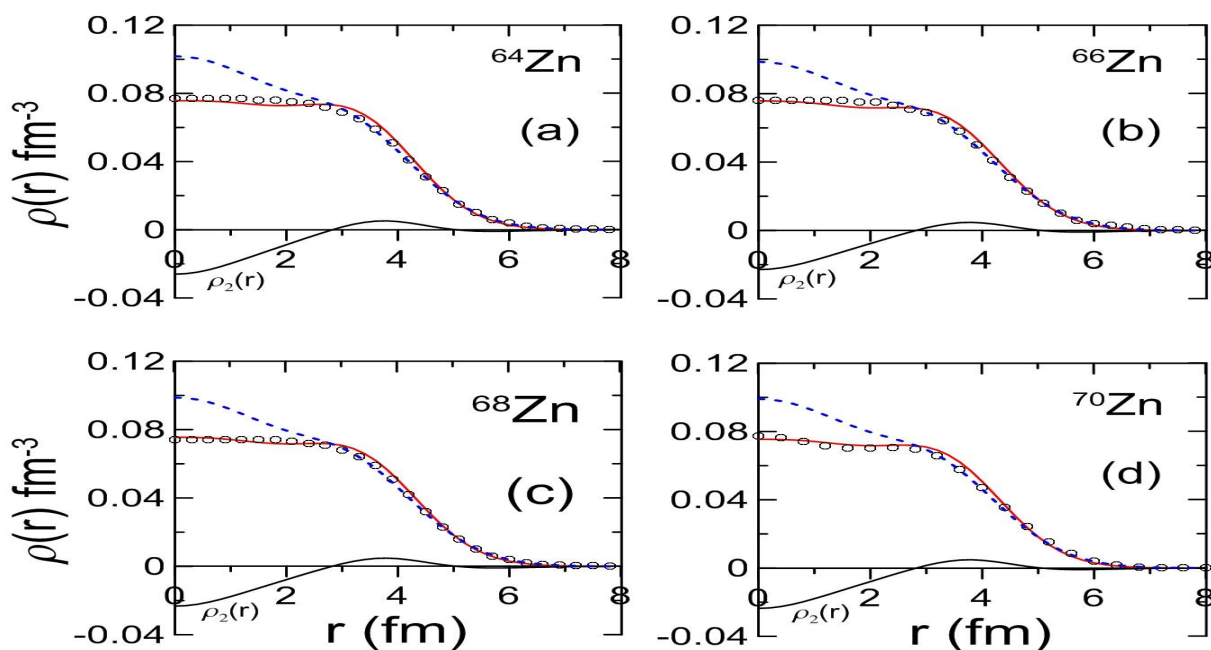


Fig. 3. Charge density distributions for Zn-isotopes. The dashed and solid curve are the computed charge densities without and with the effect of SRC's, respectively. The circles are the experimental data of 2 parameter Fermi (2PF) taken from [26].

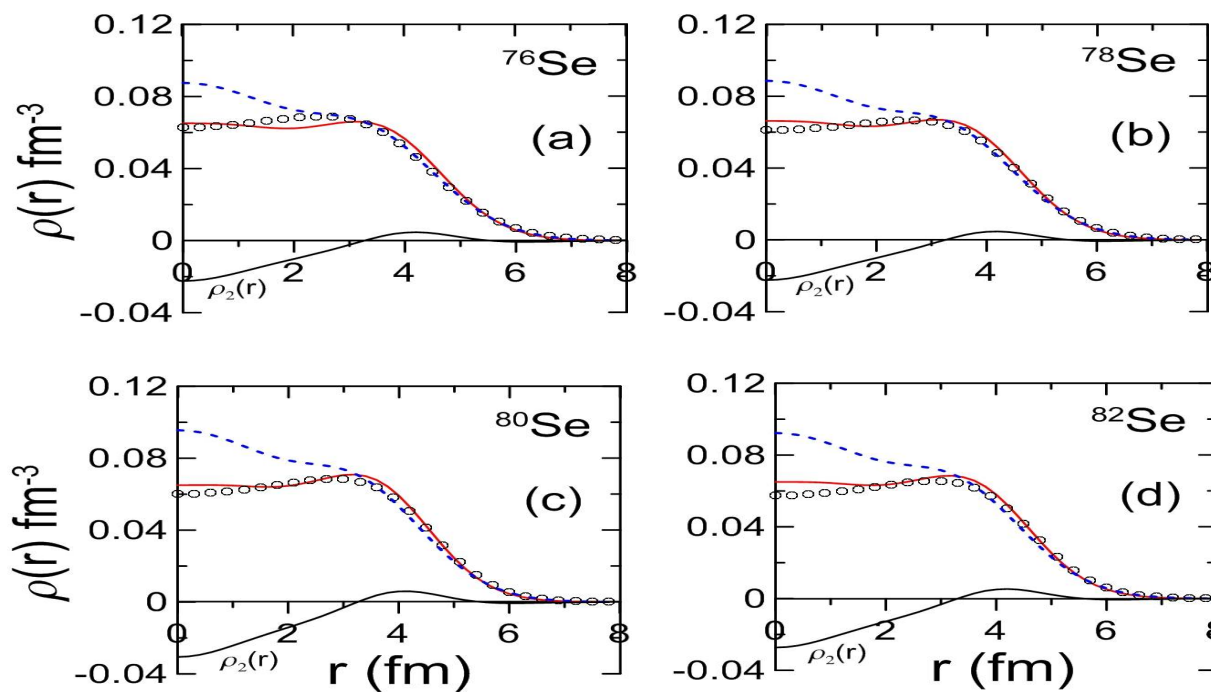


Fig.4. Same as in Fig.1 but for Se-isotopes. The circles are the experimental data of 3 parameter Gaussian (3PG) taken from [27].





Knowledge and Practices of Health Care Providers for Medication Non-Adherence in Pakistan

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Received: 20 Jan 2019

Revised: 22 Feb 2019

Accepted: 25 Mar 2019

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ABSTRACT

Background : Medication nonadherence is an intricate health care problem. Despite of the fact that efforts are being made both on individual as well as on community level to overcome medication nonadherence but this global issue remains a big challenge for the health sector. **Objective:** The purpose of the study was to gather the insights, opinions, and views of the health care providers (HCP's) concerning medication non-adherence. **Methodology:** A Cross-sectional study was conducted amongst HCP's including, consultants, registered nurses and pharmacist in Pakistan. Total 200 HCPs were enrolled through purposive sampling method. The data was collected by means of a questionnaire about clinical experience, current status and perceptions on medication nonadherence. Statistical analysis was done through SPSS version 22.0. **Results:** According to our results about 80% HCP's considered medication adherence extremely important in clinical setting. Majority agreed to the fact that medication adherence data must be provided to the HCP's. Medication adherence counselling sessions were also promoted and 75% HCP's recommended it to be upon every new prescription. Moreover, they also suggested that medication adherence campaigns and counselling session must be implemented keenly to improve clinical outcomes. Forgetfulness, increased cost, lack of trust and delayed recovery were considered as the major reasons behind medication nonadherence while education was opted as the prime source for regulating it. **Conclusion:** Medication dose monitoring, close-follow-up, proper records of medication track, single and cost effective drug dosage and effective interaction between patients and HCP can aid in reducing medication nonadherence. Within our socio-economic scenario, we need to improve adherence by implementing personalized approach based on the exclusive type of patient journey and challenges of nonadherence with the specific needs of the patient in our socio cultural setup.



**Sadaf Ahmed et al.****Keywords:** Knowledge, Practices, Medication Non-Adherence, Health Care Providers.

INTRODUCTION

According to the description provided by World Health Organization (WHO), adherence is the alterations in the patient's behavior in following prescription and taking appropriate medicine, following a proper diet plan and modifying lifestyle activities as per the recommendations of the HCP and this however, proves to be the key in disease management [1]. This dynamic procedures involves three phases i.e. initiation, implementation and non-persistence [1]. Medication non-adherence is a rising concern for health sector. The prime entity to blame might be the patients, medication, health-care providers (HCP's) or Pharmaceutical companies and socio-economic factors[2]. As a result the associated morbidity and mortality rate is increasing and as well as social cost of the drug as number of patients do not fill in the prescription even once, which in itself is a big menace to the pharmaceutical agencies and the health sector as well³. Medication non-adherence adversely affects pharmacotherapy and also reduces the intensity of improvement⁴. There are multiple reasons for nonadherence, in general lack of prescriptions, unavailability of medicines, lack of social support, increased cost, miscommunication, therapeutic alternatives, side-effect, delayed improvement and decreased literacy rate are few of them[2,5-10].

There are a number of stages of medication nonadherence, possibly patient might have not taken medication at all or he/she might have taken it once or twice and then discontinued the treatment¹¹. Usually the patients do take more or less dosage as recommended and on wrong timings. Deviation from prescribed medication and alterations in treatment plan are linked to numerous reasons that may be associated with intentional or unintentional need to modify the HCP's advice[11-13]. Among the common medication related factors leading to non –adherence are prolong therapy, fear of dependency, difficult and lengthy leaflet information. Moreover, pill color and shape have also been reported to However, pill characteristics, such as changes in color and shape, have been reported to increase medication adherence[14]. HCP-Patient interaction barriers, inadequate time period with the HCP, improper deliverance of information, insufficient explanation are major HCP associated factors promoting medication non-adherence[15]. Major drawback in the healthcare sector is physician's failure in recognition of medication non-adherent patients. Physicians do promote medication non-adherence by recommending complex drug regime without understanding the patient barriers and financial status. They even fail to explain the benefits and adverse effects of a medication effectively[16&17]. It is evident that around 70% of the non-adherence cases are associated with lack of knowledge among the physicians and the patients as well[18]. This however, indicates the need to educate physicians and also to improve health education publicly. Through this study we sought to understand the opinion of HCP's regarding nonadherence, its causes, its importance in the health sector and what can be done to improve adherence rates.

METHODOLOGY

A Cross-sectional study was conducted between March to April 2018. 200 healthcare providers (HCP's) including consultants, registered nurses and pharmacist were enrolled in the study through purposive sampling method. The objective of the study was explained to the HCPs and consent was obtained from each HCP before study initiation. All the selected HCPs had 10 to 30 years of practicing experience. Independent Ethics Committee approval was obtained before commencement of this study. All the ethical guidelines were followed and the confidentiality and anonymity of participant's data was maintained. Data was collected through a questionnaire regarding clinical experience, current status and perceptions of HCP's. The collected data was analyzed using SPSS version 22.0. Descriptive statistics was used and the variables were presented as frequency and percentages.



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RESULTS

The analysis was performed over 200 participants and each with a practicing experience ranging from 10-30 years. Table 1 shows the summary of awareness regarding medication adherence and non-adherence among HCP's. Results showed that 55% of the HCP's conveyed that there are less than 10% of the prescription that are never filled in their opinion while 25 consider it to be ranging between 10-15%. Moreover, 20% HCP's suggested that less than 10% of the patients filled the prescription at least once. HCP's perception regarding medication adherence and its importance in clinical setup was recorded and their answers rated from 0-10 on the scale. It was observed that 80% of the HCP's consider medication adherence extremely important in clinical setup and rated it highest i.e. 10 while none of them find it extremely unimportant. Around 75% HCP's recommended that Medication adherence counselling session must be conducted upon every new prescription while 50% suggested it to be placed upon follow-up visits. Around 35% HCP's mentioned that there should be minimum 90% medication adherence for improved clinical outcomes while only 20% thought that minimum medication adherence could be 50%.

In HCP's opinion patients themselves play a vital role in medication adherence (60%) while 25% consider the HCP holding the prime responsibility to improved medication adherence rate. 80% HCP's agreed to the fact that medication adherence data must be made available in the healthcare setting as this would help them to identify the non-adherent patient and to arrange elongated counselling sessions for them. According to Figure 2, 13% of the HCP's suggested that forgetfulness might be the reason behind medication non-adherence. While 10% opted the medication cost as prime reason followed by side-effects, lack of trust and priority were the other mentioned factors regulating medication non- adherence. Figure 3 shows the intervention designed for medication adherence and their influence. 12% of the HCP's suggested that education can be the best intervention for medication adherence and it also holds strong influence on the public. While 8% suggested that reminder emails or texts can help. 13% recommend reminder phone calls or letters, suggesting moderate influence.

DISCUSSION

Although the discovery of advanced medication have controlled outbreak of many prevalent conditions but medication non-adherence limits the drug impact and slows down the rate of achieving therapeutic goals [19]. Healthcare regulatory authorities and pharmaceutical companies have overlooked this crucial issue rather than addressing it[19]. Poor adherence is a rising health concern that has been treated as a humiliating fact. Despite of the fact that numerous methods for measuring medication adherence have now been introduced but the regulators and HCP remain reluctant towards any of such innovations [19]. Here, we discuss the consequences, perceptions and preferences of HCP's regarding medication nonadherence and also the possible solution to these problems [19]. One of the major factors that promote medication nonadherence is unfilled prescriptions. According to a study published in Annals of Internal Medicine around 20-30% of the prescriptions are never filled.

Moreover, 50% of the chronic disease medication are never consumed as prescribed[20&21]. It is quite natural that patients who follow the HCP's instructions and consume medication as prescribed, do not remain adherent to it for long[20&21]. The HCP's enrolled in our study had similar perception, around 25% of them reported 10-15% of unfilled prescriptions while 5% were those who reported the rate of unfilled prescriptions to be more than 30% (Table 1). As indicated in figure 1, majority of the HCP's suggested that medication adherence plays a vital role in health care setting and rated it as extremely important. There is vast literature highlighting medication nonadherence among diabetes, hypertension and asthma patients. According to a study greater would be the adherence more pronounced will be the clinical outcomes [22&24]. Likewise, on the basis of previous evidences individual beliefs, preferences and personal barriers are the factors that needs to be targeted as this would result in improvisation in medication adherence and also lead to better clinical outcomes [25&26].



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The preferences and perceptions of HCP's regarding adherence and non-adherence were also marked. Many HCP's preferred medication adherence counselling sessions and also promoted medication adherence to improve disease outcomes (Table 2). Moreover, patients were considered as the major pillar to hold responsibility for medication adherence followed by the HCP's[27]. It is a known fact that medication adherence responsibilities fall on the patient and the healthcare team[27]. They mostly preferred availability of medication adherence data in the health care setting for better understanding and more pronounced counselling of non-adherent patients (Table 2). Medication adherence data quantification is among the most required healthcare facility in the current world. In order to keep the track of patient fill, refill, discontinuation, non-persistence and other related medication taking behaviours[28]. It is evident through a number of studies that the disease management and medication adherence is significantly improved with patient counselling[29&30]. While some studies also showed contrasting results [31&32]. The reasons for medication-nonadherence were marked by the HCP's showing high, moderate and minor significance (Figure 2). Forgetfulness, speed of recovery, drug cost and side-effects were major concerns according to the HCP's. Supported by a study conducted by carter and his colleagues, they concluded that reasons for medication non-adherence can be involuntary like forgetfulness and voluntary such as fear of side-effects and medication avoiding behaviour [33&34]. While other associated reasons might be lack of patience, drug abuse, inconsistency, complex regime, illiteracy, high cost and communication gap with the HCP can also be a cause for medication non-adherence [33-36].

According to a systematic review conducted to explore interventions for controlling medication non-adherence, patient and HCP's education, patient-physician interaction and shared decision making and pharmacist support are the main leads [37]. It is proven that educational interventions improve medication adherence typically working on patient understanding. Also indicated in figure 4, education is considered as the strong influencer for medication adherence. Despite of the fact that it is mutual responsibility of patient and HCP to maintain medication adherence throughout the treatment duration. The HCPS's are recommended to focus on patient financial status and help them in gaining health with least medication costing. In addition, the HCP's should never assume regarding patient adherence. Questioning and interviewing the patient regarding medication use is necessary and in turn improves clinical outcomes.

CONCLUSION

Non-adherence is multi-layered problem and it needs to be dealt with in a number of ways in order to improve it. Major reasons for medication non-adherence as observed in our study population were cost of medications, lack of patient awareness, fear of side effects, misconceptions about effects of medication, unavailability, lack of counselling, poor doctor patient bond, social factors, mistaken influence of media, irrelevant information sources and unconvincing need for therapy were explored as factors contributing to patient non-adherence to medication. We must admit this ground reality that this is not just a minor issue that can be resolved with a slight effort. It is a gigantic problem that is linked to several other problems without a common key solution. The evidence gathered from HCPs can help develop and shape concepts for meaningful and high impact medication adherence interventions. For better implementation, all patients must be targeted regardless of whether they are adherent or not.

Future Prospects

These outcomes will be useful in development of the future awareness campaign or tools to address medication non adherence like patient counselling sessions, electronic monitoring for adherence, short messaging services, low costing programmes, patient awareness campaigns, psycho-social interactive campaigns to reduce stress and anxiety.



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None.

ACKNOWLEDGEMENTS

We would like to express our special gratitude to the entire team involved in this study. We are thankful for their support throughout the study.

Funding

This study was supported by Advance Educational Institute & Research Center.

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Table 1. HCP’s knowledge regarding medication adherence and non-adherence

	Sub-categories	%
Prescription never filled	<i>Less than 10%</i>	55
	<i>10-15%</i>	25
	<i>20-25%</i>	15
	<i>More than 30%</i>	5
Prescription filled at least once	<i>Less than 10%</i>	20
	<i>10-20%</i>	25
	<i>20-30%</i>	25
	<i>30-40%</i>	25
	<i>More than 50%</i>	5

Table 2. Indicates the preferences & perceptions of health care providers regarding management of medication non-adherence

HCP’s Preferences	Sub-categories	%
Medication adherence counselling session	Upon new prescription	75
	Upon follow-up	50
Minimum medication adherence for improved clinical outcomes	<i>50%</i>	20
	<i>60%</i>	10
	<i>70%</i>	10
	<i>80%</i>	20
	<i>90%</i>	35
	<i>Others</i>	5
Responsibility of entities to improve medication adherence rates	<i>Patients themselves</i>	60
	<i>Health care providers (HCP’s)</i>	25
	<i>Insurance companies</i>	5
	<i>Employers</i>	5
	<i>Pharmaceutical companies</i>	5
Availability of medication adherence data of each individual patient	<i>More attention could be given</i>	15
	<i>Elongated counselling sessions with the non-adherent patients</i>	80
	<i>Cannot do anything influential with it</i>	5





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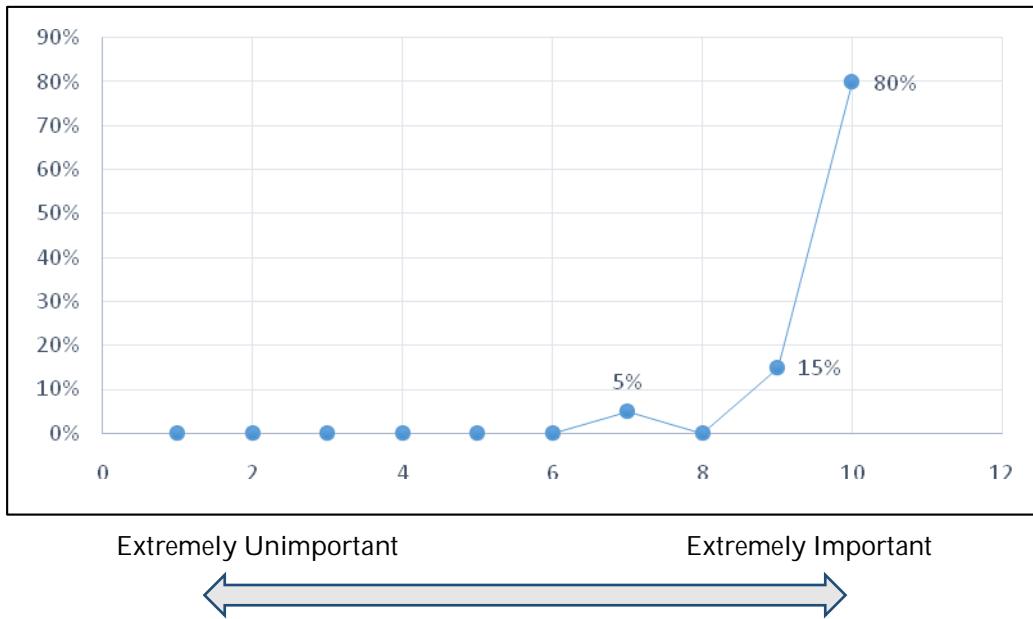


Figure 1. HCPs observation regarding medication adherence and its importance in clinical outcomes.

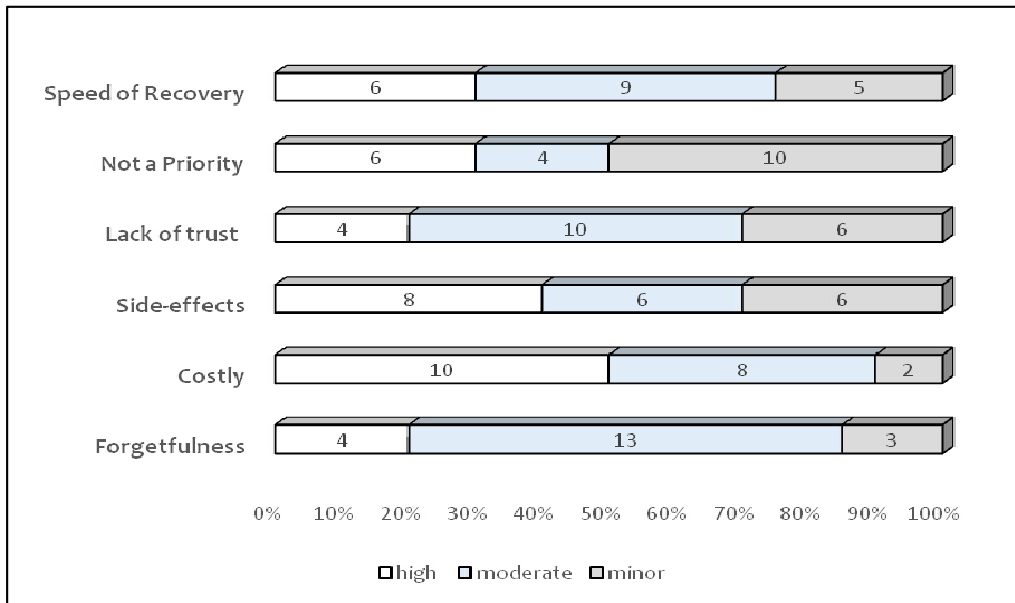


Figure 2. Expected reasons for medication non-adherence according to HCP's understanding





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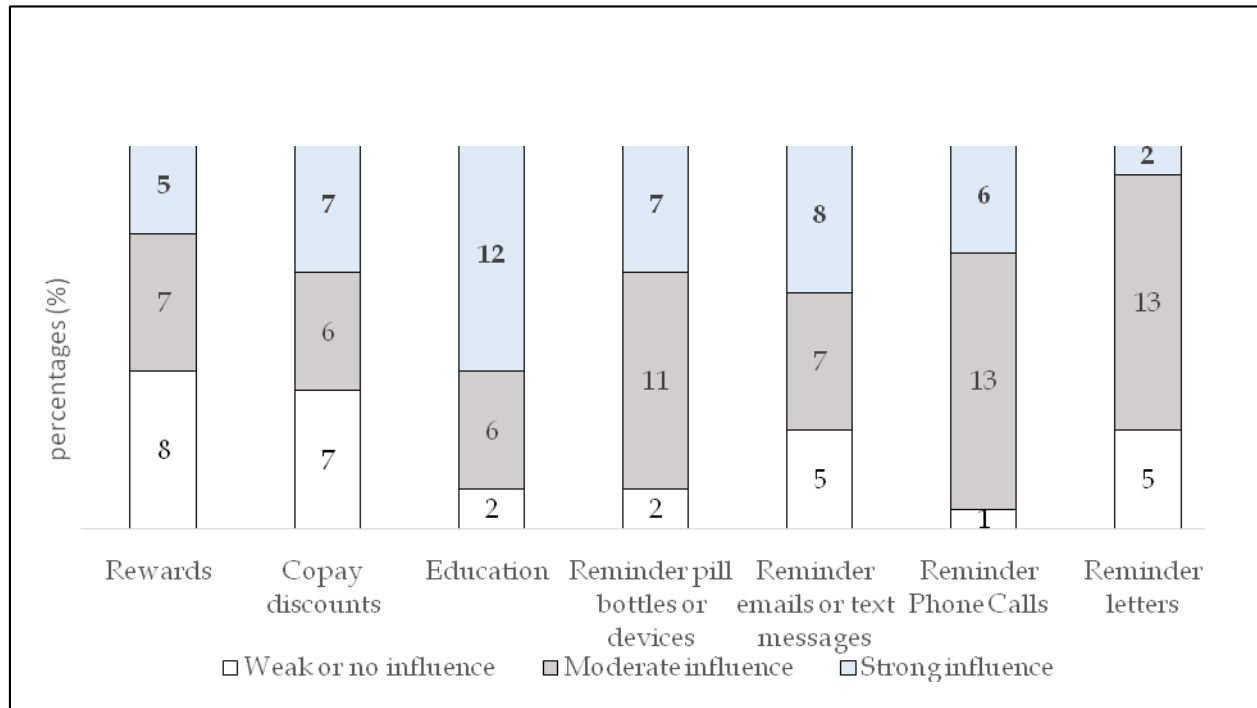


Figure 3. Interventions designed to improve medication adherence according to HCP's understanding.





An Overview of Photonic Crystal Fiber (PCF)

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Received: 21 Jan 2019

Revised: 23 Feb 2019

Accepted: 25 Mar 2019

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ABSTRACT

Photonic crystal fibers (PCFs) are a special class of optical fibers characterized by a periodical arrangement of microcapillaries that form the fiber's cladding around a solid or hollow defect core. This paper gives an overview on Photonic Crystal Fiber (PCF), fabrications, types, materials and its applications. Micro structured or Holey fiber is the alternative names of Photonic Crystal Fiber. PCF is preferred over the conventional optical fiber due to its high design flexibility. Photonic crystal fiber is an optical fiber with a periodic arrangement of low index material in a background with high refractive index material. Light can be guided inside the PCF by either modified total internal reflection or photonic band gap guidance. The discussions of photonic crystal fiber in this overview include its fabrication types, materials and its applications. "Stack and draw technique" and "Extrusion fabrication process" methods are used for the fabrication of PCF. Hence Photonic crystal fiber is a better alternative to conventional optical fiber for the above applications.

Keywords: Photonic crystal fiber (PCF), Modified total internal reflection, photonic band gap guidance.

INTRODUCTION

Optical fiber is a transmission media that provides more reliable, flexible and versatile optical channel than the atmosphere. In any communication system the most important specifications are bandwidth and signal to noise ratio (SNR) because they decide the performance of the transmission channel. Recently, optical fibers channels have loss of 0.2 dB/km at 1550 nm wavelength. The bandwidth of a single optical fiber link is approximately 50 THz. Thus fiber optic communication systems form the backbone of modern telecommunication system. Also, the optical fibers have wide range of applications in sensors[1]. Thus, an attractive alternative fiber came into existence. PCF provides excellent design flexibility by varying the geometrical dimensions of the fiber. PCFs are optical fibers with a periodic arrangement of low-index material in a background with higher refractive index. The background material in PCFs is





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usually pure silica and the low-index region is typically provided by air holes running along their entire length. In particular, by changing the geometric characteristics of the air holes in the fiber cross-section, that is, their position or dimension, it is possible to obtain PCFs with excellent design flexibility[1]. By using PCF it is possible to achieve single mode even at core diameter in the range of several tenths of μm , whereas in conventional optical fiber highly diminished core is required for single mode fiber. In optical fiber zero dispersion occurs at 1310 nm. To get zero dispersion at different operating wavelengths dispersion compensating fiber can be designed using PCF[2].

Photonic Crystal

Photonic crystals are periodic microstructures that affect the flow of photons in the same way of ionic lattice which effect on the electrons in semiconductors. Photonic crystals are periodic dielectric structures[3]. They are named as crystals due to their periodicity and photonic as they act on light. This actually occurs when the period is less than the applied wavelength of the light. Photonic crystal may prevent the flow of certain range of wavelengths in either one direction or in all directions and hence provide the possibility to guide, confine and trap the light in a cage[3]. Photonic crystal includes repeated regions with higher and lower dielectric constant which are in periodic fashion. Photons propagate through this structure. Modes are the wavelengths that are allowed to propagate and group of modes form bands[2, 3]. The disallowed bands of wavelength form photonic bandgaps. Light for some wavelength within the photonic band-gap is prohibited from propagation in any direction inside a photonic crystal. Because of this similarity with semiconductor having energy gap for electrons, photonic crystals are sometimes even called (semiconductors for photons). They can be created from almost any material, so it satisfies the material compatibility requirement[4]. Photonic crystal can be fabricated as one-dimensional [1D], two dimensional [2D] & three-dimensional [3D] photonic crystal[2, 4]. Fig.1 shows the shape of [1D], [2D] and [3D] photonic crystals. In [1D] photonic crystal, the modulation periodicity of the refractive index occurs in one axis only, while the refractive index (n) variations are uniform for other two directions of the structure. Similarly in two & three dimensional photonic crystal, the periodic modulation of the refractive index occurs in two & three axes respectively. The simplest examples of [1D], [2D], and [3D] photonic crystal are Bragg grating, photonic crystal fiber, stake of two dimensional crystals respectively[5].

Photonic Crystal Fiber (Pcf)

Photonic crystal fibers (PCFs) are a special class of optical fibers characterized by a periodical arrangement of micro structures that form the fiber's cladding around a solid or hollow defect core[2-4, 6]. Photonic crystal fibers, also known as micro structured or holey fibers generated great interest in the scientific community. There are several variations of photonic crystal fibers that are used in applications[7]. Fig. 2 classifies photonic crystal fibers into solid (a) and hollow (b) core types. Under these two types, there are many different types of photonic crystal fibers. The lattice symmetry, pitch, and hole size can be changed to suit the design of a specific fiber type. The solid core class of fibers has a high-index solid core with a lower effective index surrounding medium. The hollow core fibers have a lower index than the surrounding medium, and confinement to the center core hole is made possible by scattering and interference, combining to form an evanescent field in the cladding region[2]. We explore here just a few of the many types of specialty photonic crystal fibers available. Endlessly single mode fibers are uniquely suited to applications where widely different wavelengths are used. In common core/cladding fibers, the single mode regime is defined by the V parameter, which is the scaled frequency, i.e., $V = 2\pi a \sqrt{n_{\text{core}}^2 - n_{\text{clad}}^2} / \lambda$, where a is the core radius, and the indices of the core and cladding are n_{core} and n_{clad} . For $V < 2.4048$ ordinary fiber has a single mode, and this is desirable to avoid modal pulse dispersion effects in the multimode regime. In forever single mode fibers, the mode is designed to change the effective index, so that the indices are replaced by effective wavelength-dependent functions $n_{\text{core}}(\lambda)$ and $n_{\text{clad}}(\lambda)$, and the core radius is replaced by the hole pitch (Λ). The effective index changes with wavelength to keep the effective V parameter for the endless single mode fiber below a threshold value. To maintain a single mode photonic crystal fiber with a single central hole missing, one needs to keep the ratio of the



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hole diameter to the hole pitch small, i.e., $d/\Lambda < 0.45$. For fibers with more missing holes the ratio is smaller, e.g., for seven missing holes $d/\Lambda < 0.15$ [6]. An example of a forever single mode fiber is shown in Fig. 3 (a). Note that the holes are small as compared to the lattice pitch so that the fill factor is small for this fiber, the fiber designs with large mode areas can suppress nonlinear effects when they are undesirable. Endless single mode fibers are used to align several laser wavelengths before insertion into crystals for nonlinear parametric frequency conversion. The spiderweb fiber shown in Fig. 3 (b) has a small core that concentrates light and a very high-index contrast with the surrounding medium, which increases the local light intensity.

This fiber is designed to enhance nonlinear effects that include stimulated Raman scattering and self-phase modulation. Applications include super-continuum generation where the frequency bandwidth is more than doubled (i.e., an octave or more of light frequencies) due to nonlinear propagation in the fiber. Super-continuum generation has a bright output. The applications include spectroscopy and optical coherence tomography [1, 4, 6, 7]. The fibers can be designed to enhance selected nonlinear effects, such as Raman signal generation to amplify signals at the Raman shifted frequency, four-wave mixing signals, and nonlinear parametric processes. The hollow core fiber confines a band of light wavelengths within the air-core region, such as seen in Fig. 3 (c). In the figure blue wavelengths are confined in the core while other wavelengths leak throughout the cladding region. These fibers can transport intense laser fields that might otherwise damage a fiber by confining most of the energy in the hollow core, and they can serve as sensors that have a long path length to determine the presence of trace amounts of gases or biomolecules contained in aerosols flowing through the hollow core[5].

Guiding Mechanism

In conventional optical fiber total internal reflection process is utilized to guide the light through the fiber since core refractive index n_{core} is greater than that of cladding n_{cladding} so light is confined inside the core. In photonic crystal fiber (PCF) the two light guiding mechanisms are used. In solid core photonic crystal fibers, where light is confined in a higher refractive index region, modified total internal reflection is exploited, which is similar to the guiding process of the conventional optical fibers. While, in hollow core fibers when the light is confined in a region with a refractive index lower than that of the surrounding area, photonic band gap mechanism is used[4].

Modified Total Internal Reflection

Two dimensional photonic crystal can be used as a fiber, by making the refractive index of the core material is higher than the effective refractive index of the cladding material. An example of this type of structures is the photonic crystal fiber (PCF) which is made from silica solid core surrounded by a photonic crystal cladding with a triangular lattice of air holes. These fibers, also known as index guiding PCFs, guide light through a form of total internal reflection (TIR), called modified TIR. In solid core PCF, core consists of pure silica whereas cladding contains photonic crystal which has number of air holes that decreases the refractive index of core[7]. This modified refractive index of cladding which is less than that of core enable light to travel using phenomenon of modified total internal reflection. The cladding of PCF consists of air holes. The field of the fundamental mode, which fits into the silica core with a single lobe of diameter between zeros slightly equal (or greater) to 2Λ , is the “grain of rice” which cannot escape through the wire mesh. Whereas, the lobe dimensions for the higher-order modes are smaller, so they can slip between the gaps. When the ratio d/Λ , that is the air filling fraction of the photonic crystal cladding, increases, successive higher-order modes become trapped. A well geometry design of the fiber cross-section thus guarantees that only the fundamental mode is guide[6].



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Photonic Band gap Guiding

When photonic crystal fiber design is completely different from the traditional ones, which results from the fact that the photonic crystal cladding has greater refractive index than core. They do not rely on TIR for the guidance of photons. In fact, in order to guide light by TIR, it is necessary that the core is surrounded by a lower-index cladding material. But in photonic band gap guiding the core consists of air holes and there are no suitable low-loss materials with a refractive index lower than air at optical frequencies. Thus, light is guided due to the presence of bandgap. We know that the photonic crystal allows only those photons which have bandgap greater than that of PCF cladding bandgap. So, all those photons with band higher than PCF bandgap are evanescent in cladding and the rest propagate in air core. The first hollow-core PCF had a simple triangular lattice of airholes, and the core was formed by removing seven capillaries in the center of the fiber cross-section. In this type light is guided by using the bandgap i.e. only a particular portion can enter in cladding and rest reflect back and lost in air or hollow core. When white light is launched into the fiber core, colored modes are transmitted, thus indicating that light guiding exists only in restricted wavelength ranges, which coincide with the photonic band gap[1-7].

Fabrication Methods

One of the most important issues of any device is its fabrication process. Conventional optical fibers are usually made by fabricating a fiber preform and then drawing fiber from it with a high temperature furnace in a tower setup. The different vapor deposition techniques, for example, the modified chemical vapor deposition (MCVD), the vapor axial deposition (VAD), and the outside vapor deposition (OVD), are used for the fabrication of symmetric circular fiber preforms. Thus, the deposition can be controlled in a very accurate way only in the radial direction without significant modifications of the methods. In PCF, several parameters like viscosity, gravity, and surface tension are very important. These parameters are due to the much larger surface area in a micro structured geometry, and to the fact that many of the surfaces are close to the fiber core, thus making surface tension relatively much more important. Also in conventional optical fiber core and cladding materials with similar refractive index values, which typically differ by around (1 %), whereas designing PCFs requires a far higher refractive index contrast, differ by perhaps (50–100 %). Hence all the techniques previously described are not directly applicable to the fabrication of preform for micro structured optical fibers, whose structure is not characterized by a circular symmetry. There are two methods for the fabrication of photonic crystal fiber: stack and draw technique and Extrusion fabrication process[6].

Stack and Draw Technique

This method has become the preferred fabrication technique. Since it allows relatively fast, clean, low-cost, and flexible preform manufacture. The PCF preform is realized by stacking a number of capillary silica tubes and rods to form the desired air-silica structure. This way of realizing the preform allows a high level of design flexibility, since the control of core shape and size, as well as the index profile throughout the cladding region is possible. After the stacking process, the capillaries and rods are held together by thin wires and fused together during an intermediate drawing process, where the preform canes are drawn from preform. Then, the preform is drawn down on a conventional fiber drawing tower, greatly extending its length, while reducing its cross-section, from a diameter of (20 mm to 80–200 μm). In order to carefully control the air-hole size during the drawing process, it is useful to apply to the inside of the preform a slight overpressure relative to the surroundings, and to properly adjust the drawing speed. Temperature should not exceed 1900 C since the surface tension can otherwise lead to the air-hole collapse. Dynamics, temperature, and pressure variations are all significant parameters which should be accurately controlled during the PCF fabrication. Finally, the PCFs are coated to provide a protective standard jacket, which allows the robust handling of the fibers. Stacking method requires the handling very carefully, and the control of air-hole dimensions, their positions, and shapes in PCFs makes the drawing significantly more complex than that of conventional optical fiber [1-9].



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Extrusion Fabrication Process

Silica-air preforms have also been extruded, enabling the formation of structures not readily attainable by stacking capillaries. In extrusion a material is pushed or draw through a tool called die which is use to shape materials of desired cross-section. Extrusion process is applied to the glasses other than silica which are not readily available in the form of tubes. In this fabrication process a molten glass is forced through a die containing a suitably designed pattern of holes. Extrusion allows fiber to be drawn directly from bulk glass, using a fiber-drawing tower, and almost any structure, crystalline or amorphous, can be produced. It works for many materials, including polymers, and compound glasses. The structured preform of 16 mm outer diameter and the jacket tube are extruded. The preform is reduced in scale on a fiber-drawing tower to a cane of about 1.6 mm diameter in caning process. The cane is inserted within the jacket tube. This assembly is drawn down to the final fiber[8].

Applications of PCF

For the two main types of PCF index guiding and band gap guiding by varying hole size, space and distance between the core we can obtain required optical properties[3].

Endlessly single mode

There are two types of optical fiber according to number of modes: single mode fiber and multimode fiber. In single mode fiber only one mode can move through the fiber, while in multimode fiber many modes can propagate through the fiber simultaneously. Fabrication of single mode fiber is an expansive and it is not easy to obtain such a narrow core diameter of few micrometers. But by using single mode fiber we can overcome the problem of intermodal dispersion. So it is required to have an endlessly single mode fiber at comparatively greater radius. The first solid-core PCF which consist of a triangular lattice of air-holes with a diameter d of about 300 nm and a hole-to-hole spacing Λ of 2.3 μm as shown in fig.2 did not ever seem to become multi-mode in the experiments means it is endlessly single mode. Changing the design by including three, five or seven missing holes are also pointed to be endlessly single mode. It is noticed that when the ratio of hole size to hole spacing exceed certain value then only single mode will be propagated. Very small to very large core size can be supported regardless of wavelength. So it is possible to construct a fiber with tens of micron core size[3].

Zero Dispersion Photonic Crystal

Dispersion is a big problem in optical fiber which is required to be considered and compensated. There are two main types of dispersion they are (inter model dispersion) and (intramodal also called chromatic dispersion). Intermodal dispersion occurs because multiple modes move through the optical fiber, each mode travel with different velocity due to the different optical density in the fiber and thus they reach at the receiver at different time. Intermodal dispersion can be compensated by using single mode fiber i.e. we can use endlessly single mode fiber. Chromatic dispersion occurs due to different wavelengths of light travel with different speeds. Chromatic dispersion can be divided into two types (material dispersion) and (wave guide dispersion). Waveguide dispersion has negative dispersion coefficient so the overall dispersion must be zero at some point. For conventional fiber the zero dispersion occurs at 1.3 μm . It is possible to compensate chromatic dispersion if zero dispersion wavelengths occur at operating frequency. Due to the high refractive index difference between silica and air in PCF, and to the flexibility of changing air-hole sizes and patterns, a much broader range of dispersion behaviors can be obtained with PCFs than with standard fibers. By proper designing of photonic crystal fiber it is possible to design such a fiber which gives zero dispersion wavelengths at operating wavelength. PCF with missing air hole in solid core or missing ring as shown in the Fig. 7 can be exploited to obtain zero dispersion at different wavelengths. Hence by using different layers we can obtain zero dispersion at different wavelengths[7].





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CONCLUSION

In this review, at first we analyze the basics of photonic crystal fiber and its fabrication processes then guiding mechanisms were discussed. Various limitations and drawbacks conventional optical fiber as transmission media are discussed. Then, to overcome these problems, special PCF design was proposed. PCF also enables endlessly single mode fiber, zero dispersion at different operating wavelengths.

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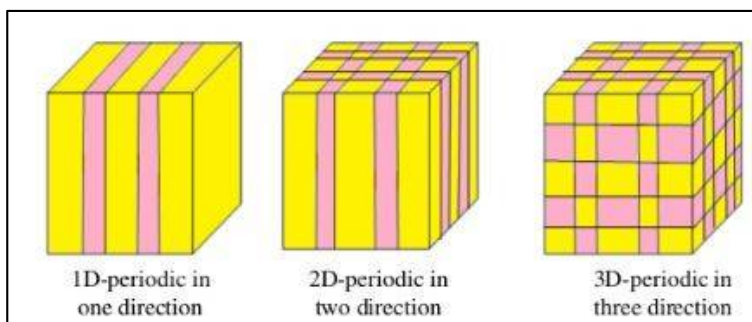


Fig. 1. Geometrical shapes of photonic crystals (a) 1D (b) 2D and (c) 3D

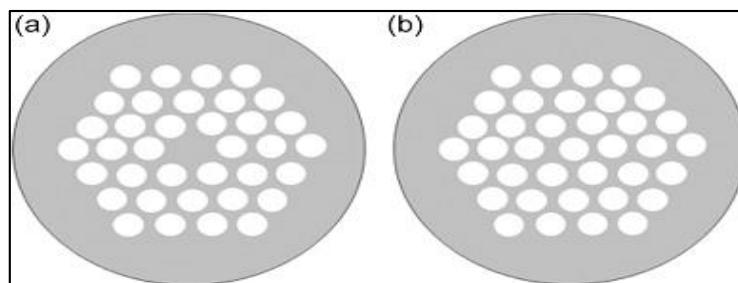


Fig. 2. Two broad classifications of photonic crystal fibers. (a) Solid core fiber cross-section. (b) Hollow core fiber cross-section.





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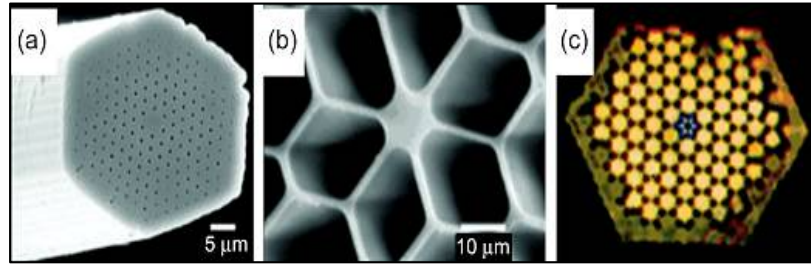


Fig. 3. Three examples of photonic crystal fibers. (a) Endless single mode fiber. (b) Spiderweb fiber. (c) Hollow core fiber.

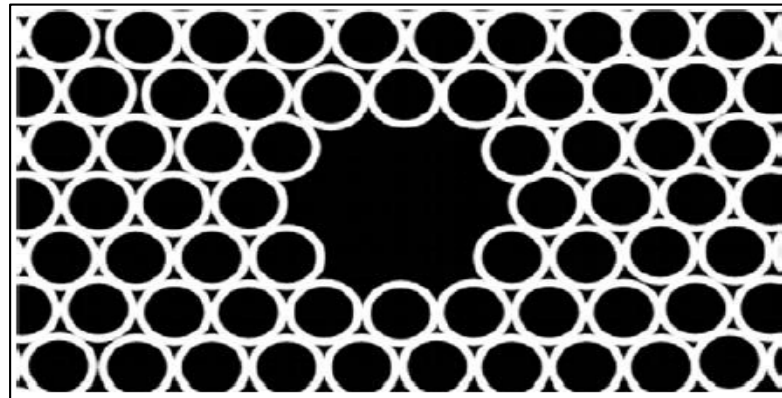


Fig. 4. Cross-section of the first hollow-core PCF, with hole-to-hole spacing of 4.9 μm and core diameter of 14.8 μm.

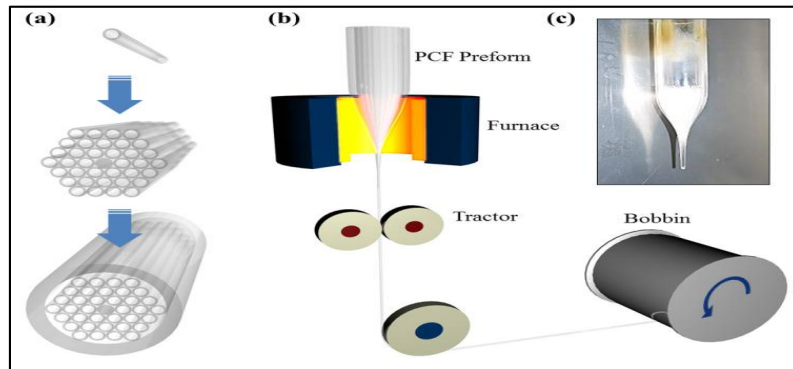


Fig. 5. Schematic of stack-and draw method. (a) Stacking of PCF preform. (b) Fiber drawing process. (c) PCF preform prepared in UM.

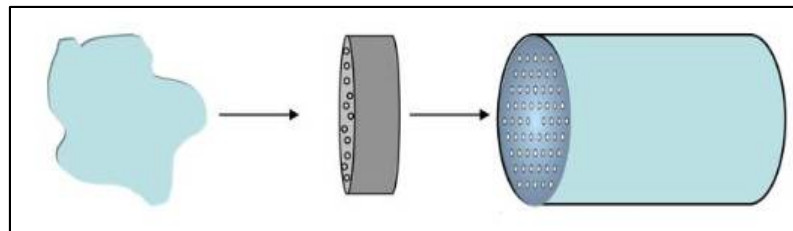


Fig. 6. Fabrication using extrusion





Argon Characteristics of an AC Driven Atmospheric Pressure Non-Thermal Plasma Jet using Two Electrodes

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Received: 17 Jan 2019

Revised: 19 Feb 2019

Accepted: 25 Mar 2019

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ABSTRACT

In this work, a home - made plasma jet system has been constructed. The system which used in this work was adiabatic electric barrier discharge (DBD) type atmospheric pressure plasma jet (APPJ). It was applied to generate a non-thermal plasma using an alternating current (AC) power supply (providing voltages 8 and 10) kV_{p-p} and frequencies up to 20 kHz) with one types of noble gas (ARGON). The system was based on two electrodes configuration, where the configuration based on on double -ring electrodes structure. Each configuration used two types of Pyrex tube with wall thickness of 0.8 mm and studying all the characteristics then when we changed to a pyrex tube with (1) mm we noticed no plasma was generated and studying all the characteristics.

Keyword: Plasma, cold, electrodes, electrical characteristics, discharge, DBD, jet.

INTRODUCTION

Plasma in nature, such as sun, aurora borealis and lightning is always fascinated by people [1]. The first investigations in electrical gas discharges are submitted by Ernst Siemens [2], who in 1857 offered a novel type of electrical discharge, which produced ozone (O₃) from oxygen or air. Two coaxial tubes made of glass are used to start the discharge in a gap between the tubes and an electric field is applied between coaxial external electrodes to produce electrical breakdown. The electric current is compelled to pass through the glass walls, acting as dielectric barriers. The discharge is ordinarily mentioned as the dielectric barrier discharge (DBD) [3]. In 1928, an American physicist Irving Langmuir [4], the Nobel laureate, proposed that the word plasma will be used to appoint that portion of an arc sort discharge, which the densities of electrons and ions are high but significantly equal [5]. This discharge carries many varied particles such as electrons, ions, excited atoms and molecules which is similar to the blood plasma method that transports red and white blood cells [6].



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Atmospheric Pressure Plasma Jets

Atmospheric pressure non-thermal plasma jet (is a great plasma type that has been actively studied through the earlier decade [7]. This device normally consists of a dielectric tube made of quartz, Pyrex, or ceramic tube with an electrode inserted inside the tube or wrapped around it [8]. Various noble gases such as argon, helium and nitrogen are fed into the dielectric tube and occasionally are mixed with a small percentage of oxygen gas to increase the reactive species [9].

Dielectric Electrode Jets

The dielectric electrode jets, as shown in figure can be operated by a power of frequency supply of 20MHz. It consists of internal and external electrode [10]. The external electrode represents the tube and it is grounded where as the internal electrode is coupled to the power supply [11]. Argon gas is fed into the tube and mixed with reactive gases [12]. The power which delivered to the plasma jet is very high (between 50 and 500W) [13].

Dielectric Barrier Discharge Jets

Consists of a dielectric tube with double metal ring electrodes which located at the external side of the tube and the seal electrodes are connected to the power supply. Different gases are used in this device like helium, argon, the second configuration as shown in figure was based on a double-ring electrode configuration, which have two aluminum rings electrode covered the external Pyrex tube. The distance between two electrodes was fixed to 12 mm and the distance between downstream electrode and the nozzle of the Pyrex tube was fixed to 4 mm. The upstream electrode was connected to power supply and the downstream electrode was grounded [14].

Measurement of the Characteristics

Measurement of the Plasma Plume Length

It's the distance between the tip of the plasma plume and the edge of Pyrex tube. This indicates that the higher gas flow rate and applied voltage lead to longer plasma plume, The voltages we studied were (8 and 10) kVp-p and the frequency was up to 20 KHz using argon gas we noticed that the plasma plume is increased by increasing the flow and also increasing the voltages also increase the plasma plume as shown in the figures

Plasma Jet Temperature Measurement

It can be measured by using the mercury thermometer where the heat sensitive part was located at various distances from the end of the tube nozzle with different gas flow rate. It is observed that the helium plasma jet temperature decrease more than argon with increased plasma plume length. In addition, with increasing argon gas flow rate, the plasma jet temperature decreased with argon in time. By increasing source voltages the temperature increases

DISCUSSION

This paper studies the electrical characterization and optical emission spectroscopy of a DBD plasma jet in argon and an argon/water vapor mixture. An experimental study is presented to characterize a room temperature plasma jet in atmospheric argon generated with pulsed atmospheric argon plasma jet is stable and cold. The plasma plume length depends on the gas type, gas flow rate and applied voltage. The reduction of Pyrex tube wall thickness has a significant effect on the plasma system operation and reduction the plasma jet temperature. The electron temperature





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for argon and helium plasma jets decreased slightly with higher gas flow rate, while growing with higher applied voltage. electron temperature decreasing with the increase of gas flow because the increasing of gas flow rate causes an increases in the number of collisions between the electrons and the gas atoms. As a result of these collisions the energy transferred from the electrons to the gas particles increases causing an increase in the gas temperature by decreasing the electron temperature. The higher gas flow rate and applied voltage lead to longer plasma plume this phenomenon is due to the effects of the electrons and ions which flow from the upstream to the downstream regions.

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Table 1. shows the relationship between the plasma plume and the flow rate at voltage at 8kVp-p.

PlasmaPlumeLength(mm)	Flow (slm)
10	1
12	2
14	3
17	4
20	5
24	6





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Table 2.shows the relationship between the plasma plume and the flow rate at voltage at 8kVp-p.

PlasmaPlumeLength(mm)	Flow (slm)
12	1
16	2
18	3
20	4
23	5
26	6

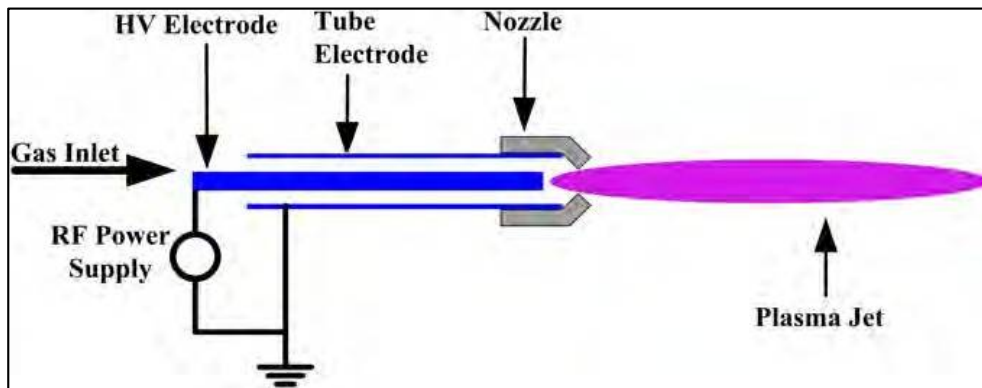
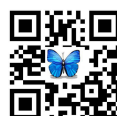
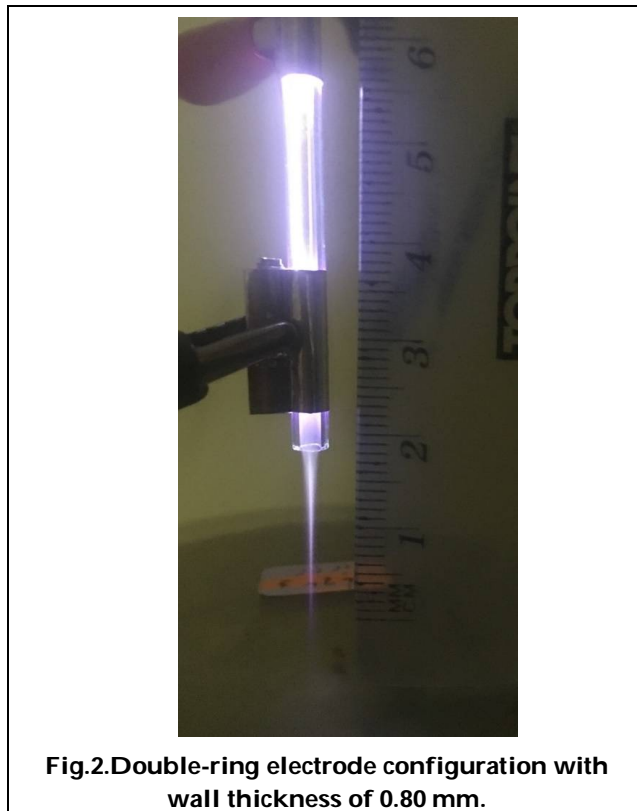


Fig.1. Dielectric ElectrodeJets





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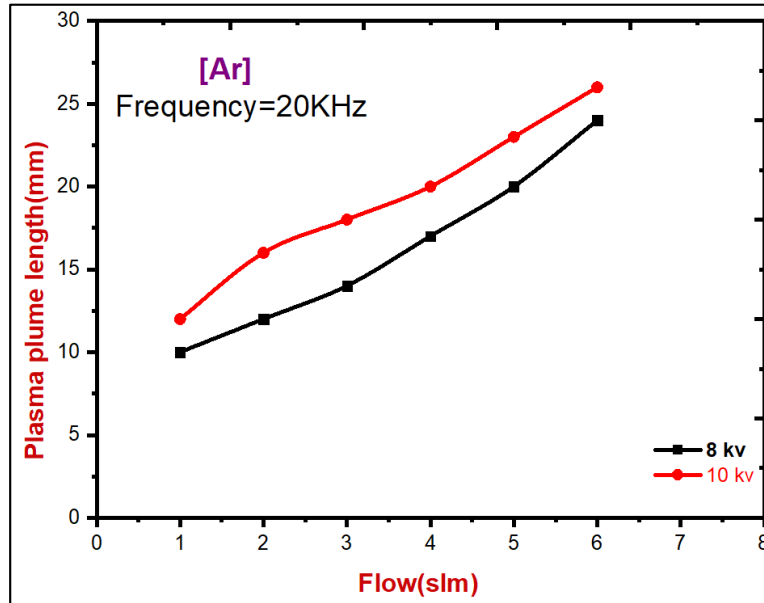
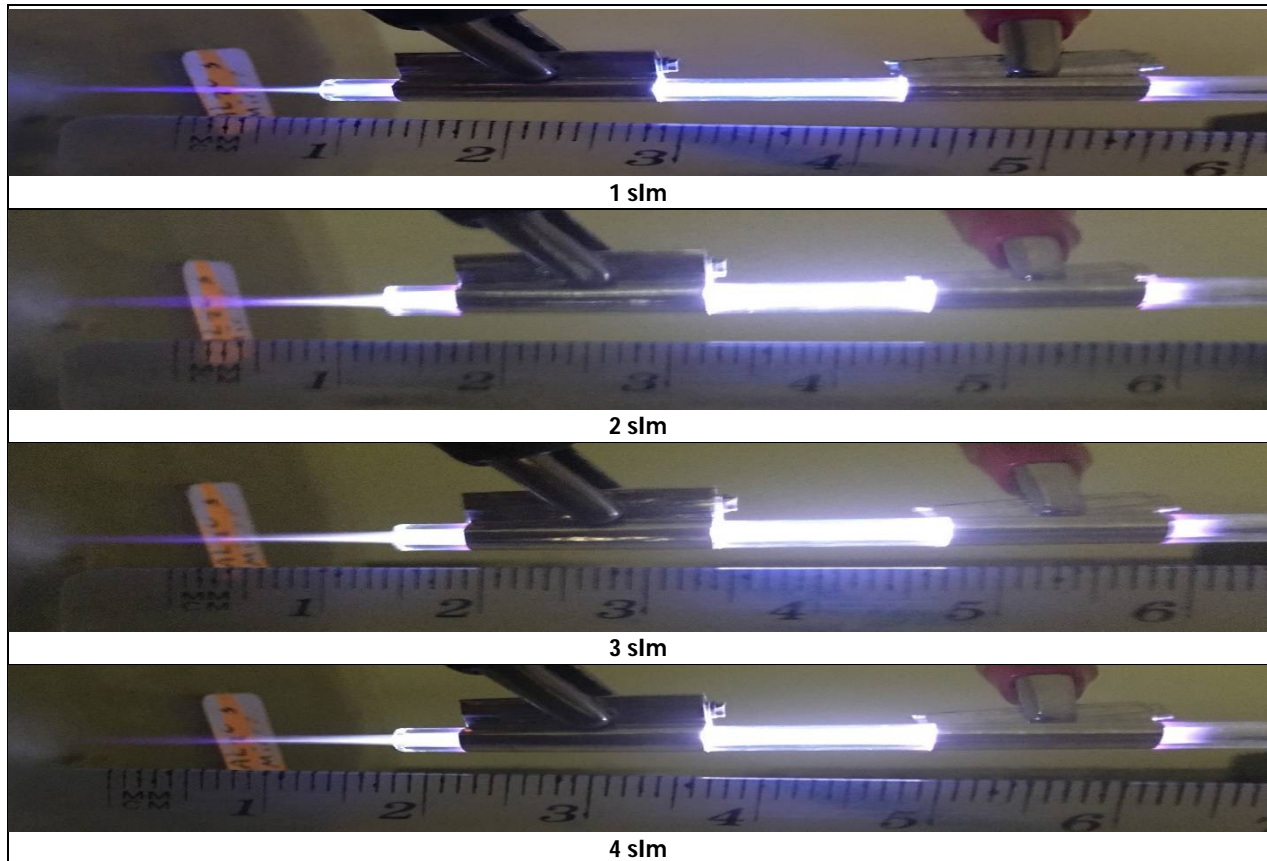


Fig .4.shows the relationship between the plasma plume and the flow.





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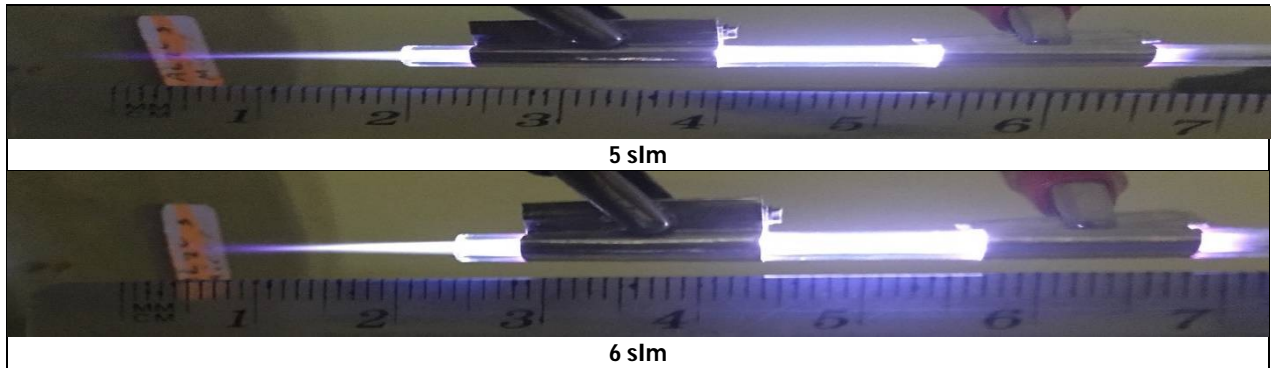


Fig.5. photographs show the plasma plumes for different argon flow rates at 8kVp-p.

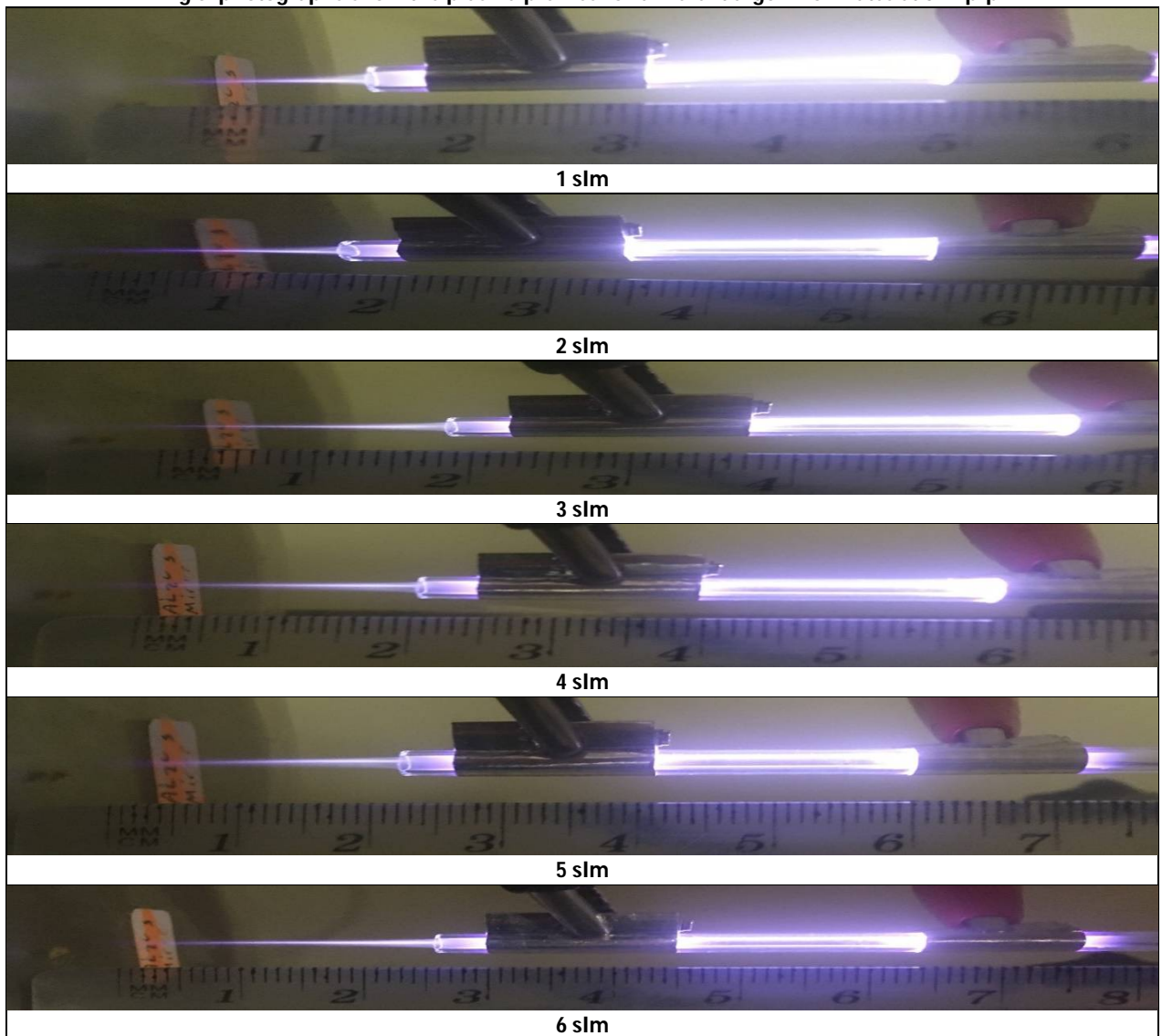


Fig.6. photographs show the plasma plumes for different argon flow rates at 10 kVp-p.





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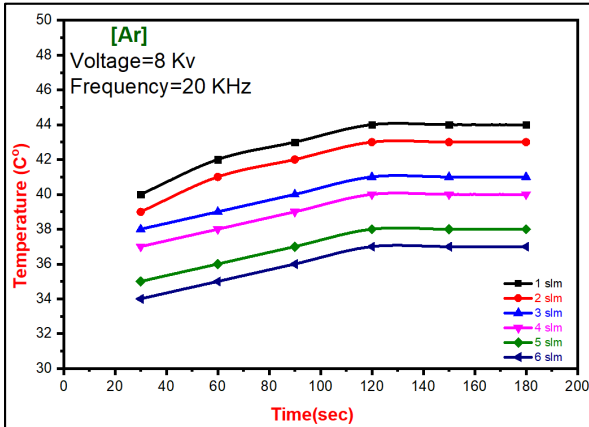


Fig.7. this figure shows the relationship between the temperature as a function of time for different Argon flow rates (voltage =8kVp-p).

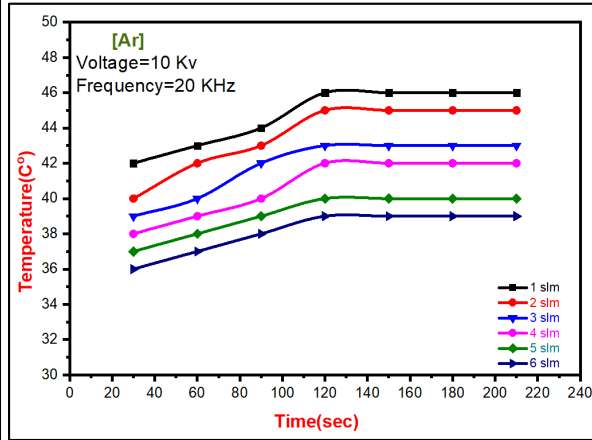


Fig.8. this figure shows the relationship between the temperature as a function of time for different argon flow rates (voltage =10kVp-p)

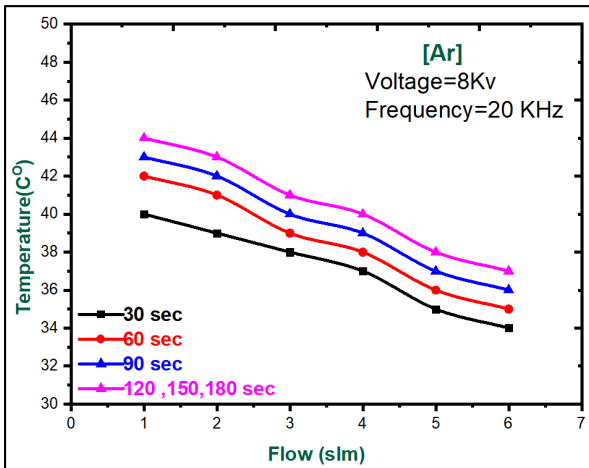


Fig.9. this figure shows the relationship between the temperature and the flow rate

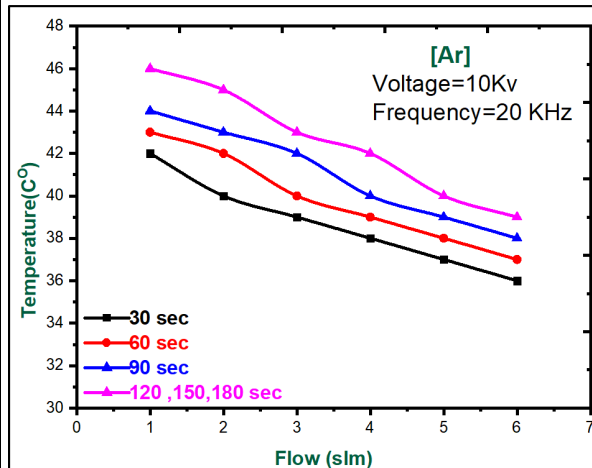


Fig.10. this figure shows the relationship between the temperature and the flow rate (voltage =10kVp-p)





Risk Factors for Hysterectomy among Patients with Placenta Previa Totalis

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Received: 22 Dec 2018

Revised: 25 Jan 2019

Accepted: 28 Feb 2019

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ABSTRACT

Objective: The aim of the study is to assess risk factors for hysterectomy among patients with placenta previa totalis (PPT). **Methods:** The medical records of all patients delivered by cesarean section (CS) for PPT were retrospectively reviewed. Eligible cases were divided into those who underwent peripartum hysterectomy (PH) and those who did not. The two groups were compared in terms of demographics, operative features and perinatal outcomes. Logistic regression analysis was used to identify risk factors associated with hysterectomy. **Results:** PH was performed in 43 (44.7%) patients with PPT. Referral patients were older when compared with those without hysterectomy ($p:0.029$). The median values for gravidity, parity, number of live children and previous CS were statistically significantly higher in the hysterectomy group ($p<0.05$). Perioperative need for blood transfusion, anteriorly placed placenta and abnormal placenta invasion were statistically significantly more frequent in the hysterectomy group ($p<0.001$). Intraoperative complication rate was higher in this group, and bladder injury was the most common complication. No significant differences were observed between the groups in terms of perinatal outcomes. In binomial logistic regression analysis; advanced maternal age (≥ 31 years), number of previous CS (≥ 2), preoperative need for blood transfusion, and abnormal placental invasion were found to be independent risk factors for PH in patients with PPT. **Conclusion:** The findings of this study suggest that placenta invasion anomaly, advanced maternal age, increased number of previous CS, and increased need for blood transfusion are important risk factors for PH in patients with PPT.

Keywords: Placenta previa, placenta accreta, peripartum hysterectomy, Cesarean section





INTRODUCTION

Placenta previa (PP) is defined as the implantation of placental some where in the lower segment, either over or very close to the internal cervical os. The reported incidence for PP varies between 3 and 4 per 1000 deliveries. It is one of the most common causes of antepartum bleeding and is associated with an increased need for blood transfusions and preterm delivery. Maternal and perinatal morbidity and mortality is significantly higher in pregnant women with PP^{2,3}. The main problem that can occur during cesarean section (CS) of these patients is severe bleeding that may result in hysterectomy. Several studies have identified similar risk factors for PP. The reported risk factors for PP are smoking, cocaine use, advanced maternal age, prior CS, recurrent abortions, male fetuses, and infertility treatment⁴. However, studies identifying the risk factors regarding peripartum hysterectomy (PH) in women with PP are relatively limited. The number of abortions as well as prior CS and placenta previa totalis (PPT) were found to be risk factors for hysterectomy in women with PP⁵. Moreover, hysterectomy was significantly higher in patients with placenta accrete⁶ and anterior located PP.⁷ In this context, here in we aimed to define risk factors for hysterectomy in cases of PPT.

MATERIALS AND METHODS

We analyzed retrospectively 104 cases of PPT treated at Al_kut Hospital for obstetrics, gynecology & pediatrics which is a tertiary referral hospital with total annual births of approximately 20,000 in Kut, Iraq. The study protocol was performed according to the principle of the Declaration of Helsinki and was carried out between January 2014 and July 2015. The study was specifically approved by the Institutional Review Board. A written informed consent was given by each participant for their clinical records to be used in this study. Of all women 43 underwent PH and 61 did not. The data of the cases were collected from hospital records and patients' files. The two groups were compared for maternal demographic characteristics, peripartum events, and perinatal outcomes. The clinical characteristics evaluated were age, BMI, past medical and obstetric history, smoking status, placental location, urgency of delivery and gestational week at birth, birth weight, Apgar scores, neonatal intensive care unit (NICU) admission, perinatal mortality, preoperative and postoperative hemoglobin concentrations, preoperative and postoperative need for blood transfusion, and intraoperative complications. Histopathology results for patients undergoing PH were also recorded. All women underwent CS due to PPT. The diagnosis of PPT was made by a perinatal logist generally accepted diagnostic criteria (the placental edge overlaps the internal os) based on a transvaginal ultrasound in the last trimester of pregnancy. An Avoluson 730 Expert scanner (GE Medical systems, Kretztechnik GmbH & OHG, Zipf, Austria) equipped with 5.0- 9.0 MHz transvaginal probe was used for ultrasonographic evaluation of the placenta.

The clinical diagnosis of placenta accrete was made if manual removal of the placenta was partially or totally impossible and no cleavage plane exists between part or the entire placenta and the uterus. The definite diagnosis of placenta accrete was confirmed by histopathological examination of the specimen in women who underwent hysterectomy. This is a high risk pregnancy unit having an experienced team. Perinatology residency training is given under the supervision of 4 clinical chiefs expert in high risk pregnancy, each with at least 20 years of experience. Perinatology assistants are obstetricians who completed 5 years of obstetrics and gynecology residencies with at least 2 before operation and at least 6 hours after cesarean delivery. Body mass index (BMI) was calculated as weight (kg)/ height² (m²) using a professional calibrated device at the admission for delivery. Gestational weeks were calculated according to last menstrual periods or were noted according to ultrasound screenings carried out on the first trimester in those who did not know their last menstrual period. Each patient was evaluated by ultrasonography and external fetal monitoring using cardiotocography. Patients were managed with bed rest and limited physical activity if remote from term and Hysterectomy decision following CS was made by at least two experienced clinical chiefs when standard medical (volume resuscitation, uterotonic drugs, Bakri balloon) and



**Maysoon Mahde saleh**

surgical (endouterine hemostatic or transuterine compression sutures, surgical uterine devascularization) treatment failure occurred for controlling postpartum hemorrhage due to morbidly adherent placenta and/or uterine atony. Transcatheter embolization had not been applied in any of the patients because no interventional radiology unit is present in our hospital. Moreover, hysterectomy without further attempts was performed in some patients with severe form of placenta accreta. Routine preoperative tests were performed in patients during the admission, vital findings and anthropometric measurements were recorded. Maternal complete blood count parameters were also taken Gestational week ≤ 34 and having the risk of preterm delivery for fetal lung maturation. All cases were also evaluated in terms of surgical methods applied to control bleeding. The need for neonatal intensive care unit (NICU) was approved by a neonatologist for some cases due to respiratory distress, low birth weight ($<2500\text{g}$), and intrauterine growth restriction (estimated fetal weight $<10\%$).

Statistical analysis

Statistical Package for the Social Sciences version 17.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Means and standard deviations for quantitative data and numbers and percents for qualitative data were computed. Kolmogorov- Smirnov and Shapiro-Wilk tests were used to assess normal distribution of the univariate variables. Non parametric methods were used to analyze the variables which did not have a normal distribution. Chi-Square or Fisher's exact tests were used for categorical variables, where applicable. Independent-samples t test was used to compare unadjusted means between groups. Non parametric variables between groups were compared through Mann-Whitney U test. The optimal cut- off points for numerical data which discriminate groups from each other were evaluated by receiver operating characteristic (ROC) analysis calculating area under the curve (AUC) as giving the maximum Youden index. Binomiallogistic regression analysis was used to identify independent risk factors for PH. The results were considered statistically significant when p values were <0.05 .

RESULTS

During the 1.5-year period of the study, a total of 28,212 deliveries occurred in our hospital. It was found that 104 of these pregnancies were complicated with PPT. PH was performed in 43 (44.7%) patients with PPT. The demographics features and clinical findings of the patients are shown in the Table 1. The mean age of the patients' was 33.0 ± 3.9 vs. 31.1 ± 4.7 years in the groups with and without hysterectomy, respectively ($p:0.029$). The two groups were similar regarding mean BMI. The median values for gravidity, parity, number of live children and previous CS were statistically significantly higher in the hysterectomy group (all $p<0.05$). Perioperative need for blood transfusion was also significantly higher in the hysterectomy group ($p<0.001$). Patients who underwent hysterectomy gave birth earlier, and accordingly median birth weights of their newborns were significantly lower than those without hysterectomy ($p<0.05$). One patient had previously undergone septum resection in the conservative treatment group. One patient in each group became pregnant following in-vitro fertilization treatment. Nifedipine as a tocolytic agent was used in three patients in both groups ($p:0.686$).

The percentage of smokers was similar in the two groups. No significant differences were observed between the groups in terms of prenatal morbidity and mortality. Anterior located placenta and abnormal placental invasion were statistically significantly more frequent in the hysterectomy group. Intra operative complication rate was higher in this group, and bladder injury was the most common complication. Planned or emergent CS deliveries were not different in comparisons between the two groups. The cut-off value of age, BMI, gravidity, parity, number of live children and previous CS was found by using ROC curve analysis, and categorical distribution of recorded data was shown in Table 2. In multivariate binomial logistic regression analysis; advanced maternal age (≥ 31 years), number of previous CS (≥ 2), preoperative need for blood transfusion, and abnormal placental invasion were found to be independent risk factors for PH in patients with PPT (Table 3).





DISCUSSION

Placenta previa is classically graded in to three types depending on how close the lower edge of the placenta is to the internal os. If the placental edge totally covers the internal os, the placenta is called as PPT. It is one of the most feared pregnancy complications because of the increased risk of massive hemorrhage. The need for hysterectomy is significantly increased in PP cases with approximately 30% relative risk [9]. A recent study suggested that a combined ultrasound and clinical scoring model may predict peripartum complication in patients with PP. However, there is a need for more comprehensive and extensive studies for its routine use [10]. In the present study we aimed to evaluate the risk factors for hysterectomy in patients with PPT. According to this study, placenta accreta, advanced maternal age, increased number of previous CS, and an increased need for blood transfusion were found to be risk factors for PH. Postpartum hysterectomy is performed when there is a life threatening hemorrhage which cannot be controlled by medical treatment during or after vaginal forces are deliveries. Although its higher morbidity rates, it can be lifesaving when performed in emergent cases with appropriate indications. PP and accreta are important risk factors for PH. To our knowledge, there are limited studies evaluating risk factors for PH in women with PP in the literature [5,9,11,12]. In those studies, previous abortion, previous CS, PPT, placenta accreta and gestational age at delivery were found to be risk factors for PH. Unlike these studies, our study is the first which evaluates risk factors for hysterectomy in patients with PPT.

Placenta accreta refers to an abnormally invasive placental implantation depending on the defect in the decidua basalis. It constitutes approximately 80% of all cases of abnormal placentation, and complicates nearly 20% of pregnancies with PP [13,14]. The incidence of abnormal placentation has steadily increased over the years. This marked increase has been attributed to the increasing prevalence of cesarean delivery in recent years. A study has reported that PP was as high as one in 533 deliveries [14]. In our study, the incidence of placenta accreta seems to be higher than reported. This may be related to the inclusion of PPT solely in to the study. The incidence of emergency PH did not change significantly in the past decade. However, the indications and intraoperative interventions have changed significantly over the years. Previously uterine atony was the most frequently reported indication for PH [15]. In recent years, abnormal placentation has become more frequently pronounced due to the increasing cesarean delivery rates [16]. A previous study conducted in our institution showed similar results [17]. Similar to that study, Çetinel found that the most common indication for emergency PH was placental insertion abnormalities in their retrospective study of 18 cases [18].

In the study of Owolabi et al., it was found that primary and repeat cesarean deliveries, advanced maternal age, obesity, and grand multi parity had a direct association with PH [19]. Uterine atony, PP/accreta, and severe obstetric hemorrhage were also found to be associated with hysterectomy. Miller *et al.*, found that advanced maternal age and previous cesarean delivery were independent risk factors for placenta accreta among women with PP [13]. Likewise, we confirmed that advanced maternal age is an independent risk factor for hysterectomy in patients with PPT. Vaginal bleeding in PP, which is mostly observed during the last months of the pregnancy, is thought to result from the maturation of the lower uterine segment. As those areas where the placenta sticks to deciduas gradually thin to prepare for birth, placental separation causing bleeding occurs. Meanwhile the lower uterine segment is not be able to contract as needed to stop the bleeding [20]. It may also cause separation of the placenta during manual vaginal examination and intercourse. PP, diagnosed with acute onset of approximately 80% of patients at around 34 weeks of pregnancy, is seen as painless bright red bleeding [21]. There is no correlation observed between the number of bleeding episodes, degree of PP and fetal survival [22]. In this study, we found that the amount of bleeding was higher in patients undergoing hysterectomy. Preoperative transfusion was an independent risk factor for hysterectomy.

Jang et al., found that anterior PP is associated with increased risk for PH in their study [7]. In accordance with this study, anterior PP was more common among the hysterectomy group when compared with the conservative group.



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However, anterior PP lost its significance in the logistic regression analysis. Our hospital is located in the middle of Al kut city. This is a referral maternity hospital where the majority of its patients are usually referred from other centers as an emergency. Consequently, the proportion of complicated cases referred to us is higher than normal. Although the present study covers a shorter period than the others, the number of patients included is larger. Some of these patients was in regular follow-up where as the others who apply in an emergency were diagnosed preoperatively. Therefore, we were not able to determine the gestational age at initial diagnosis in all patients. As is evident from the findings, the higher prevalence of elective CS in the hysterectomy group could mean that the preoperative diagnosis of placenta accreta in which a planned delivery at 34th week of gestation is recommended [23] was more frequent in this group. This explains the higher rate of preterm birth in the hysterectomy group. The higher prevalence of placenta accreta in the hysterectomy group may be seen as a selection bias. However, we included all cases diagnosed with PPT into this study during the study period. Ultrasound and magnetic resonance imaging are useful diagnostic tools in the preoperative diagnosis of placenta accreta. The main reasons for not considering the ultrasonographic diagnosis of adherent placenta in our study were that transvaginal ultrasonography was performed by different obstetricians, and preoperative diagnosis was not possible in all patients. Therefore, we also included all PPT patients regardless of the presence of a suspected diagnosis of placental accreta on antenatal visits. In addition, it should be considered that different decisions are often made under equivalent situations depending on individual surgeons.

CONCLUSION

In conclusion, the findings from this study suggest that placenta invasion anomaly, advanced maternal age, increased number of previous CS and need for preoperative blood transfusion may predict PH in women with PPT. Patients with the serious risk factors should be evaluated more carefully, and they should be delivered in a tertiary hospital with appropriate preparation for surgery. These patients should be informed in terms of hysterectomy. This approach will contribute to reducing maternal morbidity and mortality in this group of patients.

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Table 1. The demographics features and clinical findings of the patients.

	Hysterectomy group (n:43)	Conservative group (n:61)	P
Age(years)	33.0±3.9	31.1±4.7	0.029
BMI(kg/m ²)	28.7±3.8	27.9±3.5	0.262
Gravidity	3(2–9)	3(1–8)	0.014
Parity	2(1–4)	1(0–5)	0.001
D&C	0(0–5)	0(0–4)	0.073
Abortion	0(0–5)	0(0–5)	0.387
Alive	2(0–4)	1(0–5)	0.002
PreviousC–section	2(0–3)	0(0–4)	0.000
Gestationalweekatbirth	36(24–38)	37(27–39)	0.005
Birthweight(gr)	2730(470–3410)	2870(680–4500)	0.038
Apgar5	9(0–9)	9(5–9)	0.651
PreoperativeHb(g/dL)	11.5±1.4	11.6±1.1	0.640
PreoperativeHct(%)	34.2±4.2	34.7±2.9	0.411
PostoperativeHb(g/dL)	9.9±1.4	10.3±1.1	0.077
PostoperativeHct(%)	29.9±4.2	31.0±3.3	0.006
LowestdetectedHb(g/dL)	8.2±1.5	9.7±2.1	0.262
Bakriinflationvolume(cc)		186.2±64.9	

BMI; body mass index, D&C; dilatation and curettage, Hb ; hemoglobin, Hct ; hematocrit. Data presented as mean± standard deviation and median (minimum–maximum). P<0.05 is considered as statistically significant.

Table2. Distribution of the categorical variables between the two groups

	Hysterectomy group (n:43)	Conservative group (n:61)	P
Age≥31	36(83.7)	32(52.5)	0.001
BMI≥28.5	26(60.5)	24(39.5)	0.034
Gravidity≥3	37(86)	36(59)	0.003
Parity≥2	31(72.1)	25(41)	0.002
Alive≥2	28(65.1)	23(37.7)	0.006
PreviousC–section≥2	25(58.1)	11(18)	0.000
Smoker	5(11.6)	5(8.2)	0.562
GWatbirth≤36	31(72.1)	27(44.3)	0.005
Male gender	20(46.5)	32(52.5)	0.550
IUGR	3(7)	4(6.6)	1.000
Complication	6(14)	0	0.004
NICU	10(23.3)	11(18)	0.513
Perinatal mortality	1(2.3)	3(4.9)	0.641
Placenta location			0.000
Anterior	34(79.1)	17(27.9)	
Posterior	9(20.9)	44(71.1)	
Pre optx	42(97.7)	22(36.1)	0.000





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Post optx	28(65.1)	12(19.7)	0.000
Urgency	7(16.3)	14(23)	0.404
Abnormal Placenta invasion	40(93)	10(16.4)	0.000

BMI; body mass index, GW; gestational week, IUGR; intrauterine growth restriction, NICU; neonatal intensive care unit, Tx; transfusion. Data are represented as n(%). P<0.05 is considered statistically significant.

Table 3. Logistic regression model for risk factors of peripartum hysterectomy in case of PP totalis

Outcome: hysterectomy	$\beta\beta$	S.E.	Wald	P	OR	95%CI
Age \geq 31	3.851	1.297	8.809	0.003	47.035	3.698–598.165
No. of previous C-section \geq 2	3.357	1.406	5.700	0.017	28.692	1.824–451.293
No. of live children \geq 2	2.704	1.486	3.311	0.069	14.942	0.812–275.007
Anterior located placenta(+)	1.808	1.000	3.267	0.071	6.101	0.859–43.349
Pre op blood transfusion(+)	3.203	1.576	4.131	0.042	24.618	1.121–540.552
Abnormal Placental invasion(+)	4.730	1.325	12.739	0.000	113.333	8.438–1522.235

SE; standard error, OR; odds ratio, CI; confidence interval. P<0.05 is considered statistically significant.





Morphological and Morphometrical Features of Testes and Penis in Indigenous Tom Cat

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Received: 20 Jan 2019

Revised: 23 Feb 2019

Accepted: 25 Mar 2019

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ABSTRACT

The present study involved five adult tom cats to investigate the anatomical features of penis and testicles in indigenous tom cat. All animals were opened up, and the genital organs were sighted and photographed, in situ. Images of the excised genital organs were taken with a digital camera and also dissecting microscope used for penile spine, corpus cavernosum, corpus spongiosum and urethra illustration, the penis directed caudo-ventrally and was composed of three parts (body, root and glans), originated from two crura, it has a white color with rounded structure. The mean length and diameter of the penis was 4.00 ± 0.26 cm, 0.68 ± 0.08 cm respectively. The penis showed a single of corpus cavernosum, and the corpus spongiosum housed the penile urethra, the cone shape glans penis was completely enclosed by a winding architecture prepuce, with several cornified papillae was located on the dorsal and lateral surface. The white colored testes have an oval shape located outside of the body covered with a scrotum. The Os penis was attached to and continuous with the liberated end of the corpus cavernosum penis and was completely articulated at the tip of the penis, the mean testicular weight, length and diameter were 1.35 ± 0.43 g, 2.15 ± 0.34 cm, 1.25 ± 0.15 cm respectively.

Keywords: tom cat, penis, testicles, Anatomy.

INTRODUCTION

The standard reproductive tract in male mammals consists of the testes and related epididymis, accessory sex glands, ductus deferens, urethra, and penis^[1]. The anatomical structures of the penis and associated structures of the male reproductive tract have developed quickly and separated in many mammalian orders. Numerous explanations have been known and hypotheses advanced to give information the notable difference in the penile and bacular structures within and between mammalian species^[2]. In domestic cats the sexual maturation occurs between eight and twelve months that agreement with the appearance of sperm in the ejaculate. Males preserved in controlled lighting do not



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exhibit seasonal reproductive behavior, and animals reserved in substeamy or steamy climate are fertile and sexually active during the year^[3]. The testes are oval to rounded paired organs, situated outside the abdominal cavity, in a sac fold, scrotum, responsible to the manufacture of sperm and secretion of male sex hormones, especially testosterone. It was surrounded by a capsule called the tunica albuginea, consisting of dense connective tissue^[4]. The tomcat penis consisted of root, body, and glans. The body of the penis is consisted of one distinct corpus cavernosum. The cranial part of the penis has a bone Os–penis and this is regarded as a part of corpus cavernosum^[5]. Review of literature showed a seldom studies deals with the macroscopical structure of the penis of the tom cat in Iraq. Therefore, this study intends to investigate the anatomical structures of the penis and testes of indigenous tom cat.

MATERIALS AND METHODS

Five adult tom cats weight ($2500 \pm 221.7g$), they were obtained from the Department of Surgery and Obstetrics at the Faculty of Veterinary Medicine, University of Baghdad after her death due to unsatisfactory accidents were used for this study. The study was carried out at the department of Anatomy & Histology, University of Baghdad. All animals were opened up, and the genital organs were sighted and photographed, in situ. Images of the excised genital organs were taken with a digital camera and also dissecting microscope used for penile spine, corpus cavernous, corpus spongiosum and urethra illustration, the length, diameter, circumference, and weight of the genital tract were recorded using a ruler, thread, and weighing scale (Shimadzu AW320, Germany), in addition, the length of Os penis was also reported according to the method described by [6]. The gross anatomical features of all penises were described. Statistical analysis has been done by using The SPSS statist version to analyze the data^[7].

RESULTS

Macroscopic appearance revealed that the penis of the tom cat was composed of three parts (body, root and glans), it was attached to the ischial arch, originated from two crura, directed caudo-ventrally it has a white color with rounded structure. The mean length of the penis was $4.00 \pm 0.26cm$, while the mean penile length was $0.0962 \pm 742\%$ as a percentage of body length, the mean diameter was $0.68 \pm 0.08 cm$ (Fig.1&2). The vertical section of the body of penis showed that the penis consisted of paired corpus cavernosum at the level of middle part of the penis body, while at the level of distal body part became a single corpus cavernosum due to uniting the paired corpus cavernosum which separated from corpus spongiosum by a septum, corpus spongiosum housed the penile urethra (Fig 3,b3), and the crone shape glans penis was completely enclosed by the prepuce, which has a winding architecture internally and smooth externally, with several cornified papillae (penil spine) were located on the dorsal and lateral surface of the posterior quarter end of the corpus penis. The ventral urethral groove has been showed at the level of the glans penis with urethral orifice (Fig. 4). The mean length of the glans penis in the tom cat was observed, and made up only a minimal portion of the whole penile length with a ratio of 1:5 to the corpus penis. The pointed protrusion of the distal end of glans penis was observed (fig4). The Os penis observed in the tom cat was attached to and continuous with the liberated end of the corpus cavernosum penis and was completely articulated at the tip of the penis, the mean length of the Os penis was $1.46 \pm 0.20 cm$. The paired testes of the tom cat have an oval shape located outside of the body between the thighs covered with tunica albuginea, tunica vaginalis and externally by scrotum. The longitudinal axis of the testes was horizontal and the epididymal border was lateral (Fig 5). Testes have a white color, the mean of left and right testicular weight, length and diameter were $1.35 \pm 0.43g$, $2.15 \pm 0.34cm$, $1.25 \pm 0.15cm$, $1.36 \pm 0.33g$, $2.10 \pm 0.24cm$, $1.20 \pm 0.19cm$ respectively. The mean testicular weight was $0.054 \pm 0.172\%$ as a percentage of body weight, ductus deferens was extended from the testes towards the abdominal cavity. There was no enlargement in distal part of ductus deferens at the level of the urinary bladder neck and it was dorsally entered to the dorsal wall of the intra pelvic urethra (Fig.6).





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DISCUSSION

The results of the microscopical features of the present study revealed that the penis of the tom cat was composed of three parts (body, root and glans), attached to the ischial arch, originated from two crura with white colored rounded structure of caudoventrally direction which has a length and diameter about 4.00 ± 0.26 cm, 0.68 ± 0.08 cm respectively, the current study was coincided with^[8]in dog,^[9]in camel, ^[10]in gazelle and^[11]in porcupine, ^[12]in ram and buck and^[13]in Arabian Oryx, however,^{[8], [12], [10]}. The result noticed that the direction of penis was crainoventrally and the corpus cavernosum was paired separated by a septum instead of single, while^[11]demonstrated different results associated with length and diameter, were 5cm,1cm respectively and described a black color glans penis with cornified papillae and two nail structures at the tip of glans penis. In addition ^[14]in rodent Observed that the glans penis had blind sac below the urethra called vertesa cculus urethralis formed a balloon-like swelling during the erection mechanism. ^[15]in European hedgehog have a compatible results as the current study, but they noticed that the penil length was 5.46 ± 0.36 cm with cranial direction towards the umbilical region, the sigmoid flexure and urethral process had been illustrated, while^{[16], [17]} in pig and^[18]in Babirusa showed a round penis in middle and flat at the tip in addition to the urethral process, slit-like urethral orifice and sigmoid flexure.

Where as the average length, width and thickness of free part of the penis were 4.0 cm, 0.60 cm and 0.30 cm respectively. In the past decade^[19]in greater cane rat demonstrated the similar results of the current study, however they declared that the penis initially directed cranio-ventrally and after made a bend at the junction with glans penis causing a latter to project caudo-ventrally, the glans penis has a club shape and the anterior parte with 1/4 posterior of corpus penis have a cornified papillae, in addition said the mean length and diameter were 4.46 ± 0.45 cm, 0.58 ± 0.08 cm respectively, But^[20]in monkey mention incompatible results when described the penis, said, this organ has a triangular bottom shape glans penis.^[21]in rabbit,^[22]in guinea pig and ^[23]in rat, these authors have the same opinions as^[19]associated with the penis direction. However, ^{[19], [22]}had no algometry between the body size and penil size. The white color paired testes have an oval shape located outside of the body covered with a secretum with longitudinal axis of horizontal and the epididymal border was lateral, the mean testicular weight, length and diameter were 1.35 ± 0.43 g, 2.15 ± 0.34 cm, 1.25 ± 0.15 cm respectively, while the mean testicular weight was $0.054 \pm 0.172\%$ as a percentage of body weight, ductus deferens was extended from the testes towards the abdominal cavity There was no enlargement in distal part of ductus deferens at the level of the urinary bladder neck and it was dorsally entered to the dorsal wall of the intra pelvic urethra.

These results were in compatible with ^[15]in European hedgehog that stated the oval testes of pinkish brown color are intra-abdominal, as there are no true scrotal sacs, but the protrusions of scrotal pouches were in contact with the abdominal muscles in the pubic region. The longitudinal axis of the testes was horizontal and the epididymal border was dorsal, while the free border was ventral. The average length, diameter, and weight of the testes were 1.72 ± 0.04 cm, 1.11 ± 0.03 cm, and 1.25 ± 0.05 g, respectively. The mean testicular weight was $0.17 \pm 0.002\%$ as a percentage of body weight. While ^[24]recorded the testis length in ram were 5.83 ± 0.165 cm, in addition^[25]observed the average length of left and right testis in Marwari goats were 10.59 ± 0.26 and 10.16 ± 0.24 cm, respectively. The results of the current study were incompatible with ^[26]in one-humped camel bull, Uda Ram and Red Sokoto buck, whom reported that The testis of the three animals are oval in shape and the first one dark-brown color lies obliquely with their long axes cranioventrally. Both Uda ram and Red Sokoto buck of vertical long axis with whitish-yellow in the Uda ram and white to red in the Red Sokoto buck. While the left and right mean testicular weights of OCB, UR and RSB were 83.50 ± 11.07 g, 83.50 ± 6.33 , 155.43 ± 21.42 g, 163.45 ± 24.69 g, 43.40 ± 5.76 g, 42.20 ± 5.43 g respectively.^[27] in dog and^[28] In goat observed dissimilar information associated with testicle color and measurement, in dog a white reddish colored testicles were noted, while in goat irregularly elliptical and rounded to oval testicles illustrated, after that the same author in goat declared that the average percentage of the testicular weight to the body weight of the right and left testicle were 0.18 ± 0.005 and 0.20 ± 0.007 respectively and the average length of the right and left testicle were 5.59 ± 0.203 cm and 5.95 ± 0.195 cm, respectively.





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CONCLUSION

In conclusion, this study described the anatomical structures of the penis and testis of the tom cat in Iraq. The results also showed the differences between the penis and testis of cat and other animals and provided acknowledge of reproductive system in carnivorous.

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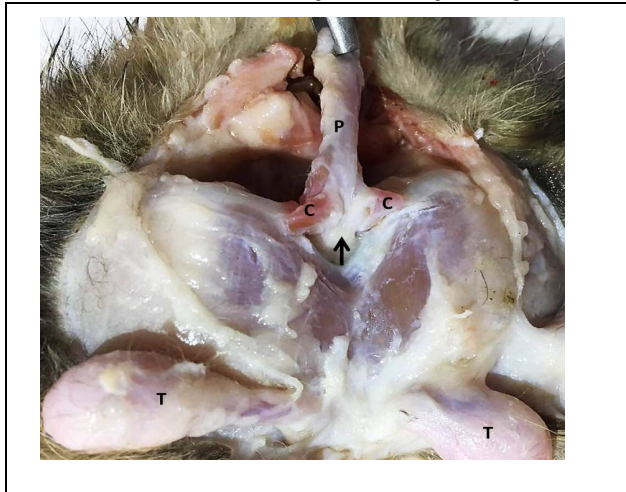


Figure 1. Anatomical view of the penis in situ showed, Root (black arrow), two crura (C), penis (P) & testes (T).

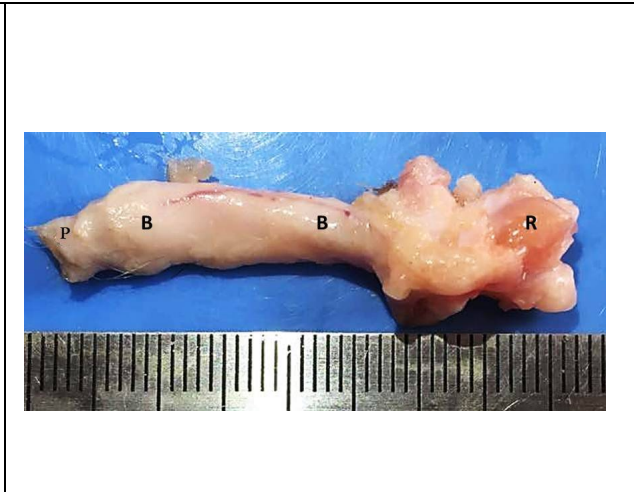


Figure 2. Anatomical view of the penis showed, crura(R), penis body (B) & prepuce (P).



Figure 3a. cross section of the distal part of the penis showed, (paired corpus cavernosum (Cc), corpus spongiosum (Cs), urethra (U) & septa (S)



Figure 3b. cross section of the distal part of the penis showed, urethra (black arrow), corpus spongiosum (Cs),ingle corpus cavernosum(Cs), blood vessel (orange arrow) & septa (S).





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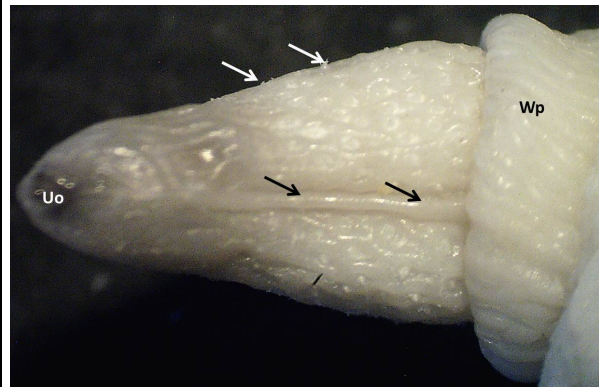


Figure 4. section of glans penis showed, spine (white arrow), urethral orifice(Uo), urethral groove (Black arrow), (winding prepuce (Wp)

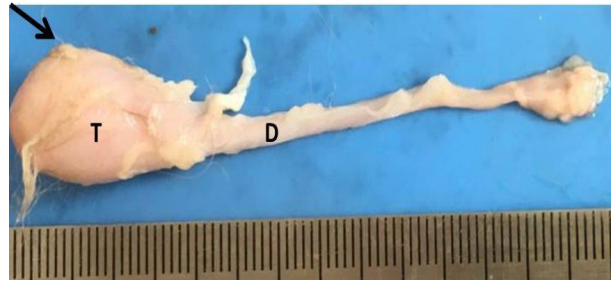


Figure 5. Anatomical view of the testes showed ductus deferens (D), epididymis (arrow), testes (T).

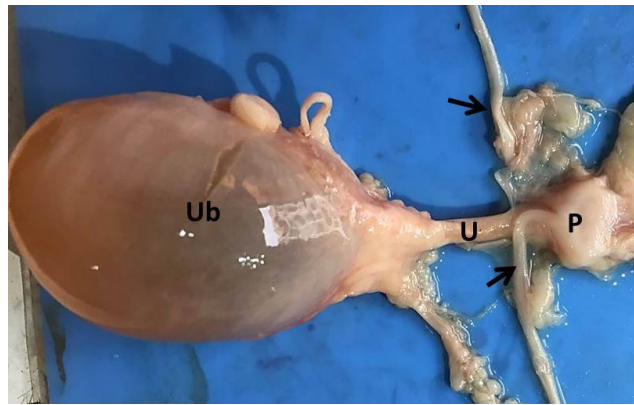


Figure 6: Anatomical view showed ductus deferens (black arrow), urinary bladder(Ub), ureter (U) & prostate (P).





Enlightening Women Health using Machine Vision Technique for Cervical Cancer Detection

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Received: 18 Jan 2019

Revised: 20 Feb 2019

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ABSTRACT

Abnormal cell growth causes cancer to human body which invades and spreads to inner parts of the human body. Cervical cancer appears in the lower part of uterus. Increase in population and poor economical status are the major reason for unattended the cancer disease at the right time. Automatic and fast identification of cervical cancer can be possible with the help of machine vision algorithm with higher accuracy. This work enables the segmenting the cancerous part of the image is appropriately done and multi support vector machine algorithm is employed for accurate classification.

Keywords: Cervical cancer, Image processing, SVM, Women health

INTRODUCTION

The reproductive system in female has cone shaped cervix that interfaces vagina and the uterus. Cervical cancer is the second major threatening cause which disturbs the women lives. World health organization (WHO) says that 1, 30,000 women are affected by cervical cancer [1]. In our country [2 -3], 80, 000 women dies every year due to cervical cancer. Statistical analysis says that one woman dies for every 2.5 minutes with cervical cancer. Annually 500,000 cases were found in developed countries. Detection of cervical cancer is very challenging because it affects women health without any symptoms. Proper screening and effective treatment increases the life of women. Medical imaging technique plays a vital role in early cancer detection for improving the health status of women. The main objective is to detect the cancerous tissue with image processing algorithms and to classify the segmented portion as normal or



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diseased. The various stages of cancer has been identified which is very helpful to take the treatment immediately. Artificial neural network detects the diseased part very quickly with more accuracy compared to the available manual screening such as PAP smear and Liquid Cytology based (LCB) test. Numerous automatic classification of this disease has been attended by various researchers. Unfortunately, those procedures took maximum classification time. The proposed work avails faster diagnosis or classification without compromising the accuracy. Experiment results represent significant reduction in classification time compared to the existing works by maintaining higher accuracy, sensitivity and specificity.

This paper is organized as follows: Section II discusses about the current scenario of the techniques. Section III describes the proposed work. Section IV shows the experimental results and Section V provides the conclusion of the improve methodology. Das et al., [4] has developed automated method of detecting uterine cervical cancer. The region of interest method is involved here to extract cancer. This ROI is gross in nature and extends its interest region in vaginal parts. Royan et al. [5] worked on automatic classification for normal and abnormal cervical cells with artificial neural network and linear vector quantization. This work attains 90% of accuracy with better classification. The experimentation is carried out with 80 image data sets. Karthigai Lakshmi et al. [6] have presented seven class classifier for feature extraction and feature selection to diagnose cervical cancer. Two level cascade system is developed for automatic detection of cervical cancer cells in images captured via liquid based cytology slides which is described by Su et al. [7]. Yung et al. [8] have suggested semi-automatic cytology method for identifying the cancer cell. This automated system was developed for morphological measurements and evaluation of cervical cells. In order to detect three tissues named as Columnar epithelium (CE) which is irregular in shape, Squamous epithelium which has smooth texture and pink in color and transient texture known as Aceto white (AW), agglomerative clustering method is used by Hiri Gordon [9]. Meng et al. [10] has discussed about K means segmentation algorithm using CHAMP software for image classification. This algorithm is useful to classify two different objects with dissimilar color distributions. Yaswanth et al [11] has presented a fully automated segmentation technique to detect cancer cells which are classified to micro tissue and macro tissue. Faster and reliable segmentation method using Laplacian of Gaussian filter is developed for Pap smear images by Biju [12]. Wasswa et al. [13] has classified cervix cancer from Pap smear images. They used Weka segment classifier and a sequential elimination method using fuzzy C classification

Proposed Work

The proposed work is represented in Fig.1. The proposed methodology computes the cancerous cells image texture analysis with accurate boundary detection and faster computation. Gaussian filtering method has been intensively applied in image and computer vision algorithms. Adaptive Gaussian filter is employed to adapt the noise variance characteristics in received images and to find local variance in an effective manner. Gaussian filter behaves as an effective low pass filter which removes noises from the normal distribution condition. Independent level set method enables the speed up function to control movement of the curve. This also tracks and controls the movement propagating interfaces. This procedure is efficient in segmentation to tag the blurry boundaries with strong tag lines. Statistical texture analysis and its features are calculated using gray level co-occurrence matrix (GLCM). Statistical distributions in combination of image intensities are analyzed here. Statistical intensities are grouped into first, second and higher order statistics. GLCM method is helpful in finding second order statistics. SVM classification is designed to separate group of training image into two classifications, named as normal and abnormal. SVM constructs optimum hyper plane separation based on its kernel function. Image feature vector lies on one side of hyper plane belongs to class -1 referred as abnormal and class +1 said as normal stage.





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EXPERIMENTAL RESULTS

Matlab software is used for simulating the image processing algorithms and classification algorithms. The tested results are exhibited in this section. Figure 2 gives the details of image reading. Figure 3-7 shows the classification of various stages of cancer. Table 1 presents the diagnosis or detection of cervical cancer nucleus by several statistical parameters. Table 2 lists the geometric measures of the proposed method. Table 3 presents the comparison of the proposed method with the existing methods. The proposed method shows the improved accuracy compared to the recent techniques.

CONCLUSION

This work involves both texture and geometrical features for the purpose of classification using support vector machine algorithm. This work involves segmenting geometric and texture information. Experimental results show 97% of accuracy with few milliseconds of classification time.

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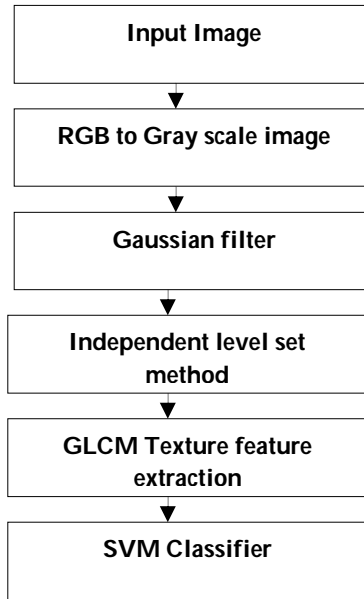


Figure 1. Flow diagram of the proposed methodology

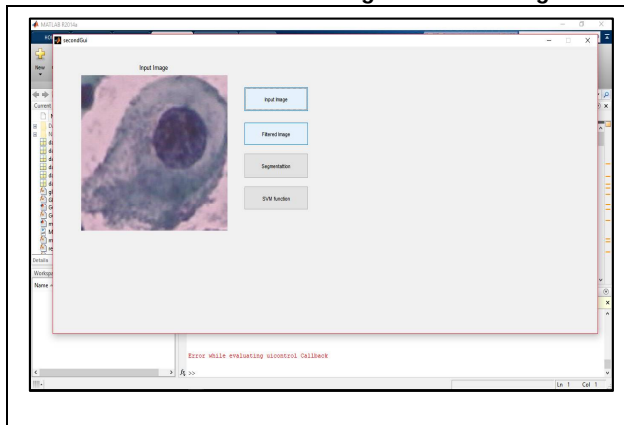


Figure 2 Reading input image

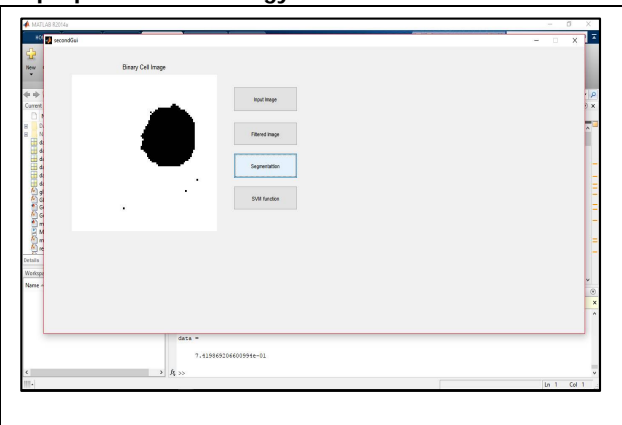


Figure 3. Filtered image

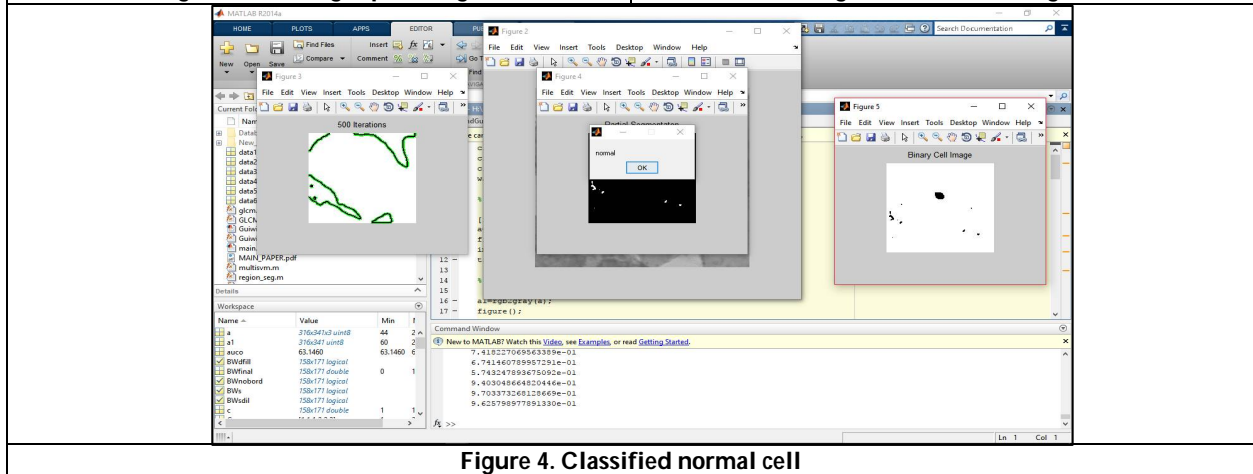


Figure 4. Classified normal cell





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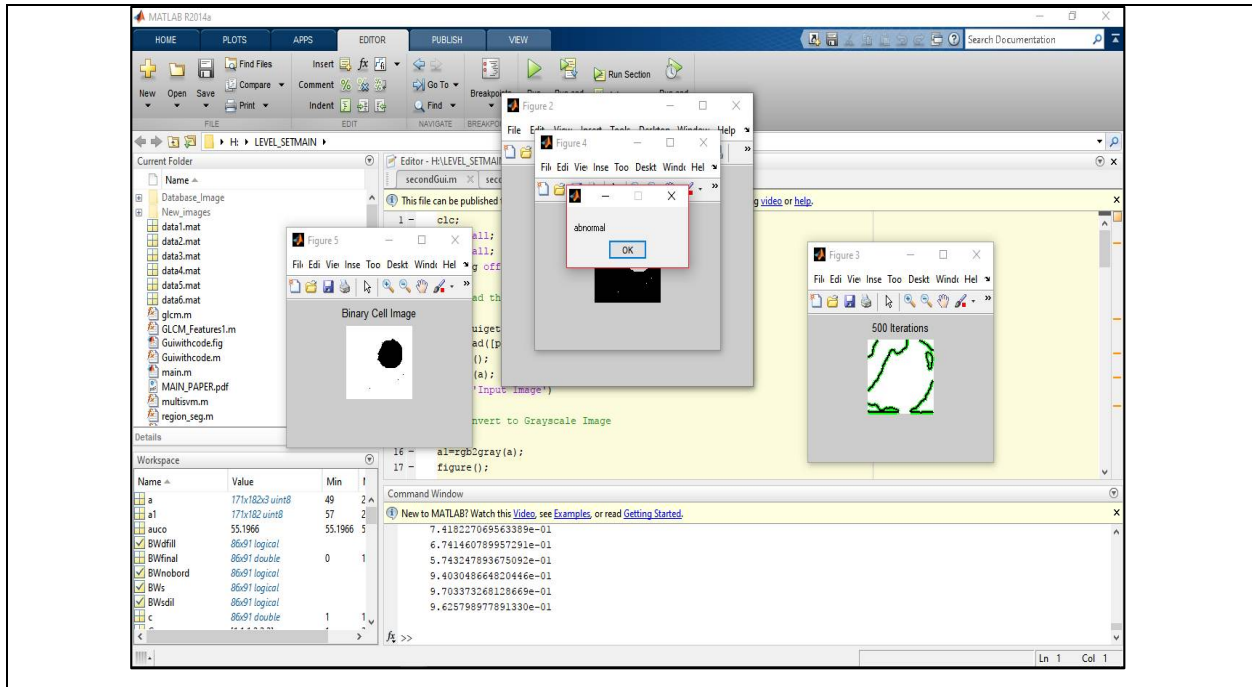


Figure 5. Classified abnormal or cancerous cell

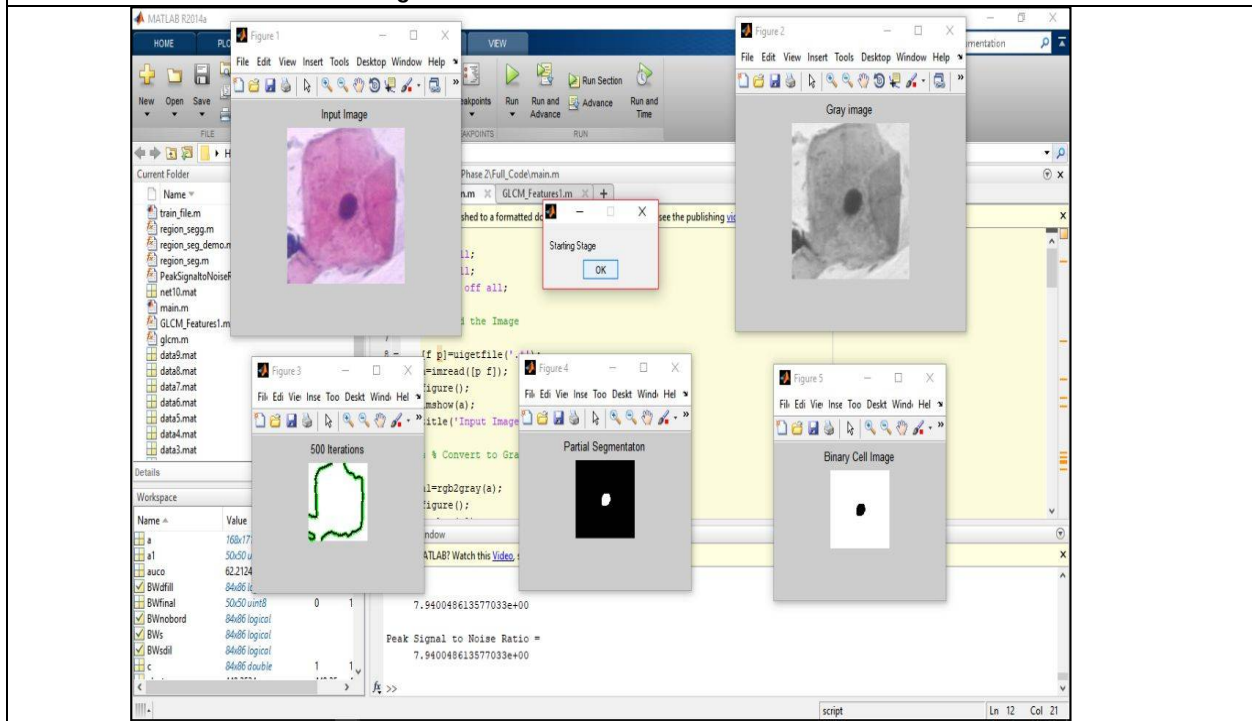


Figure 6. Classified starting stage of cancerous results





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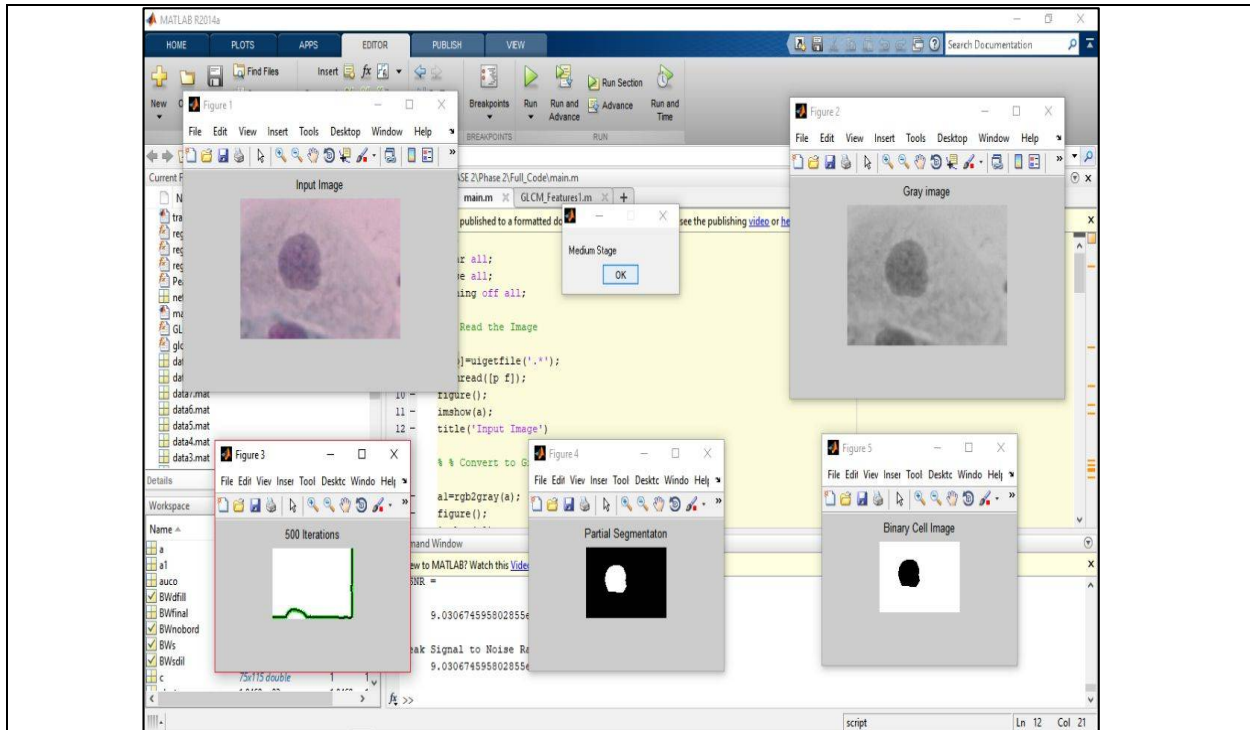


Figure 7. Classified middle stage of cancerous cells

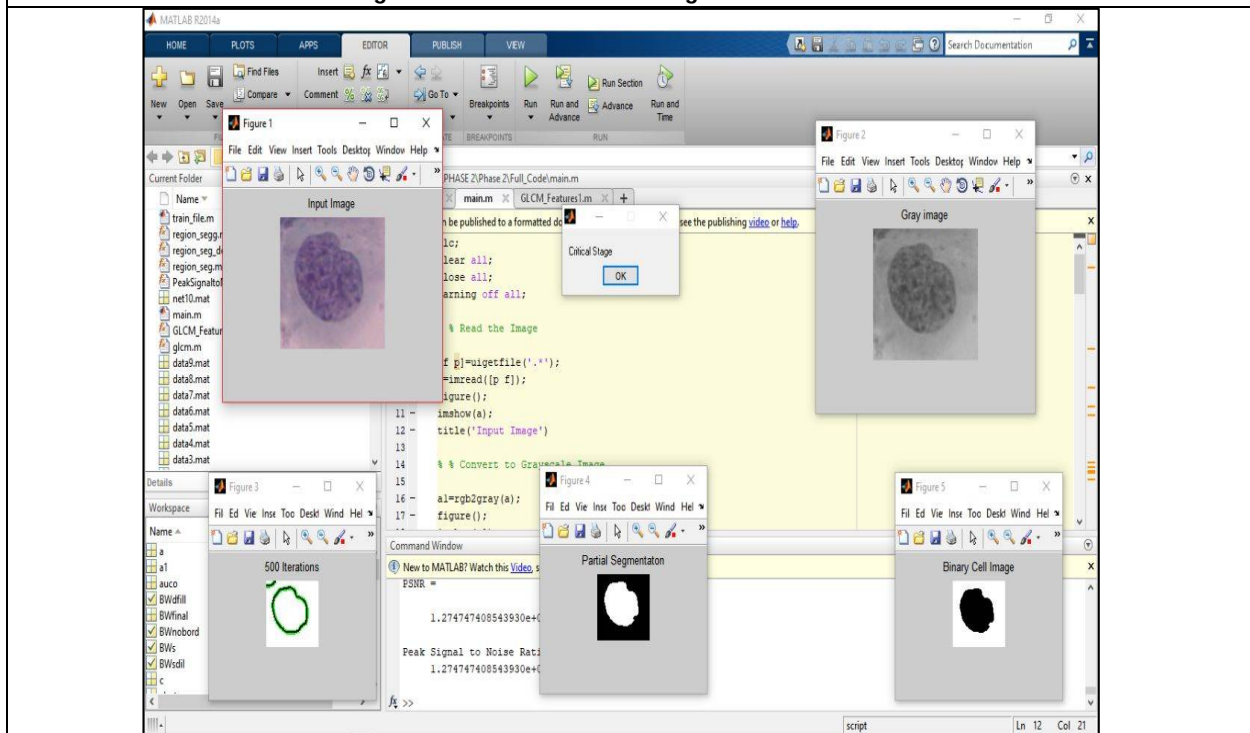


Figure 8. Classified critical stage of cancerous cells





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Table 1. Statistical performance measures of disease segmentation at various stages

	Parameter	Energy	Entropy	Homogeneity	Contrast	Correlation	PSNR
Stages							
Normal		9.70636e-01	9.305e-02	9.899e-01	5.618e-01	3.639e-01	
Abnormal		7.41994e-01	5.367e-01	9.594e-01	2.2735e+00	7.829e-01	
Starting stage		9.4036e-01	1.695e-01	9.835e-01	9.231e-01	5.428e-01	7.9403e+00
Middle Stage		7.9604e-01	4.470e-01	9.665e-01	1.874e+00	7.7110e-01	
Critical stage		4.209e-01	1.037e+00	8.879e-01	6.276e+00	7.259e-01	6.45e+00

Table 2. Geometric features of segmentation

Features	Cell area	Nucleus area	Nucleus/Cell ratio	Cell Eccentricity value	Nucleus Eccentricity value
?	674893	32844	0.053	0.9124	0.495

Table 3. Performance Comparison of various classifiers

Performance	Logistic Regression	Naive Bayes	Kmeans	Proposed
Precision	94.213	93.451	94.6749	96.572
Accuracy	92.562	91.950	94.3753	97.349
Sensitivity	91.227	89.259	93.3691	94.237
Specificity	2.364	4.763	0.000	0.0000





An Evaluation of the Relationship between the Theory of Planned Behavior and Plagiarism: An Academic Investigation

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Revised: 23 Feb 2019

Accepted: 25 Mar 2019

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ABSTRACT

In an age of digital world plagiarism is increasing. It threatens the credibility of educational institutions and it also affects the intellectual growth of students. The present study has used the theory of planned behavior to examine plagiarism in Pakistan. This theory has been widely used in different settings to study cheating and academic dishonesty. The study hypothesizes the positive relationship of plagiarism with positive attitudes towards plagiarism, subjective norms and perceived behavioral control. Data was collected from the University of Karachi and stratified sampling was used. 231 students from all social sciences departments participated in the study. Data analysis was performed through SPSS software and correlation statistical technique was used. Negatively weak but statistically significant relationship was found between positive attitudes towards plagiarism and plagiarism while no statistically significant relationship was found with subjective norms and perceived behavior control. The results show that Pakistan needs other theoretical constructs and variables to study plagiarism in educational institutes. Findings are further briefly discussed in Pakistani context.

Keywords: Planned behavior, Academic dishonesty, Subjective norms, perceived behavioral control, Plagiarism.

INTRODUCTION

Plagiarism is a very common yet complex phenomenon. It does not only exist in educational institutes but its presence can be found in every walk of life. The increasing use of information technology and internet has made plagiarism easier than before. The work on academic dishonesty, cheating and plagiarism is found in different

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disciplines and different perspectives are used to study it. Educationalists study it in the terms of educational context, developmental psychologists have used psychological variables to understand the psychology behind plagiarism, library scientists have studied it differently, and media and software houses have also worked on plagiarism (Ercegovac & Richardson, 2004). Hence the literature found on plagiarism is dominated by these fields. The present study offers a sociological analysis of this issue. Technology has many positive sides but it also has some darker sides. Copy-paste syndrome is found everywhere in the world and it is spreading quickly. It includes the copying of vast and easily available material on Google. It has also made plagiarism easier. Students around the world use Google or internet to find the answers of their questions. As a result of which they start relying on this material. Everything they need is available on internet and it is just one click away. Educational institutes realized this issue and started working to combat it. It does not only affect the intellectual growth and critical thinking of an individual but it also affect the quality of the scientific material produced by the people (Kulathuramaiyer & Maurer, 2007).

Plagiarism is like a plague in institutions and organizations (Kulathuramaiyer & Maurer, 2007). Educational institutions aim to provide quality education and to improve the proficiency of their students. Organizations make sure to improve the quality of their work and employees. Research and originality helps both institutions and organizations to achieve their goals. Plagiarism on the other hand plagues these goals. Hence we are in dire need to address the issue of plagiarism in our communities and also in our research. Kulathuramaiyer and Maurer (2007) also highlighted different techniques and software available for plagiarism detection. Despite of all these available software it is difficult to detect “professional” plagiarists. The question why students plagiarize is of utmost importance. Despite of growing awareness and knowledge people are still doing it. Addressing the root cause of a problem helps us to find its solution. There are number of reasons behind plagiarism but some of them are: students are not fully aware of the meaning and seriousness of plagiarism, they believe that there are least chances of detecting their plagiarism, they do not believe on penalties, they do it for excitement seeking or experimenting without getting caught, they do not want to put their own effort in the work and sometimes they do it because they ‘do not care’ (Liddell, 2003).

Students in Pakistan are encouraged to copy material from books and other sources. In early years of education we follow a syllabus which does not include original thinking and creativity. A part from this there are different educational systems under one institution. Public, private and O levels schools are following different teaching strategies and they even use different books to teach. Same happens with colleges also. Teachers encourage and reward the students who imitate them. This is creating confusion in our environment. When these students enter in to university they face completely different environment. Here they are introduced by plagiarism but they cannot fully comprehend the true idea behind it because they have been plagiarizing their whole life. This creates plagiarism even more serious in our environment because our whole system plays its part in it. Plagiarism can be defined by many ways but the most common definition of plagiarism is the copying of someone else’s work and presenting it as your own without acknowledging the sources (Fusch, Ness, Booker and Fusch, 2017). The major types of plagiarism are discussed below:

- Intentional Plagiarism: It is a type of plagiarism in which someone intentionally takes other people’s work or ideas and presented them under his/her own name (Cheema, Mahmood, Mahmood, & Shah, 2011).
- Unintentional Plagiarism: It is a type of plagiarism which is not planned. Sometimes we confuse our own ideas with the ideas of others. It is mostly due to the availability of vast material on the topic (Maurer, Kappe, & Zaka, 2006).
- Accidental Plagiarism: It is a type in which plagiarism occurs due to the lack of awareness of plagiarism. Wrong referencing, improper citations and poor paraphrasing might cause it (Maurer, Kappe, & Zaka, 2006).
- Self-Plagiarism: Using your own work in more than one place is known as self-plagiarism (Maurer, Kappe, & Zaka, 2006).



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Higher Education Commission of Pakistan HEC has formulated effective policies to combat plagiarism in Pakistan. All the institutes which are recognized by HEC follow this policy. This policy is easily available on HEC's official website. This policy is applicable on students, teachers, researchers and staff members. It is the responsibility of an institute to spread awareness about this policy among its members. HEC will derecognize any institute which will violate this policy. Any individual who found guilty of plagiarism will be reported to HEC. The plagiarism standing committee will then start a proper investigation of the case. There are different penalties for students, teachers, researchers and staff members. The guilty teacher, researcher or staff member will be removed from the office and his/her name will be added in a black list. This individual will not be able to find job in any other academic or research organization. In case of moderate plagiarism an individual will be granted lower grade and his/her name will be added in black list. In case of minor plagiarism such as copying of few paragraphs a warning will be issued to a person. Research grant and scholarship offers will not be applied to the offender. If plagiarism is found in thesis then the student is responsible for it, not a supervisor. That particular student will be expelled from an institute and will not be able to get an admission in any other institute for some period. He/she will be failed in that course. An offender will be charged with fine. In case of first offence or minor plagiarism a written warning will be issued against offender. An offender will not be awarded with the degree. An offender will not be eligible for any research grant and scholarships. The committee might also give extra punishments to the offenders.

Literature Review

Many theorists have tried to describe behavior and its components. But their empirical evidences are not sufficient. Psychologists throughout the history have worked on behavior and have highlighted its predictors. Icek Ajzen also gave his theory on behavior, known as the theory of planned behavior. The theory of planned behavior is the modified form of the theory of reasoned action. Its main focus is on intention to perform particular behavior as intention is the strong force behind behavior. Intention describes the willingness of an individual towards behavior. According to this theory three components can predict human behavioral intention, which in turn is linked with human behavior (Ajzen, 1991). These three components are attitude towards behavior, subjective norms and perceived behavioral control. Ajzen added perceived behavioral control in the modified version in 1985. Attitude towards behavior is describes as person's own view about that particular behavior, how favorably an unfavorably an individual thinks about a behavior. Subjective norms are defined as views about a behavior in a person's surroundings, what his/her family, friends and closed ones think about a behavior. Perceived behavioral control is the control which a person has over the action. It can include all the resources which individual possesses to perform a particular behavior (Madden, Ellen, & Icek, 1992). According to this theory favorable attitude towards a behavior is related to behavioral intention. Further a person is more likely to engage in a behavior if his closed ones have positive views about it. Perceived behavioral control is also linked to behavioral intention.

A person will perform certain acts if he/she has an easy access towards it. The theory of planned behavior talks about behavioral intention which leads to the actual behavior depending upon the control of actual real circumstances (Mayhew, Hubbard, Finelli, & Harding, 2009). Behavioral intention is also the motivational force behind actual behavior (Beck & Ajzen, 1991). It describes the willingness of a person to perform actual behavior. This theory has been tested in different contexts to predict human behavior and it is widely used to describe cheating behavior in academia. Beck and Ajzen (1991) have used the theory of planned behavior to know more about the model's efficiency to predict the cheating, shoplifting and lying behavior. Apart from predictor variables that are attitude, subjective norms and perceived behavior control, this study has added perceived moral obligation or responsibility to enhance the predictability of model. Their results showed that theory of planned behavior is best in predicting cheating behavior in exams than shoplifting and lying. The other variable that was added in the model predicted lying and was not efficient in predicting cheating and shoplifting. Imran and Nordin (2013) have also used the theory of planned behavior to describe the underlying psychological factors of academic dishonesty. Their results showed that all variables of the theory are statistically useful in predicting an intention towards academic dishonesty and intention is also related to the actual behavior of academic dishonesty. Favorable attitudes and subjective norms are



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related to academic dishonesty. If a culture of academic dishonesty prevails in an institute then students will consider it normal and acceptable. Perceived behavior control is also directly related to intention and academic dishonesty. If students perceived difficulty in dishonest actions then they are more likely to avoid it. According to the study of Stone, Jawahar and Kisamore (2010) the structural model of TPB is best to predict academic misconduct. Attitude, subjective norms and perceived behavior control are related to intention and intention is also related to actual cheating behavior in academia. Cheating behavior does not only occur due to attitude but behavior of others also affects it. Peer behavior makes it a norm in an institute and students start taking it less seriously. Further more if the chances of getting caught are less then students are more likely to cheat. Camara, Ziskin, Wimberley, Dabbour and Lee (2016) conducted a study to predict student's intention to plagiarize. Results showed that positive attitudes towards plagiarism was positively related to intention to plagiarize, subjective norms were also positively related to intention to plagiarize and perceived behavioral control also predicted the behavioral intention to plagiarize. That means negative attitudes towards plagiarism reduces the intention to plagiarize, views of our closed ones affect our intention to plagiarize and perceived difficulty/easiness also affects our intention to plagiarize. By controlling all these factors we can control plagiarism in academia.

Moss, White and Lee (2017) conducted a systematic review to find out psychological causes of plagiarism. Their study included the review of 83 empirical papers. The findings which are related to the present study are the psychological explanations behind attitude, subjective norms and perceived behavioral control. Plagiarism is not just limited to academic world only, it is found in other fields also. In academia when students do not value knowledge and learning then they are more likely to have positive attitude towards plagiarism. Therefore students should focus on intellectual growth rather than grades. This way they will be motivated to work hard and avoid plagiarism in it. Norms also affect plagiarism. When something is normal in our surrounding and we observe it constantly then we are more likely to do the same thing. Similarly the views of our closed ones also affect our views towards a particular behavior. Perceived behavioral control is determined by the student's intellectual ability to hide plagiarism and certainty of not getting caught by an institution. Mayhew, Hubbard, Finelli, Harding and Carpenter (2009) have also conducted a study on college cheating. Results showed that TPB predicts the cheating behavior. Stone, Kisamore and Jawahar (2007) have also worked on the prediction of academic dishonesty. They have taken the theory of planned behavior to predict academic dishonesty and have also added personality variables, adjustment and prudence in their study. Results showed that all three components of TPB that are attitude towards behavior, subjective norms regarding behavior and perceived behavioral control are related to intention to behavior.

Hence this theoretical model predicted academic dishonesty. Both personality constructs were also proved to be the predictor of academic dishonesty. Similar findings were found by Whitley (1998). According to his study the students who have positive favorable attitude towards cheating are more likely to cheat. Students who consider cheating normal and live in an environment where cheating prevails are more likely to cheat. Students who perceived themselves as capable of cheating and cheating is easy for them are more likely to cheat. Al-Dossary (2017) conducted a study on cheating in Saudi Arabia. According to it TPB model successfully predicts the cheating behavior. Subjective norms were the powerful predictor of cheating. Subjective norms also influence attitude towards cheating. That means the views of our closed ones also affects our attitudes towards a particular behavior. Mustapha, Hussin, Siraj and Darusalam (2016) have also used the modified version of the theory of planned behavior to study cheating intentions among Malaysian students. They have added an element of religiosity in their study to find out its influence on cheating intentions. There are very few studies which have used religiosity because it has mostly been used by western people and is always dominated by Christianity. Islam condemns dishonesty in all matters and cheating is always looked down upon in Islam. Religiosity is different from religion; it refers to the practices, attitudes and beliefs which prevails in a community. Their results showed that attitudes and subjective norms are significantly related to cheating while there were no statistical significant relationship was found with perceived behavioral control and Islamic religiosity.



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1. Favorable attitude towards plagiarism is positively related with the plagiarism.
2. Subjective norms are positively related with plagiarism.
3. Perceived behavioral control is positively related with plagiarism.

MATERIALS AND METHODS

The present study has adopted quantitative research methodology and is explanatory in nature. The universe of the study is Karachi University and data were collected from 231 students. Stratified sampling technique is used and only social sciences departments are added in to the final sample. Questionnaire was used as a data collecting instrument. Attitudes and subjective norms are measured by Attitude towards Personality ATP questionnaire (Mavrinac, Brumini, Bilić-Zulle, & Petrovečki, 2010). Perceived behavioral control is measured by three item scale used by Stone, Jawahar&Kisamore (2010). Plagiarism is measured by plagiarism survey (Hosny & Shameem, 2014). Other relevant items were also added in plagiarism scale. All the responses are self-administered and are measured on 5 point Likert scale except the plagiarism scale which is measured on the 3 point scale. The necessary changes were made in original scales. The data is analyzed through SPSS software and correlation statistical technique is used.

RESULTS AND DISCUSSION

An analysis of the demographic variables shows that 84% students who participated in this study were females and 16% were males. 78% respondents who took part in this study had English as a medium of education in matriculation while 20% had Urdu and only 2 respondents had chosen other languages as their medium of education in matriculation. The table shows that 68% respondents have taken their current courses in English, 31% have chosen their courses in Urdu and 3 respondents have their courses in other language. 28% people in this study felt comfortable with English, 69% people were comfortable with Urdu and 2% were comfortable in other languages. The variables taken in the study were treated as scale variables but for the purpose of data presentation researchers had categorized the scores of variables into three levels. Table 1 highlights that level of plagiarism was low in 34% of respondents, moderate in 48% of respondents while high in 18% of the respondents. Around 58% of respondents considered themselves as moderate in perceived behavioral control while 32% were low and 11% high in respondents. Subjective norms were moderate in 79% of respondents whereas approximately 13% were low and 8% were high in subjective norms. Around 80% of the respondents considered themselves as having moderate level of positive attitude towards plagiarism while approx. 10% had high level and 10% had low level of positive attitude towards plagiarism.

Table no. 2 indicates that there was negatively weak but statistically significant relationship between positive attitudes towards plagiarism and plagiarism ($r = -.153$, $N=231$, $p= 0.020$) hence hypothesis no. 1 was accepted. Negatively weak relationship was found between plagiarism and subjective norms ($r = -0.103$, $N=231$, $p= 0.118$). Extremely weak and statistically no significant relationship was found between plagiarism and perceived behavioral control ($r = -0.091$, $N= 231$, $p= 0.167$), hypothesis no. 3 was rejected. The findings of the study pointed that the statistically negative and significant relationship was found between positive attitudes towards plagiarism and plagiarism. This means that students who have negative views about plagiarism were more likely to plagiarize. In Pakistan plagiarism is a new phenomenon. HEC (Higher Education Commission) has introduced several policies on plagiarism but these policies do not apply on any thesis or research article which was published before 2007. This means that mixed attitudes towards plagiarism were found in our society. Those who had plagiarized before 2007 were part of academia and HEC itself. Those same people are teaching the subject of "Social Research" to the students. The word plagiarism carries negative connotations in Pakistan but students due to other several reasons do



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plagiarize. The other finding which showed that there was no statistically significant relationship between perceived behavioral control and plagiarism was consistent with the findings of Mustapha, Hussin, Siraj and Darusalam (2016). The model of the theory of planned behavior is not sufficient enough to describe plagiarism in Pakistan. Although this model has been successfully used by many other researchers and in many different countries but it has not provided expected results in our setting. The developing countries are facing different problems and the magnitude of the problems is different in different settings. In schools students are encouraged to imitate teachers and it is often considered as a form of respect. The same practice continues in college and universities. The concept of plagiarism in Pakistan is introduced at university level. These students then deal with the ethical dilemmas in universities. The findings of Rathore, Waqas, Zia, Mavrinac and Farooq (2015) showed that most of the medical students and faculty members in Pakistan had favorable attitude towards plagiarism and plagiarism was acceptable to them. They also suggested that lack of research writing and knowledge is one of the causes of plagiarism. The same research was conducted by Shirazi, Jafarey and Moazam (2010), they also concluded that limited and shallow knowledge of plagiarism existed in Pakistan. Although these studies were conducted on medical students but it does provide us some insights of plagiarism culture in Pakistan's context. This lack of knowledge about referencing and citation leads towards unconscious plagiarism. They believed that plagiarism occurs due to lack of knowledge and training in Pakistan. This ignorance and lack of knowledge can only be dealt with proper education and research training.

Limitations and Directions for Future Research

The data in this study is collected from one public university only. It does not provide the comprehensive view of the whole country because it does not include the data of other private and public universities. The future researchers must include other institutes in their study. Plagiarism is a relatively new phenomenon in our research. Therefore different methodologies should be used to study the issue of plagiarism in our environment. Researchers can take help from both qualitative and quantitative research methodologies to further explore this topic. Why students plagiarize is the real question. This area requires more research. Variables other than the theory of planned behavior can be used in research to find out the causes of plagiarism. The effect of plagiarism on intellectual growth should be studied and highlighted by future researchers.

CONCLUSIONS

Plagiarism is an ongoing phenomenon which is constantly threatening our institutions. Today Pakistan and all developing countries are facing a number of problems. Some of them are economic problems, technological problems, educational crisis, and corruption etcetera. Plagiarism plays an important role in solving many of these issues. The present study has used the theory of planned behavior to study plagiarism in Pakistan. This theory has been widely used in the literature of cheating, plagiarism and academic dishonesty. These theoretical constructs along with the plagiarism were tested for the first time in Pakistani context and due to the difference in our plagiarism culture these variables did not show any significant relationship with plagiarism. The findings of this study showed no clear relationship between the theoretical constructs and plagiarism. Hence Pakistan needs new variables and dimensions to study plagiarism and academic dishonesty in various educational institutes. Therefore researchers suggest the use of new theoretical constructs and variables for the further exploration of this topic.

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Table 1. Characteristics of Subjects (n = 231)

Indicators	Frequency	Percent	Cumulative Percent
Medium of Education in Matriculation			
English	181	78.35	78.35
Urdu	48	20.77	99.12
others	2	0.86	99.98
Total	231	99.98	
Medium of Education in Current Courses			
English	157	67.96	67.96
Urdu	71	30.73	98.69
others	3	1.29	99.98
Total	231	99.98	
Ease in Language			
English	66	28.57	28.57
Urdu	161	69.69	98.26
Others	4	1.73	99.99
Total	231	99.99	
Level In Plagiarism			
Low	79	34.19	34.19
Medium	111	48.05	82.24
High	41	17.74	99.98
Total	231	99.98	
Level In Perceived Behavioral Control			
Low	73	31.60	31.60
Medium	133	57.57	89.17
High	25	10.82	99.99
Total	231	99.99	
Level In Subjective Norms			
Low	29	12.55	12.55
Medium	183	79.22	91.77
High	19	8.22	99.99
Total	231	99.99	
Level In Positive Attitudes Towards Plagiarism			
Low	24	10.38	10.38
Medium	183	79.22	89.60
High	24	10.38	99.98
Total	231	99.98	





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Table 2. Correlations among variables

		Plagiarism	Positive attitude towards plagiarism	Subjective norms	Perceived behavioral control
Plagiarism	Pearson Correlation	1	-.153*	-.103	-.091
	Sig. (2-tailed)		.020	.118	.167
	N	231	231	231	231
Positive attitude towards plagiarism	Pearson Correlation	-.153*	1	.565**	.358**
	Sig. (2-tailed)	.020		.000	.000
	N	231	231	231	231
Subjective norms	Pearson Correlation	-.103	.565**	1	.427**
	Sig. (2-tailed)	.118	.000		.000
	N	231	231	231	231
Perceived behavioral control	Pearson Correlation	-.091	.358**	.427**	1
	Sig. (2-tailed)	.167	.000	.000	
	N	231	231	231	231





ISSN: 0976-0997

Indian Journal of Natural Sciences

Instruction to Author(s), 2018

NAAS RATED JOURNAL 3.56

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Prediction of Soil Properties Using Image Processing with ANN (Artificial Neural Network) for Crop Cultivation

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Received: 16 Jan 2019

Revised: 20 Feb 2019

Accepted: 25 Mar 2019

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ABSTRACT

Agriculture is the key development for the rise of sedentary human civilization, and agriculture science depends on the cultivation of land for growing of crops whereby farming of domesticated species created food surpluses to breed animals, plants and humans to provide food, fiber, and other products to sustain and enhance life. Soil properties are a significant physical quantity that plays a substantial role in agriculture. In this proposed work, the system is developed with image processing and Artificial Neural Network (ANN) to identify the properties of the soil image and to determine the suitable crop for cultivation. This work aims testing of soil which is useful to know the type of soil and the properties like fertility and acidity of the soil, and predicting the crop yield at a particular soil and thereby recommending suitable crop for that soil.

Keywords: Artificial Neural Networks, Back Propagation, Gray Level Co-Occurrence Matrix.

INTRODUCTION

Agriculture is the science and art of cultivating plants and livestock. Agriculture plays a crucial role in the life of an economy and most countries have an economy that is dependent on crop cultivation of agriculture either in a small or big way. Farming offers food for societies and gives somethings for trades. Nowadays, crop cultivation is



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decreasing rapidly. The farmers with wide ranges of techniques such as precision and sustainable agriculture to face challenges in the field. To overwhelm this problematic, observing the crop water necessities, hotness, and moisture of the gardening land. Moisture content in soil is one of the main components which play an important role in the yield of crops. If the moisture content is more in the soil it will cause respiration problem in the plants. If it is less it will cause dehydration problem. So the moisture content should be appropriate for the proper growth of the plant. The main concept of this work is testing most of the soil and find which crop is suitable for that field using image processing techniques. This method increases the crop production which will not be detrimental to farming by saving a lot of manpower energy, time and most importantly money. The main phase to track later obtaining the images is to sieve the images for whichever noise existing. The filtered images are then subjected to edge detection techniques, whereby each particle is separated from adjacent particles and identified. The diameter of each particle is determined and the grain, size, distribution of the sample to be determined. Soil belongings depend not solitary on the cereal, mass, spreading of atoms but also on numerous further aspects like mineral components, physical orderings, ecological history, etc.

Literature Survey

John Carlo PUNO et al (2017) discussed the soil nutrients and pH level using Image Processing and Artificial Neural Network to efficiently identify the nutrients and pH level of soil with the use of Soil Test Kit (STK) and Rapid Soil Testing (RST) of the Bureau of Soils and Water Management. They used Artificial Neural Network to hasten the performance of image processing in giving an accurate result. The system captured image data, trained 70%, and tested 15% and 15% for validated with the tool of MATLAB. The results showed the qualitative level of soil nutrients and pH level of the soil. Katariya et al (2015) proposed a farming pitch which is completed by a robot without charitable any human maintenance. For designing a spontaneous system, that offer appropriate tracking over the white line next to the robot model in which the robot differentiates white and black or dark outward and follows the only white path. With reference to this perception, successful to implement a white line on a farm where really the necessity to work remaining outward is treated as a black surface due to the brownish color of the soil. Also deliver stay to the robot at equivalent space to do the farming effort like pesticide spraying, water supplying, persevering, releasing of seed, exactly and spontaneously the solar plant offers the supply basis to the entire robot which creates it eco-friendly.

Swarup et al (2013) proposed a technique based on Smart sensors. The sensors based observing units for farming have been used to raise the harvest of plants through observing the ecological circumstances (parameters) and hence supplying the essential data to the farmers. The projected scheme was mainly developed for the betterment of farmers. The usage of wireless sensor network through the wired one supports for positioning it in whichever form of atmosphere for observing, constructing it malleable and healthy. The usage of the FPGA component assists the system for reconfigurability and re-programmability according to different environmental conditions. Sanjukumar et al (2013) discussed the Soil moisture content based irrigation method with the flow sensor. The consumer can effortlessly predetermine the stages of the Wetness and is frequently modernized about present rate of all Constraints on LCD presentation and planned to combine soil constraints specifically soil pH and soil electrical conductivity in the system. Viscarra Rossel R A, Webster R (2012) tested the prediction of using tree-based technique for 24 soil properties. They used the root mean squared error (RMSE) to measure the inaccuracy for predictions, the mean error (ME) to measure bias and the standard deviation of the error (SDE) to measure imprecision. And also used the ratio of performance to deviation (RPD), which is the ratio of the standard deviation of the observed values to the RMSE of the predictions.

The existing systems used KNN and SVM algorithm to perform this work. KNN will be providing the nearest value and also has a few limitations like poor run-time performance when the training set is large. It is known irrelevant or redundant features contribute to the similarity and classification of data. Two other disadvantages of the method are: Distance-based learning is not clear and computation cost is quite high (need to compute the distance of each query



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instance to all training samples). SVM will be in the form of true or false and each time we need to say the comparison of the dataset.

MATERIALS AND METHODS

The proposed system is a software-based application which can be used to find the pH value of the soil sample. And this work aims at predicting the moisture of the soil, and also the crop yield at the particular weather condition and thereby recommending suitable crop for that field. It involves the following steps.

Step 1: Collect the soil satellite images for agricultural crop monitoring.

Step 2: Categorize the image found on Soil form, wetness content, climate states, pH rate, natural nitrogen, etc.

Step 3: Execute satellite image dispensation with reverence to textural and spatial characteristics.

Step 4: Examine crop shapes with the support of earlier data and plot them with present calculate records.

Step 5: Observe crop produce and notify methods for growing it.

Step 6: Suggest gainful crops for every soil type.

As per the requirement of the system as automated, that wants to make software which process and give some intermediate results. So the people who want to test the soil can easily test it by using the software. Digital image processing is a term in which digital images will be taken and calculate some values from it to perform a particular operation on it. By using the digital image processing we have implemented our system based on the user need. The system works as by taking the image file of soil sample as input with some constraints and gave pH and the constituents of soil as output.

SYSTEM FRAMEWORK

Figure (1) shows the overall framework of the proposed system.

Image uploading

First, the input of a soiled image is uploaded; figure (2) shows the uploading of a soiled image.

Image Filtering

Image filtering is used to change the pixel values of an image, so the colors of the image are altered without changing the pixel positions; here the input soil image is filtered with the predefined filters. (ieusing the special function that can be used with filter). The input image is filtered by applying an unsharp masking filter to a grayscale image. The unsharp masking filter has the effect of making edges and fine detail in the image crisper. Figure (3) displays the filtered image.

Image Pre- Processing

The aim of pre-processing is to improve the image data to suppress unwanted distortions or to enhance some image features which is important for future processing. For this thresholding method is used which a technique of inverse filtering. The resultant preprocessed image is given in figure 4.





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Image Masking

An image processing technique that is used to remove the background from which photographs those have blurred or fuzzy edges, transparent or hair portions. But it retains the original photo quality. A mask image is simply an image where some of the pixel intensity values are zero and remaining are non-zero. The particle Analysis tool is used to generate the mask image. Figure 5 shows the masked image.

Artificial Neural Networks Processing (ANN)

This section deals with ANN which is used to find the soil type. An artificial neural network is an interconnected group of nodes, akin to the vast networks of neurons in a brain. The neural network is a framework working together with multiple machine learning algorithms to process complex data inputs. Image preprocessing, data reduction, segmentation, and recognition are the process used in managing images with ANN. A soil image can be represented as a matrix; each element of the matrix containing color information for a pixel. A non-natural neuron mimics the working of a biophysical neuron not a biological neuron model with inputs and outputs. The system arranged by joining the output of some neurons to the input of further neurons developing a managed biased graph. The masses as well as the tasks that calculate the activation can be modified by a process called learning which is governed by a learning rule.

Back Propagation

Back propagation is a specific method used for training a multi-layer neural network. It is also called the generalized delta rule. It is a gradient descent method that reduces the output value of total squared error. The back propagation takes place in three different stages during training neural network.

1. Feedforward of the input pattern.
2. Calculation and Backpropagation of the associated error.
3. Adjustments of the weights. After the neural network is trained, the neural network has to compute the feed forward phase only. The output is produced by the network immediately even if the training is slow.

Gray Level Co-Occurrence Matrix

A statistical way to investigate surface that gives thought to the spatial association of pixels is known as the gray-level co-occurrence matrix (GLCM) and is referred to as the gray-level spatial dependence matrix. These data offer facts about the surface of an image. A co-occurrence matrix also a co-occurrence distribution is a matrix that is distinct atop of the image to be the issue of co-occurring pixel point (that may be grayscale data or colors) at an assumed offset. It is moreover to describe the matrix through two dissimilar images. That matrix is used for color mapping. To capture the properties of a texture, Co-occurrence Matrices formula is used and the numeric features computed from co-occurrence matrices can be used to represent and compare textures. Standard features from a normalized co-occurrence matrix are

$$\text{Energy} = \sum_{i,j=0}^{N-1} P(i,j)^2$$

$$\text{Entropy} = - \sum_{i=0}^{n-1} \sum_{j=0}^{n-1} p(i,j) \log_b p(i,j)$$





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$$\text{Contrast} = \sum_{i,j=0}^{N-1} P_{i,j} (i - j)^2$$

$$\text{Homogeneity} = \sum_{i,j=0}^{N-1} P(i,j) / R$$

Algorithm

Step 1: Upload an input image.

Step 2: Perform image filtering.

Step 3: Perform Image preprocessing.

Step 4: Image Masking is performed.

Step 5: Perform ANN Process

Step 5.1: Train the data set.

Step 5.2: Perform testing by loading a soiled image and compare with trained data.

Step 5.3: Calculate the similarity value for the input image and trained data by using Euclidean distance formula.

Step 5.4: If test image = train data then go to Step 6 (Similarity value equals) Else train the test image by using reverse propagation till it equals trained data.

Step 6: Display the properties of the soil by using the formulas (3.1. To 3.4)

Step 7: Stop

RESULTS AND DISCUSSION

Figure (6), (7) and (8) shows the ANN Processing results and soil type and its properties like contrast, homogeneity, entropy, energy and dissimilarity values of input soil image using the proposed system. First, the data are trained and its properties are stored. Next testing is done with ANN by first giving a test soil image and it is compared with the existing trained data. The Main advantage of ANN is if the similarity is 100 percent then only it will stop the testing process. If it found any dissimilarity then it will go for reverse propagation to train the input and continue this process till it finds accurate results. Likewise, first, the data are trained and tested. Using this proposed system the user can upload an image and get back the soil properties as a result.

Performance Analysis

The performance of ANN is compared with the existing algorithms KNN and SVM algorithms. Our proposed system is found to be high in performance in finding the soil properties due to ANN process when compared with existing algorithms. (It will continue its training process till it gets accurate results.) The comparison of ANN, KNN, and SVM for various types of soils is given in table (1, 2, 3) and as graphs in figures (9, 10, 11).

CONCLUSION AND FUTURE ENHANCEMENT

The proposed system is an automated technique to estimate the moisture content in the soil. The system finds the moisture content along with data which gives information about whether the input soil is moisture deficient or contains correct moisture content. This model is based on a digital image processing technique where digital





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photographs of the soil samples are used for soil pH determination. Soil pH tells us the acidity as well as basicity present in the soil because if the soil is very acidic then it affects the growth of the plants. This work simplifies and reduces the manual work and predicts the moisture of the soil which is used to recommend a suitable crop for that. Further, fundamental research is needed to characterize the variation and uncertainty inherent in text mining techniques. In this paper, the use of both LDA (Linear Discriminant Analysis) and PLS (Partial Least Square Regression) did not give consistent results with different training and test set selections. These differences need to be formally characterized ideally and described with a probabilistic model that further enhances understanding of the contributors to accidents.

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Table 1. Comparison of Soil type 1

Soil Properties \ Techniques	ANN	KNN	SVM
Contrast	299.44	210.12	1
Homogeneity	0.11	3.75	0
Entropy	3.54	8.24	1
Energy	8.04	5.6	0
Dissimilarity	12.53	25.21	1





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Table 2. Comparison for Type 2

Techniques Soil Properties	ANN	KNN	SVM
Contrast	91.01	120.23	0
Homogeneity	0.13	2.73	0
Entropy	3.02	7.56	1
Energy	0.001	2.36	1
Dissimilarity	7.31	26.25	1

Table 3. Comparison for Type 3

Techniques Soil Properties	ANN	KN	SVM
Contrast	202.3	252.3	0
Homogeneity	0.43	3.65	0
Entropy	2.77	8.22	1
Energy	0.01	3.62	1
Dissimilarity	6.45	32.6	1

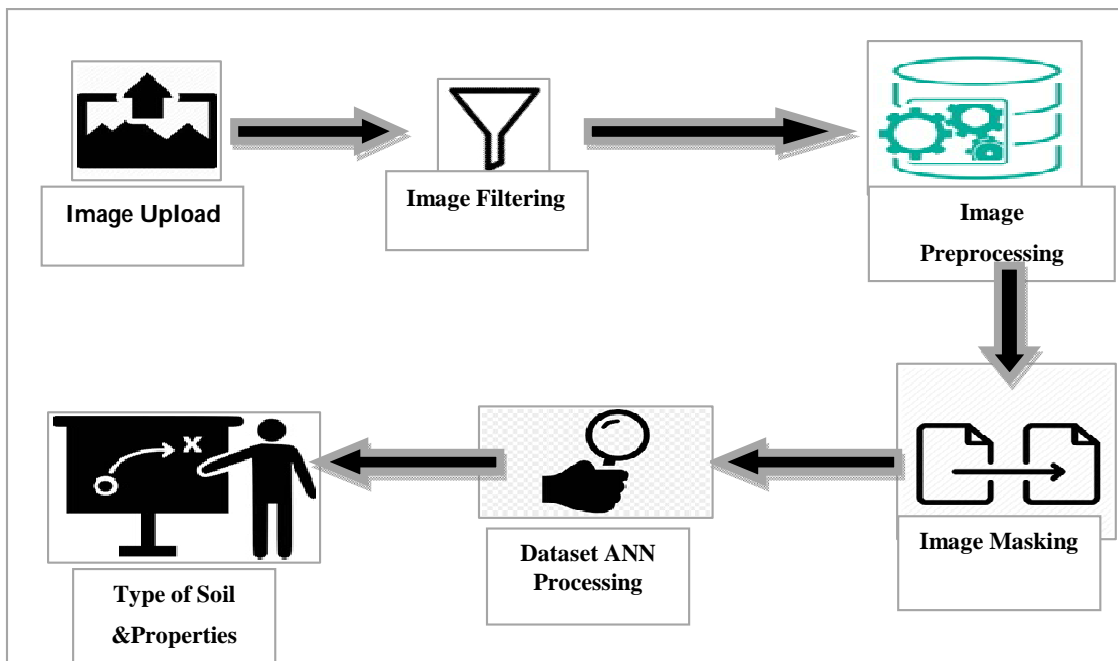


Figure 1. The framework of the Proposed system





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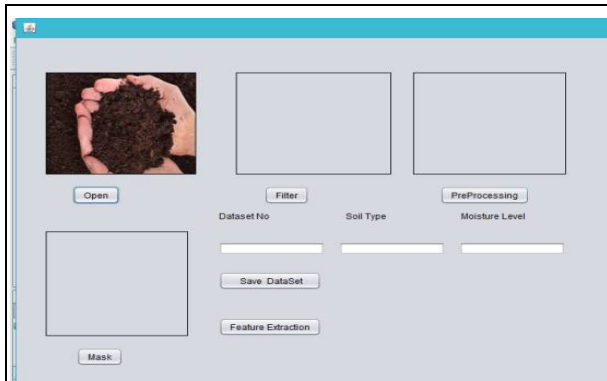


Figure 2. Input soil image

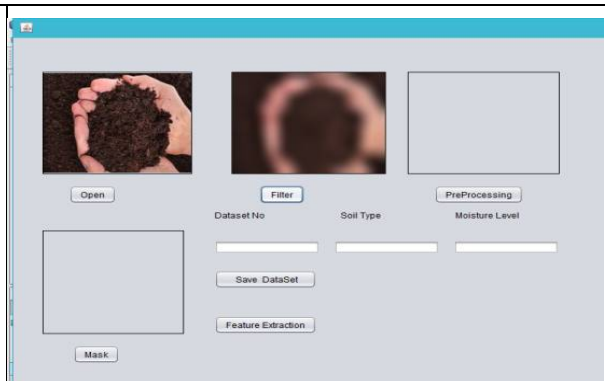


Figure 3. Image Filtering

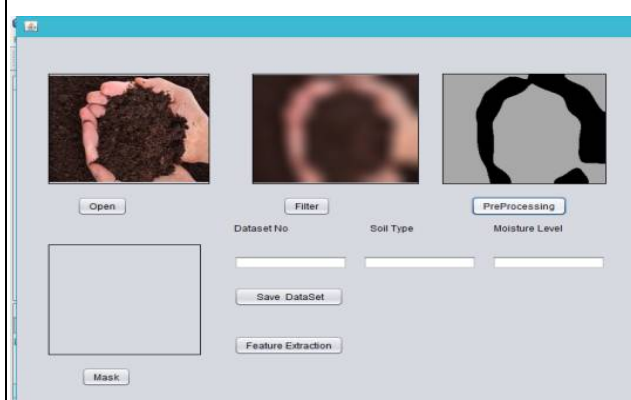


Figure 4. Image Preprocessing

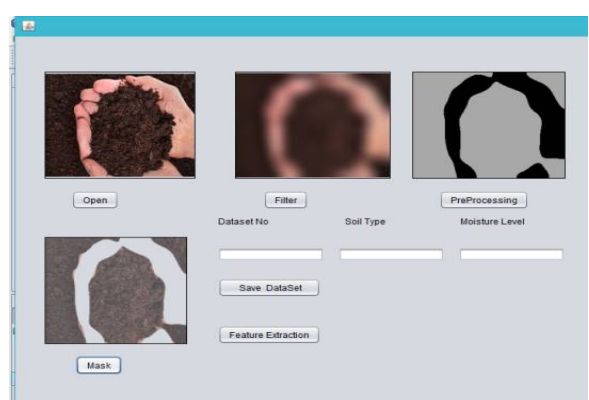


Figure 5. Image Masking

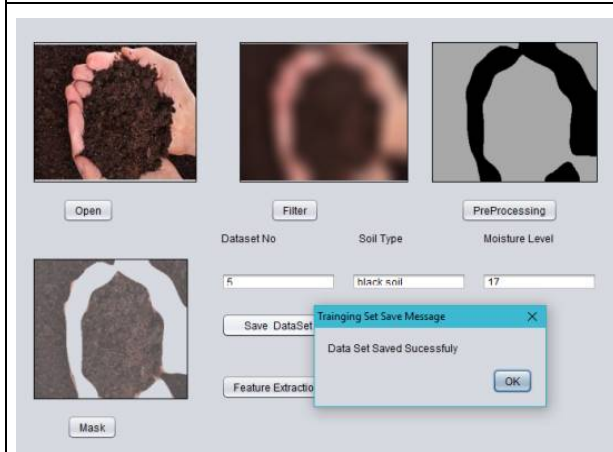


Figure 6. Dataset and ANN processing

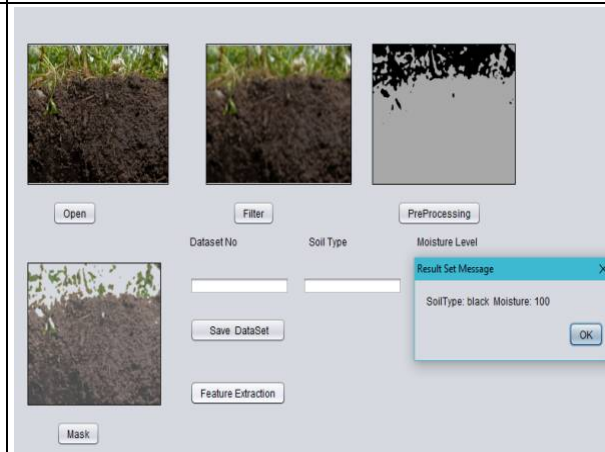


Figure 7. Result





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id	SoilType	Contrast	Homogeneity	Entropy	Energy	Dissimilarity
2	1	299.494324796	0.11433300824	3.54953415327	8.04520480044	12.5337520201
3	2	91.0174714022	0.13603920986	3.02091711721	0.00144886079	7.31597566980
4	3	202.338755934	0.43642836416	2.77192509493	0.01206746058	6.45035835584
5	5	124.732645545	0.18848799137	3.25752289188	0.00102540109	6.93992762177
6	6	122.428664332	0.16755181404	3.23346705376	0.00109051023	7.37267843615
7	8	73.0176317517	0.36113513956	3.32328495622	0.00100963049	4.48406965733

Figure 8. Sample Test Result by using ANN

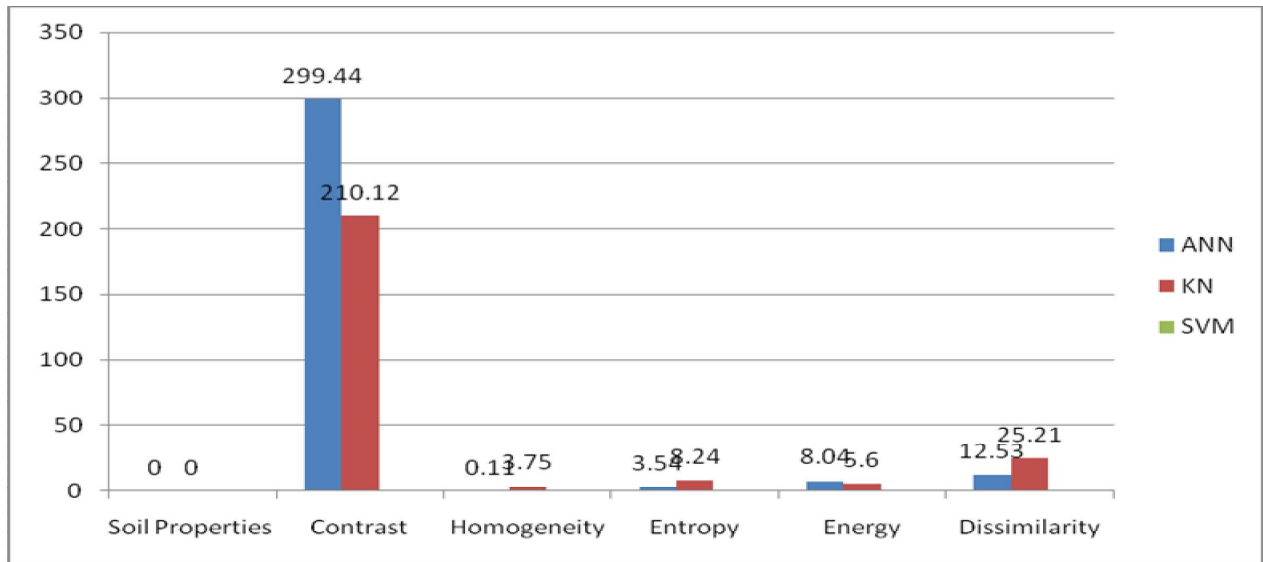


Figure 9. Comparison chart for Soil type 1

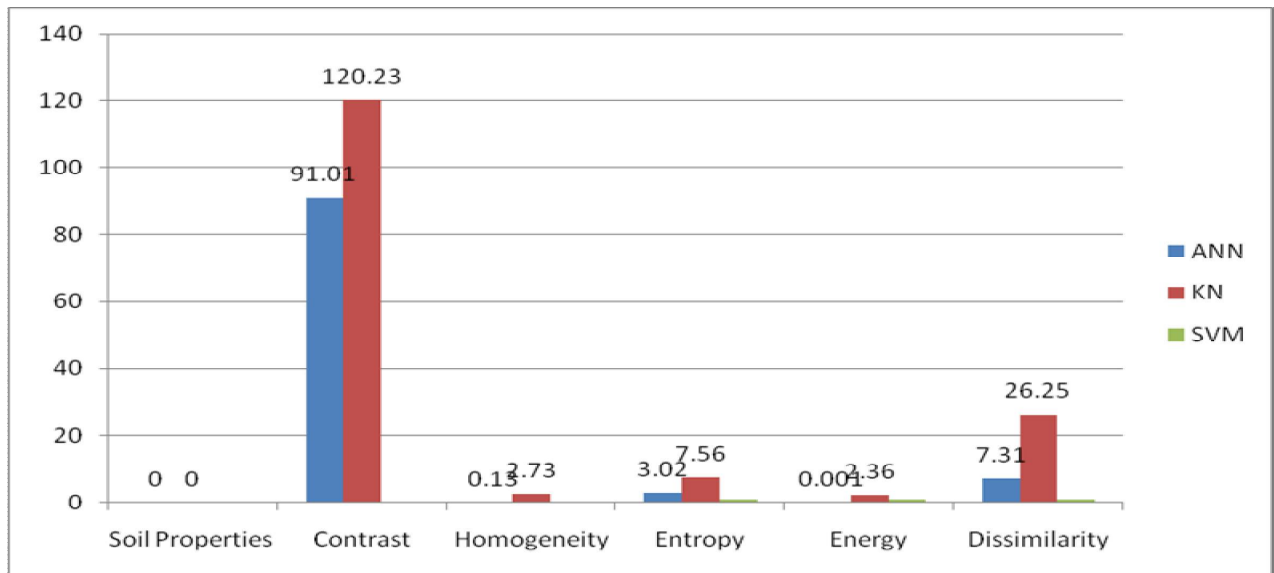


Figure 10. Comparison chart for Soil type 2





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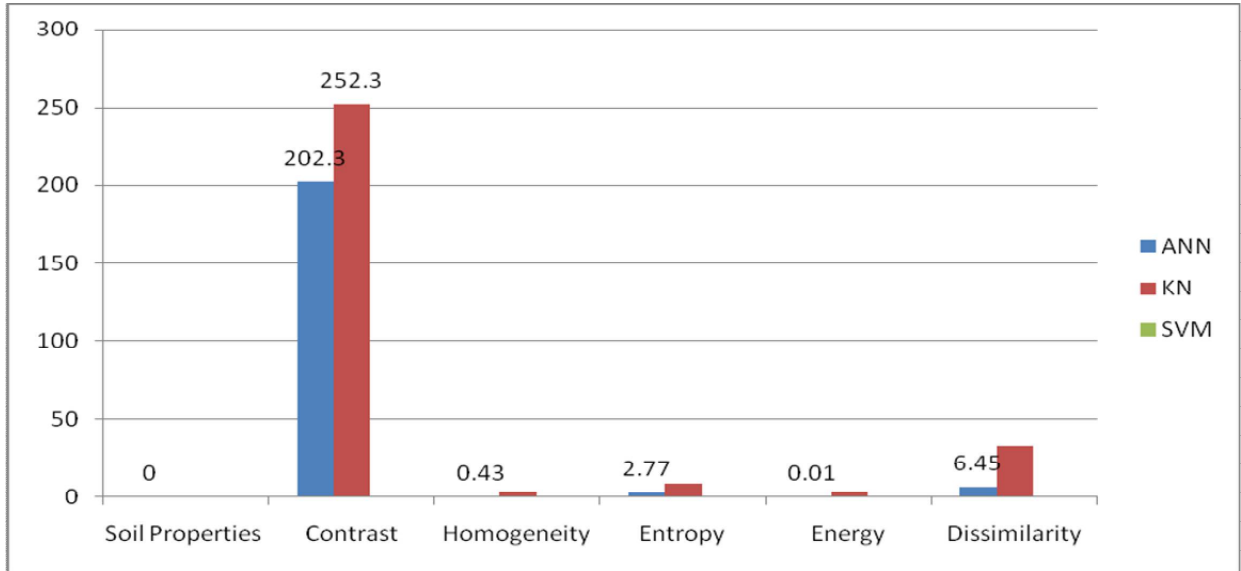


Figure 11. Comparison chart for Soil type 3





Impact of KAP on Spinal Stenosis Patients in Ongkharak, Thailand

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Received: 19 Jan 2019

Revised: 22 Feb 2019

Accepted: 26 Mar 2019

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ABSTRACT

Modern society, prevalence of spinal stenosis remains high in different communities. There is lacuna on knowledge and attitudes of spinal stenosis and pain management practices. This study aimed to explore the level of knowledge and attitudes of patients with spinal stenosis and their pain management practices. Fifty-six subjects were selected by using simple random sampling from Ongkharak hospital area. The instruments composed of three parts: Demographic Data, Knowledge and Attitudes of spinal stenosis and Pain Management Questionnaire, and patients Regarding Pain Management caring behavior Questionnaire. The findings indicated that spinal stenosis patients had very low level of knowledge and negative attitudes pain management whereas the level of practice was moderate. Therefore, it is necessary to improve the patient's knowledge and attitudes on spinal stenosis and their pain management practices in the study area. Upon KAP application most of the respondents showed a significant change in their attitudes on pain management.

Key words: Spinal Stenosis, Knowledge and Attitudes, Practice, and Pain Management.

INTRODUCTION

Back pain, lower back pain is a common problem encountered in general, working and elderly population. Several studies have revealed that the prevalence of back pain remains high in common population. According to the United Nations estimates, between 2010 and 2040, 8 to 14 folds of population aged 65 or older will grow from 16 to 25 percent in developing regions. (Population Reference Bureau, 2010). Prevalence studies suggest that up to 50% of the population currently have 65% low back pain (Bressler et al., 1999). These tend to emphasize the need for the physical therapists to manage the aging spinal cord. The impact of the lower back pain in the older population is very high and has functional limits (Scudds and Robertson, 1998; Weiner et al., 2003; Tong et al., 2007), mental health conditions and depression in the elderly (Weiner et al., 2003; Meyer et al., 2006), and deficits in balancing with associated



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increased fall risk (Yagci et al., 2007). Elderly population must attain significant knowledge, how to change behavioural attitudes and pain management practices on spinal stenosis. Unfortunately, knowledge deficit on spinal stenosis in common population of the study area. Pain management could play an important role on inaccurate assessment and pain management. Sometimes, health professionals or targeted population often underestimates the pain severity. Worldwide, most of the patients are administered with analgesics. Low levels of knowledge and attitudes are alarming the necessity of KAP in the study area. This could be due to educational backgrounds, attitudes and lacking of regular pain management practices. Hence it is necessary to deploy the knowledge on pain management practices. Therefore, study is worthwhile to examine the knowledge and attitudes of elderly population and their pain management practice. The finding the investigation would furnish baseline information for future improvement on pain management in the study area.

METHODOLOGY

A 5-point scale (Likert-type) attitude questionnaire was constructed; a primary pilot study was conducted and tested (Reddy & Buncha, 2017). The data was collected from 56 local respondents in Ongkharak, Nakhon Nayok.

RESULTS AND DISCUSSION

The subjects mean age of the samples was 58 years (SD = 4.06), ranges 49 to 58. Most of them subjects were female (91%), males (9%), Buddhists (97%), others (3%) 93.8 % of the subjects had primary education level, high school (4.2%) and 2% with bachelor degree. Three-fifths of the nurses had previous pain experience. Among the elderly population 68.91% had previous low back pain, 12.09% moderate pain and 19.0% had severe pain. Some of them attended for the pain management program (38.98%), 61.02% of the subjects never attended for the pain management program. The above table elucidates the distribution of respondents based on the knowledge and attitudes towards pain management. Here specific parameter defined as very low, moderate and high. Majority of the respondents expressed their perceptions and behavioural attitudes towards pain management as follows very low (67.86%), moderate 17.86% and high at 14.28% respectively.

The above table explores the distribution of respondents based on the practice towards pain management. Here specific parameter defined as daily once, daily twice, weekly monthly and if I have free time. Majority of the respondents practicing pain management systematically weekly once (33.92%), daily twice 25%, If I have time (16.10%), daily once (14.27), monthly once (10.71). It shows the deficit of systematic practice in spinal stenosis pain management. The above table elucidates the distribution of respondents based on KAP application on practice towards pain management. Here specific parameter defined as daily once, daily twice, weekly monthly and if I have free time. Majority of the respondents practicing pain management systematically daily twice 55.36%, daily once (33.93%), weekly once (10.71), If I have time (0), monthly once (0). It shows the enormous improvement on systematic practice in spinal stenosis pain management. Most of the respondents significantly responded towards the application of KAP on spinal stenosis pain relief program.

Limitations of the Study

Study was executed through questionnaire-based survey could generalize some of the aspects and respondents may change their attitudes after the survey. Majority of the respondents are females, conducted at Ongkharak area. Hence, this could limit the study up to some extent. Study has been made to minimize problem by oral interview with respondents. Thus, further study recommended to initiate with complications in application of KAP on spinal stenosis pain management.





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CONCLUSION

The finding of this study evidenced that elderly population of the study area were had very low level of knowledge and attitudes in spinal stenosis pain management and had moderate level of practices to reduce or manage the spinal stenosis pain. Furtherly, a significant positive response was received from the subjects upon post sessions of KAP on spinal stenosis pain management.

Recommendations

Lack of pain reducing or pain management program may contribute to the very low level of knowledge and attitudes of elderly population in the study area. Therefore, the public health officials need to initiate training programs on back pain and spinal stenosis pain management at targeted population. Although, subjects mentioned about the pain management practices were moderate or negligible. Need to educate and concentrate on behavioural acts of the subjects. Public health care administrators can use the findings of this study to request to policy makers for allocating budgets for training the elderly population at the study are with reference to spinal stenosis pain management in order to enhance the quality of life of the population. A repeated study could be initiated with more male population, urban and rural population.

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Table 1. Distribution of subjects based on the knowledge and attitudes towards pain management

Level of knowledge	Frequency	Percent (%)
Very low (score 1-3)	38	67.86
Moderate (score 4-6)	10	17.86
High (score 7-10)	8	14.28





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Table 2. Distribution of subjects based on the practice towards pain management

Practice frequencies	Frequency	Percent (%)
Daily once	8	14.27
Daily twice	14	25
Weekly	19	33.92
Monthly	6	10.71
If I have free time	9	16.10
	56	100

Table 3. Distribution of subjects based on the posttest of KAP application on practice towards pain management

Practice frequencies	Frequency	Percent (%)
Daily once	19	33.93
Daily twice	31	55.36
Weekly	6	10.71
Monthly	0	0
If I have free time	0	0
	56	100





Genetic Association of Adiponectin Gene Polymorphism rs2241766 (+45T/G) SNP and Dyslipidemia in Coronary Heart Disease Patients

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Received: 17 Jan 2019

Revised: 21 Feb 2019

Accepted: 25 Mar 2019

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ABSTRACT

Background: Adiponectin (ADIPOQ) is a hormone with insulin-sensitizing, anti-inflammatory, and anti-atherosclerotic effects. It is a potent regulator of lipid metabolism and an indicator of metabolic disorders. Adiponectin is encoded by *ADIPOQ* whose polymorphisms are associated with type 2 diabetes mellitus (T2DM), obesity and coronary heart disease. Hyperlipidemia is a major risk factor for many diseases including coronary artery disease (CAD) and arteriosclerosis. **Aim:** To assess the association of rs2241766 SNPs of adiponectin gene with changes of serum lipid profile in the investigated patients. **Methods:** This is a case-control study carried out on 300 individuals. Anthropometric measurements and blood tests were performed and recorded. The levels of cholesterol, triglyceride and HDL were measured and analyzed by enzymatic method. LDL-cholesterol was calculated by indirect method using Friedewald equation. *ADIPOQ* rs2241766 polymorphisms were genotyped by a PCR-RFLP (Polymerase Chain Reaction – Restriction Fragments Length Polymorphism) assay. **Results:** A statistically significant correlation was identified between patients and control group. IHD was more prevalent between 60- 69 year old cases and prevalence of CAD was found higher in males than females. In regard to the lipid profile, patients had significantly higher serum Cholesterol and triglycerides, LDL-C and lower levels of HDL-C compared with the controls. Patients with the rs2241766 GG/GT genotype had significantly lower of HDL-C ($P=0.008$) than TT carriers. However, for patients with the (TG+GG) carriers had a significant decrease HDL-C ($P=0.002$) with significant increases of triglyceride ($P=0.017$) and VLDL-C ($P=0.024$) levels comparison with TT carriers. **Conclusions:** Adiponectin gene polymorphism of rs2241766 is involved in changes of serum lipid concentration.

Key words: Adiponectin, hormone, triglyceride, patients, serum, coronary, heart.



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INTRODUCTION

Adipose tissue has been identified as an active secretion organ that triggers a variety of proteins, collectively called adipokines. Adiponectin (ADIPOQ) is the most abundant adipokine (Pineda-Tenor et al., 2014). It is a peptide hormone with insulin-sensitizing, anti-inflammatory, and anti-atherosclerotic effects (Yamaguchi et al., 2017). The concentration of adiponectin in plasma is 5 to 30 $\mu\text{g} / \text{ml}$ (Sargolzaei et al., 2018), containing approximately 0.01% of all plasma proteins (Cui et al., 2016). Thus it exceeds other adipokines 100 fold and most hormones three fold (Wang et al., 2017). It circulates in humans as a trimer, a hexamer, and a high molecular-weight (HMW) forms (Song et al., 2016). Adiponectin is encoded by *ADIPOQ* which has been mapped on chromosome 3q27 (Liu et al., 2018). In this chromosome region, Genome-wide scans evaluated as a susceptibility locus for type 2 diabetes mellitus (T2DM), obesity and coronary heart disease (Sun et al., 2017). The mechanism of how the polymorphism of adiponectin gene related to CAD risk is still unclear. The adiponectin protein plays an important role in the prevention of cardiovascular disease through the metabolic effects of anti-inflammatory, anti-atherosclerosis (Murphy et al., 2018; Yamaguchi et al., 2017) and by controlling the metabolism of lipid and glucose (Ghadge et al., 2018; Ruan & Dong, 2016).

CHD is a complex chronic disease characterized by pathological changes in both the coronary arteries and the myocardium, and incorporates multiple different phases and clinical syndromes (Dweck et al., 2016). Hyperlipidemia is still a major risk factor for many diseases including coronary artery disease (CAD) and arteriosclerosis (Valensi et al., 2016). HDL-c was found to be positively correlated with adiponectin levels (Lee et al., 2018). On the other hand, Serum triglycerides and apolipoprotein B (ApoB), the main apolipoproteins of the triglyceride rich VLDL negatively associated with adiponectin levels (Liu et al., 2017). Dyslipidemia is a complex metabolic disorder in which genetic and environmental factors interact to produce homeostatic abnormalities (Manning et al., 2012). The abnormal lipid level is usually associated with variations in the structures and functions of genes associated with lipid metabolism. Variations in the composition of the genes may ultimately change their function that affects fat synthesis and metabolism, causing dyslipidemia (Zhang et al., 2012). Several single-nucleotide polymorphisms (SNPs) have been identified in *ADIPOQ* (Luis et al., 2018; Liang et al., 2017). One of the most common polymorphisms in the *ADIPOQ* gene is the 45T> G substitution. The G allele in exon 2 of the *Apm1* gene has been shown to be associated with obesity, adverse lipid profiles and IR (Pineda-Tenor et al., 2014).

MATERIALS AND METHODS

Study design

A case control study was conducted on 300 individuals aged 40-70 years. The subjects included 150 patients diagnosed with CHD at the Heart Disease Center in Najaf and 150 healthy control Subjects. All data was collected from December 2017 to February 2018. Examination of lipid profile is carried out in the laboratory of Biochemistry department, University of Kufa, College of Medicine.

Phenotype measurements

Data was collected on age, gender, height and weight. Body mass index (BMI) was calculated by dividing the weight measured in kg by height per square meter. Obesity was defined as $\text{BMI} \geq 30 \text{ kg/m}^2$. 3 ml of blood was collected in a normal tube without anticoagulants, and then serum tubes were centrifuged at 2000 x C for 10 minutes. Cholesterol, triglycerides and HDL were measured and analyzed enzymatically. LDL-cholesterol was calculated indirectly using the Friedewald equation as the total cholesterol minus HDL minus one-fifth triglycerides level. Final results are reported at mg / dL concentration.



**Zaman Dakhil Kamil et al.****Genotyping of ADIPOQ polymorphisms**

The ADIPOQ 45T>G polymorphism was genotyped by a PCR-RFLP (Polymerase Chain Reaction – Restriction Fragments Length Polymorphism) assay. The polymerase chain reaction (PCR) was performed in a 25 µl reaction mixture containing 12.5 µl GoTaq® Hot start Green Master Mix (Promega), 1 µl of each primer, 3 µl genomic DNA and 7.5 µl nuclease-free water. The Polymorphic fragments of the +45T/G were amplified using specific primers (Sabouri et al., 2011): forward 5'–GAA GTA GAC TCT GCT GAG ATG G–3 and the reverse primer 5'–TAT CAG TGT AGG AGG TCT GTG ATG–3, which resulted in a 372-bp product. The PCR amplification conditions were as follows: initial denaturation at 95°C for 4 minutes, 35 cycles, denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, extending at 72°C for 30 seconds, and final extension at 72°C for 7 minutes. PCR products were digested by SmaI restriction enzyme (10U) at 37°C for 3 hours (Promega) and separated on 2% agarose gel electrophoresis. Thus, after digestion, homozygotes for the wild-type (T) allele present a 367bp fragment, homozygotes for the G allele have 2 fragments of 215bp and 153bp respectively and heterozygotes present all 3 fragments 372, 215 and 153bp fragments.

Statistical analysis

All analyzes were performed using SPSS version 20.0 (SPSS Inc., Chicago, IL). The difference in mean values between patients and control group was tested by t-test of the student. The ANOVA test was used to compare numerical variables across genotypes. The 5% level of the P value was considered significant.

RESULTS**Demographic Characteristics**

Anthropometric measurements of 300 individuals of both genders who participated in this study are shown in Table 1. The age of the participants ranged from 40 to 69 years old, with a mean of 55 (± 12 ; SD) years for patients and 48.16 \pm 7.45 for control. IHD was more prevalent between 60- 69 year old cases Figure 1. According to the results of coronary angiography, 64% of males and 36% of females were diagnosed with CAD. Therefore, the prevalence of CAD was found to be higher in males compared with females Figure 2. There was a significant difference among patient and control group in their age ($p < 0.001$) and BMI ($p < 0.0024$). However, the groups did not differ significantly in their sex ($P=0.087$). In regard to the lipid profile, patients had significantly higher serum Cholesterol ($P < 0.001$) and triglycerides ($P < 0.001$) compared with the controls. However, the IHD patients had significantly higher serum levels of LDL-C ($P < 0.001$) and lower levels of HDL-C ($P=0.0001$) in comparison with the control group (Table 1).

Lipid profiles and the ADIPOQ polymorphism

The frequency of the minor allele G for the ADIPOQ rs2241766 polymorphism in CHD (24.67%) patients was significantly (OR=2.92, CI 95%= 1.73-5.24, $P=0.001$) higher than those of the control group (15.33%). When the co-dominant model was considered for the genotypes of the rs2241766 SNP, a trend of a significant decrease of HDL-c ($P=0.008$) level was clear as the G allele appeared in the three groups, whereas BMI ($P=0.112$), cholesterol ($P=0.703$), triglyceride ($P=0.054$), VLDL-c ($P=0.057$) and LDL-c ($P=0.390$) values did not exhibit significant variation in patients with TT, TG, GG genotypes (Table 2). Results being more evident, when the data were analyzed under the dominant model. Thus, a significant decrease of HDL-c ($P=0.002$) concentration with significant increases of triglyceride ($P=0.017$) and VLDL-c ($P=0.024$) levels, were obtained in carriers (TG+GG) of the G allele in comparison with those of the wild type allele (TT). Other parameters failed to highlight significant variations (Table 3).



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DISCUSSION

This study was conducted to assess the association of rs2241766 SNPs of adiponectin gene with changes of serum lipid profile in the investigated patients. Adiponectin is a potent regulator of lipid metabolism and an indicator of metabolic disorders (Pineda-Tenor et al., 2014). Dyslipidemia is closely related to the occurrence and development of CHD. It has been demonstrated that dyslipidemia could account for around 50% of the population-attributable risk for CHD (Wang et al., 2016). The combination of elevated triglyceride and low HDL-c concentrations, commonly considered as atherogenic dyslipidemia (Valensi et al., 2016). Studies in different populations have shown that genetic variables as well as environmental factors affect blood lipid level; and variations in the AdipoQ gene are among the genetic factors associated with blood lipid levels. High levels of triglycerides in humans have been linked to atherosclerosis, and thus, the risk of heart disease (Joffe et al., 2011; Standl et al., 2012). In the current study, elevated levels of triglyceride, cholesterol and LDL-c, with a decreased concentration of HDL-c were observed in patients with CHD. These results reflect an abnormal lipid profile that remains as a potent risk factor for CHD.

The analysis of serum lipid concentrations in relevance to the genotypes of rs2241766 (T>G) SNP of adiponectin gene under the codominant model exhibited only significant decreases of HDL-c level as the G allele increases among the 3 genotype groups. However, serum lipid concentrations were changed evidently when the analysis was carried out under the dominant model. Hence, a decreased levels of HDL-c with elevated concentrations of triglycerides and VLDL-c were obtained in carriers of the G allele (TG or GG) compared with those of the wild type allele (TT). The picture seemed to be parallel with the dyslipidemia associated with T2DM (Arca et al., 2012). The remarkable observation is the appearance of significant changes of lipid concentrations in the group of patients but not the control group. Such findings solidify our suggestion for the role of the adiponectin +45T/G SNP in the development of CHD, possibly by modulating lipid metabolism through the directing towards decreasing HDL-c concentration. Similar findings were reported by Yan et al. (2013) who showed that carriers of the GG+TG genotypes had lower levels of HDL-c than those of the TT genotypes. Slightly higher plasma triglyceride and insulin levels were reported in the G carriers in European population (Jiet al., 2018). In contrast, the current findings are in agreement with previous study that showed no significant genotype effect of the adiponectin 45T/G polymorphism on cardiovascular risk factors including lipid profile (Lee et al., 2012).

The main mechanism of adiponectin in modulating lipid and glucose metabolisms is the activation of adenosine monophosphate (AMP)-activated protein kinase and stimulation of peroxisome proliferator-activated receptor (PPAR)- α , which leads to increase glucose uptake and oxidation of fatty acids in skeletal muscle as well as decreased hepatic glucose output (Jiet al., 2018). Data of the analysis of BMI values in relevance of the three groups of the genotypes revealed insignificant association of the +45 G/T SNP with the BMI values. The current results are in agreement with several previous studies that illustrated the absence of such association (Du et al., 2012; Ranjith et al., 2011 & Bouatia-Naji et al., 2006). Conversely, other studies have suggested the G allele of the rs2241766 SNP to be associated with a higher risk of obesity (Hsueh et al., 2018; Ranjith et al., 2011; Sutton et al., 2005 & Menzaghi et al., 2002).

CONCLUSIONS

Adiponectin gene polymorphism of rs2241766 is involved in dyslipidemia of patient's with coronary artery disease (CAD).



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Table 1. Biochemical and clinical characteristics of study individuals

Parameters	Control persons (Mean±SD)	IHD patients (Mean± SD)	P value
No(M/F)	150(83/67)	150(98/52)	0.087
Age(y)	48.16±7.45	55±12	< 0.001
BMI(kg/m)	26.63±4.32	28.011± 3.43	0.101
Cholesterol(mg/dl)	180.69± 22.78	193.86± 22.73	< 0.001
triglyceride(mg/dl)	133.11± 23.43	159.16 ± 28.60	< 0.001
VLDL(mg/dl)	26.62± 4.68	31.79± 5.74	< 0.001
LDL(mg/dl)	100.83± 20.09	113.40± 23.96	< 0.001
HDL(mg/dl)	53.24± 10.32	48.67± 8.97	0.0001

Table 2. Characteristics of the carriers of the three genotypes of the IHD patients analyzed under codominant model for rs2241766 (T>G) SNP in adiponectin gene

	TT (N=84) (Mean±SD)	TG (N=58) (Mean±SD)	GG (N=8) (Mean±SD)	P value
BMI(kg/m ²)	27.92± 3.91	28.04± 2.94	28.54± 0.94	0.113
Cholesterol (mg/dl)	192.53±22.60	195.77± 23.29	194.37±17.816	0.704
Triglyceride (mg/dl)	154.86±29.61	166.53± 27.19	162.12±17.80	0.055
VLDL-c (mg/dl)	30.97± 5.92	33.30± 5.43	31.66±4.40	0.058
LDL-c (mg/dl)	111.05±24.74	116.28±22.34	117.33±23.48	0.390
HDL-c (mg/dl)	50.51±8.9	46.03± 8.62	45.37±9.85	0.008





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Table 3.Characteristics of the carriers of the two genotypes of the IHD patients analyzed under dominant model for rs2241766 (T>G) SNP in adiponectin gene

	TT (N=84) (Mean±SD)	TG+GG (N=66) (Mean±SD)	P value
BMI(kg/m ²)	27.49± 3.69	28.53±2.78	0.059
Cholesterol (mg/dl)	192.53±22.60	195.60±22.59	0.410
Triglyceride (mg/dl)	154.86±29.61	166±26.16	0.017
VLDL-c (mg/dl)	30.97± 5.92	33.10±5.32	0.024
LDL-c (mg/dl)	111.05±24.74	116.41±22.29	0.171
HDL-c (mg/dl)	50.51±8.9	45.95±8.70	0.002

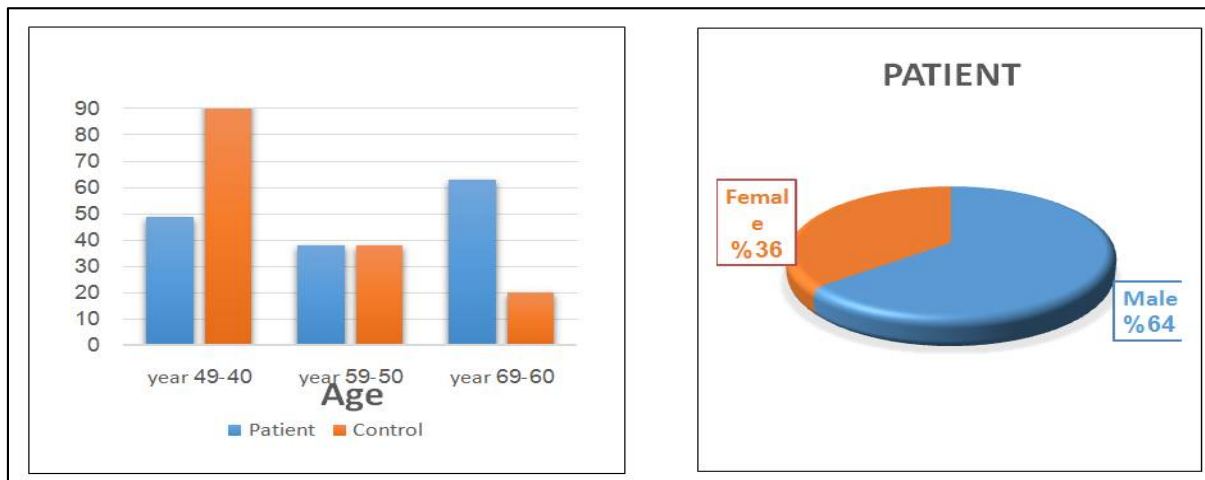


Figure 1. BMI distribution of study individuals

Figure 2. Gender distribution of study individuals





Knowledge Attitude and Practices of Diabetic Patients in Ongkharak, Thailand

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Received: 19 Jan 2019

Revised: 20 Feb 2019

Accepted: 26 Mar 2019

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ABSTRACT

Diabetes, patients diagnosed with progressive long-term conditions should be involved in behavioral change to avoid problems. According to the American Diabetes Association, the objectives of behavioral change are described in diabetes medical care standards: treat the blood sugar better, regular physical exercise, and medical appointments per week by adherence to drugs and diet, self-monitor blood sugar. Fifty-one subjects were selected by using simple random sampling from Ongkharak area. The instruments composed of three parts: Demographic data, knowledge and attitudes of diabetes, management questionnaire, and patients caring behavior questionnaire. The finding of this study provided a concrete evidence on attributes of diabetic population of the study area. Study revealed that subjects had very low level of knowledge and attitudes on diabetes management, walking and exercises. Most of the population has moderate level of practices to manage the diabetes severity on daily basis. A significant positive response was received from the diabetic subjects upon post sessions of KAP on diabetes management.

Keywords: Diabetes, Knowledge and Attitudes, Practice, and Diabetes-Management.

INTRODUCTION

Diabetes, patients diagnosed with progressive long-term conditions should be involved in behavioral change to avoid problems. According to the American Diabetes Association, the objectives of behavioral change are described in diabetes medical care standards: treat the blood sugar better, regular physical exercise, and medical appointments per week by adherence to drugs and diet, self-monitor blood sugar. Promotion of such behavioral changes is based on self-management programs in diabetes care and control. Diabetes self-management programs are shown to



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improve the majority, knowledge, attitude, learning and health care outcomes. However, the causes of the development of health education interventions in literature are not gradually explored, especially to show how diabetes education intervention affects the effects of diabetes. Improved knowledge, attitude, preventative learning and results, and potentially and effectively facilitate academic interventions for patients benefiting from self-preservation management. The review examines multiple causes of variations in clinical or health effects such as glycated Hemoglobin (A1C), Low-Density Lipoprotein Cholesterol (LDLC), Functional Ability (FC), and Poor Perceived Health (PPH).

Knowledge, Attitude and Practice (KAP) about a disease can also significantly affect the behavior and ultimate treatment of health care, such as lifestyle management is a significant component of KAP's of most disease's management on patients. KAP, affected by literacy and awareness levels. Worldwide, the decision of the individual's decision is usually a decision between the patient and well-wishers. In a study from the cancer center in the United States, different steps have been taken to diagnose and diagnose relative families on the treatment of the patient, including treatment, such as the doctor, treatment protocol and care path. The attitude to disease and religious perception is some of the factors that influence a person's KAP. This is a potential source of conflict between different family members and / or patient and his / her families. However, such a family influence on treatment in some parts of the world, including Asia, is even more prominent (Ghannadi *Set al*, 2016; Zhang AY, Siminoff LA2003, Hobbs G*Set al*, 2015). Differences in such KAP cannot affect the relevant patient decisions but a physician can significantly affect treatment decisions. A study in the USA has shown that doctors are often under strain by family views in prescribing certain medications. Therefore, all relevant shareholders, including family members, should take a decision on a particular treatment. Disease caused by cancer, such as cancer therapy, is important because cancer treatment often requires a long time. Unless the family is motivated to continue such extended therapy, many patients are difficult to overcome their own treatment. In India, the attitude of other family members is widely affected by a patient's treatment decision. A negative and unlawful attitude can spoil the proper care path, a good family attitude can encourage faster healing through various means. The importance of family in influencing clinical care in Indian scenario is shown to diseases such as HIV, eating disorders and alcohol abuse. Such a holistic approach is often called family therapy (Hobbs G*Set al*, 2015; Franz CE*et al*, 2007).

Worldwide, Low levels of knowledge and attitudes are alarming the necessity of KAP on diabetes in the study area. This could be due to educational backgrounds, attitudes and lacking of precautionary practices. Hence it is necessary to instill the knowledge on diabetic management. Therefore, study is worthwhile to examine the knowledge and attitudes of diabetic population. Investigation could provide baseline information on diabetic knowledge of the patients.

METHODOLOGY

A 5-point scale (Likert-type) attitude questionnaire was constructed, a primary pilot study was conducted and tested (Reddy & Buncha, 2017). The data was collected from 51 local respondents in Ongkharak, Nakhon Nayok.

RESULTS AND DISCUSSION

The subjects mean age of the samples was 55 years (SD = 3.90), ranges 50 to 58. Out of fifty-one 38 were female (74.5%), males (25.5%), Buddhists (91%), others (9 %), 76.45 % of the subjects had primary education level, high school (21.55%) and 2% with bachelor degree. Among the diabetic population 67.91% are suffering with diabetes in the past 10 years, 13.09% between 5-9 years 19.0% less than 5 years. Some of them attended for the diabetic management program (27.51%), 71.49 % of the diabetic population had never attended for the diabetic management program.



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The above table describes the distribution of subjects based on the knowledge and attitudes towards diabetes management. Here specific parameter defined as very low, moderate and high. Majority of the respondents expressed their perceptions and behavioural attitudes towards diabetes management as very low (60.79%), moderate (29.41 %) and high at (9.80 %) respectively. The above table elaborates the distribution of respondents based on the exercises and walking practice as part of diabetes management. Here specific parameter defined as daily once, daily twice, weekly monthly and never. Majority of the respondents practicing systematically weekly once (31.37%), never 27.45%, daily twice (17.65%), daily once (13.73%), monthly once (9/80%). Results of the study shows and evidenced that daily walking and exercises are lacking as a part of their diabetes management.

The above table elucidates the distribution of respondents based on KAP application on practice towards diabetes management. Here specific parameter defined as daily once, daily twice, weekly monthly and never. Majority of the respondents started practicing exercises and walking as a part of diabetic control management daily once 62.75%, daily twice (29.41%), weekly once (7.84), never (0), monthly once (0). After the KAP post session most of the diabetic patients started practicing the exercises and walking. Thus, KAP implementation has the significant influence on the diabetic patient's behaviour changes at study area. Subjects showed significant response towards the application of KAP on diabetic management

Limitations of the Study

Study was executed through survey questionnaire. Thus, it could be possible of generalizes some aspects and respondents may change their opinion after the survey. Extracted the information from Ongkharak area. Hence, this could limit the study up to some extent. Study have been made to minimize problem by oral interview with respondents.

CONCLUSION

The finding of this study provided a concrete evidence on attributes of diabetic population of the study area. Study revealed that subjects had very low level of knowledge and attitudes on diabetes management, walking and exercises. Most of the population has moderate level of practices to manage the diabetes severity on daily basis. A significant positive response was received from the diabetic subjects upon post sessions of KAP on diabetes management.

Recommendations

The public health officials need to initiate training programs on diabetes management in targeted population. Need to educate continuously and surveillance on behavioural aspects of subjects. Administrators can use the study findings to include in public health policies and as an evidence to policy makers.

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Table 1. Distribution of subjects based on the knowledge and attitudes diabetes severity

Level of knowledge	Frequency	Percent (%)
Very low (score 1-3)	31	60.79
Moderate (score 4-6)	15	29.41
High (score 7-10)	5	9.80
	51	100

Table 2. Distribution of subjects based on the exercise, walking practices

Practice frequencies	Frequency	Percent (%)
Daily once	7	13.73
Daily twice	9	17.65
Weekly	16	31.37
Monthly	5	9.80
Never	14	27.45
	51	100

Table 3 Distribution of subjects based on the posttest of KAP application on exercise and walking practices as a part of diabetes management.

Practice frequencies	Frequency	Percent (%)
Daily once	32	62.75
Daily twice	15	29.41
Weekly	4	7.84
Monthly	0	0
Never	0	0
	56	100





Profile of Diabetes Mellitus Type One in Pediatrics, the Experience of a Single Institute

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Received: 21 Jan 2019

Revised: 23 Feb 2019

Accepted: 26 Mar 2019

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ABSTRACT

Background: The two major forms of diabetes are type 1, previously called insulin-dependent diabetes mellitus (IDDM) or juvenile-onset diabetes, and type 2, formerly called non-insulin-dependent diabetes mellitus (NIDDM) or maturity-onset diabetes. **Materials and Methods:** Cross section study of 50 patients with T1DM information (sex /symptom /family HX/ height /weight /investigation) and all information was obtained from medical file of the patients in AL-zahraa Teaching Hospital, the centre of diabetes. **Results:** By reviewing the data of the patients, 30 female (60%) and 20 male (40%), the mean age of diagnosis ten years, most common presenting symptom polyurea, polydipsia and weight loss, 38% has a positive family history, 36% have positive antigliadin Ab. **Conclusion** Most of the patient presented with polyurea, weight loss, polydipsia, increased appetite, abdominal pain, headache, family history present among one-third of the patient.

Keywords: Diabetes mellitus, children, Iraq.

INTRODUCTION

Diabetes mellitus type 1 (also known as type 1 diabetes) is a form of diabetes mellitus in which not enough insulin is produced [4]. This results in high blood sugar levels in the body [1]. The classic symptoms are frequent urination, increased thirst, increased hunger, and weight loss [4]. Additional symptoms may include blurry vision, feeling tired, and poor healing [2]. Symptoms typically develop over a short period [1]. The cause of type 1 diabetes is unknown [4]. However, it is believed to involve a combination of genetic and environmental factors [1]. Risk factors include having a family member with the condition [5]. The underlying mechanism involves an autoimmune



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destruction of the insulin-producing beta cells in the pancreas [2]. Diabetes is diagnosed by testing the level of sugar or A1C in the blood [5][6]. Type 1 diabetes can be distinguished from type 2 by testing for the presence of auto antibodies [5]. There is no known way to prevent type 1 diabetes [4]. Treatment with insulin is typically required for survival [1]. Insulin therapy is usually given by injection just under the skin but can also be delivered by an insulin pump [7]. A diabetic diet and exercise are an important part of management [2]. Untreated, diabetes can cause many complications [4]. Complications of relatively rapid onset include diabetic ketoacidosis and nonketotic hyperosmolar coma [5]. Long-term complications include heart disease, stroke, kidney failure, foot ulcers and damage to the eyes [4]. Furthermore, complications may arise from low blood sugar caused by excessive dosing of insulin.[5] Type 1 diabetes makes up an estimated 5–10% of all diabetes cases [8]. The number of people affected globally is unknown, although it was estimated that about 80,000 children develop the disease each year [5]. Within the United States, the number of people affected was estimated at one to three million [5][9]. Rates of disease vary widely with approximately one new case per 100,000 per year in East Asia and Latin America and around 30 new cases per 100,000 per year in Scandinavia and Kuwait [10] [11]. It typically begins in children and young adults [1].

MATERIALS AND METHODS

Cross section study of 50 patients with diabetes, 30 female, 20 male, age 2-17 years, information (sex /symptom / family HX/ height /weight /investigation) and all information were obtained from medical file of the patients in AL-zahraa Teaching Hospital, a centre of diabetes. The researcher designed a questionnaire. Consent was taken from the college of medicine, Wasit University. Data were entered into Statistical Package for Social science (SPSS) program version 19 for Windows 7. Quantitative variables were summarised by finding mean and qualitative variables were summarised by finding frequencies and percentages.

RESULTS

Demographic data of the patients shown in table no 1 to 6. One regarding age, sex, and clinical presentation

DISCUSSION

Most of the patient presented with polyuria 94%, weight loss 88% polydipsia 82%, increase appetite 50%, abdominal pain 44%, headache 40%. Also, this can be explained [10]. In the absence of adequate insulin secretion, persistent partial hepatic oxidation of fatty acids to ketone bodies occurs. Two of these three ketone bodies are organic acids and lead to metabolic acidosis with an elevated anion gap. Lactic acid may contribute to the acidosis when severe dehydration results in decreased tissue perfusion. Hyperglycemia causes an osmotic diuresis that is initially compensated for by increased fluid intake. As the hyperglycemia and diuresis worsen, most patients are unable to maintain the large fluid intake, and dehydration occurs. Vomiting [11] as a result of increasing acidosis, and increased insensible water losses caused by tachypnea can worsen the dehydration. Electrolyte abnormalities occur through a loss of electrolytes in the urine and transmembrane alterations resulting from acidosis. As hydrogen ions accumulate as a result of ketoacidosis, intracellular potassium was exchanged for hydrogen ions.

Serum concentrations of potassium increase initially with acidosis then decrease as the kidney clears serum potassium. Depending on the duration of ketoacidosis, serum potassium concentrations at diagnosis may be increased, normal, or decreased, but intracellular potassium concentrations were depleted. A decreased serum potassium concentration is an ominous sign of total body potassium depletion. Phosphate depletion also can occur as a result of the increased renal phosphate excretion required for elimination of excess hydrogen ions [12]. Sodium depletion is also common ketoacidosis, resulting from renal losses of sodium caused by osmotic diuresis and from gastrointestinal losses from vomiting. Patients with DKA present initially with polyuria, polydipsia, nausea, and vomiting. Abdominal pain occurs frequently and can mimic an acute abdomen. The abdomen may be tender from



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vomiting or distended secondary to paralytic ileus. The presence of polyuria, despite a state of clinical dehydration, indicates osmotic diuresis and differentiates patients with DKA from patients with gastroenteritis or other gastrointestinal disorders. Respiratory compensation for acidosis results in tachypnea with deep (Küssmaul) respiration. The fruity odour of acetone frequently can be detected on the patient's breath. An altered mental status can occur, ranging from disorientation to coma. Laboratory studies reveal hyperglycemia (serum glucose concentrations ranging from 200 mg/dL to >1000 mg/dL). Arterial pH is below 7.30, and the serum bicarbonate concentration is less than 15 mEq/L. Serum sodium concentrations may be elevated, normal, or low, depending on the balance of sodium and free water losses. The measured serum sodium concentration is artificially low, however, because of hyperglycemia. Hyperlipidemia also contributes to the decrease in measured serum sodium. The level of blood urea nitrogen (BUN) can be elevated with prerenal azotemia secondary to dehydration. The white blood cell count is usually elevated and can be left-shifted without implying the presence of infection. Fever is unusual and should prompt a search for infectious sources that may have triggered the episode of DKA.

CONCLUSION

Most of the patient presented with polyuria 94%, weight loss 88% polydipsia 82%, increase appetite 50%, abdominal pain 44%, headache 40%. Associated with a positive family history in about 38% (19). Hypothyroidism found only in 4% (2). Antigliadin AB (association with celiac disease) positive in 36% (18) of the patient.

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Table 1. Demographic Data

Variables	Item	No	Percentage
Age	<10 years	21	43%
	>10years	29	57%
Sex	female	30	60%
	male	20	40%
appetite	increase	25	50%
	normal	25	50%
Weight loss	Yes	44	88%
	No	6	12%
Abdominal pain	Yes	22	44%
	No	28	56%
headache	Yes	20	40%
	No	30	60%
polyurea	Yes	47	94%
	No	3	6%
Polydipsia	Yes	41	82%
	No	9	18%
Family history	Yes	19	38%
	No	31	62%
Antigliadin AB	Yes	18	36%
	No	32	64%

From 50 patient who has been diagnosed to have T1DM (19) had a positive family history, and from (19) patient with a positive family history (10) were female & (9) were male.

Table 2. The relation between sex and family history

Sex	Family history		P value
	Positive	Negative	
female	10	20	0.7
male	9	11	

From 50 patient who has been diagnosed to have T1DM (18) had the positive antigliadin antibody, and from (18) patient with positive antigliadin AB (12) were female & (6) were male.

Table 3. Relations between sex and antigliadin AB

Sex	Antigliadin AB		P value
	Positive	Negative	
female	12	18	0.4
male	6	14	

From 50 patient who has been diagnosed to have T1DM (21) had early onset before ten years old (12 were female, and 9 were male) & (29) had started after ten years (18 were female, and 11 were male).





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Table 4. Relation between sex & on set

Sex	Early on set <10 years	On set > 10 years	P value
female	12	18	0.8
male	9	11	

From 50 patient who have been diagnosed to have T1DM, (8) were below six years old (4 of them had HBA1C >7.5% & 4 had HBA1C <7.5%) (30) were between 6-12 years old (21 of them had HBA1C >7.5% & 9 had HBA1C <7.5%). (12) Were between 13-18 years old (7 of them had HBA1C >7.5 % & 5 had HBA1C < 7.5%).

Table 5. Age & HBA1C value.

Age	Patient no.	HBA1C		P value
		>7.5 %	<7.5 %	
< 6 years	8	4	4	0.8
6-12 years	30	21	9	
13-18 years	12	7	5	

From 50 patient who was diagnosed to have T1DM, Family history was positive among (19) of the patient, among them (12) had HBA1C >7.5% & (7) had HBA1C <7.5%. Family history was adverse among (31) of the patient, among them (20) had HBA1C >7.5% & (11) had HBA1C <7.5 %.

Table 6. HBA1C and family history

HBA1C	Family history		P value
	Patient with real history (19)	Patient with a negative history (31)	
>7.5%	12	20	0.4
<7.5%	7	11	

