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CONTENTS

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EDITORIAL
MEDICAL SCIENCE LANGUAGES ACROSS HUMAN HISTORY

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The Edwin Smith Papyrus is written in *ancient Egyptian language* around 1,600 BC. The earliest foundations of Ayurveda medicine is written in *Sanskrit* dating from about 600 BC. The foundational text of *Chinese* medicine is the *Huangdi neijing*, (Yellow Emperor's Inner Canon), written 5th century to 3rd century BC. The oldest written sources of western medicine are the Hippocratic writings from the 5th and 4th centuries BC written in *Greek*. During the Middle Ages (800–1,500 AD), *Arabic* was the language of medicine in most parts of the world. Scholars from different parts of the world were gathered in '*Bait ul Hikma*' at Baghdad. They translated scientific works from Greek, Syriac, Pahlavi, and Sanskrit into Arabic. After Renaissance (14th AD), in Europe, both Greek and Arabic works were translated into Latin. *Latin* supplanted Greek as the preeminent medical language and remained so until the early 1800s. Then followed the era of the national medical languages. Since the 1950s, *English* has been the principal language of the medical profession. The global language of medicine in the 21st century is English.

Keywords: Ancient Egyptian language, Sanskrit, Chinese, Greek, Latin, Arabic, Medical English

The Edwin Smith Papyrus is one of the oldest surviving texts of medical literature (medical papyri) and is the world's oldest known surgical document. The Edwin Smith (Surgical) Papyrus is written around 1,600 BC in the hieratic script of the ancient Egyptian language. It is traced to about the 16th to 17th century BC but actually it is the only surviving copy of part of a much older treatise on trauma surgery from about 3,000 to 2,500 BC.[1] The Atharvaveda, a sacred text of Hinduism dating from the Early Iron Age, is one of the first Indian texts dealing with medicine. The Atharvaveda also contains prescriptions of herbs for various ailments dating from about 600 BC. The foundational text of Chinese medicine is the Huangdi neijing, (Yellow Emperor's Inner Canon), was written during 5th century to 3rd century BC.[2]

The oldest written sources of western medicine are the Hippocratic writings from the 5th and 4th centuries BC. Catarrh (downflow), diarrhoea (throughflow), dyspnoea (bad breathing) and melancholic (pertaining to black bile) are the remnants of Greek legacy. At the beginning of the first century AD, Aulus Cornelius Celsus wrote *De Medicina* in Latin. He imported a few Greek terms directly and he Latinized other Greek words, writing them with Latin letters. So, in his and other Latin texts some of these words are the original Greek ones, while others are Latin equivalents introduced by Celsus and his successors.[3]

The Early Middle Ages or Middle Ages in Western Europe is the period of history after the fall of the Western Roman Empire which is called the Dark Ages (the period between about 500 and 1,000 AD). That was marked by economic, intellectual, and cultural decline in the western Europe –a period of intellectual darkness and barbarity.[4,5] During this period, world saw a new phenomenon –the Islamic Golden Age (800–1,550 AD) during which Islamic Empire grew and *Arabic* became the language of medicine in major parts of the world. Many Islamic physicians made outstanding discoveries in all aspects of medicine by building upon the knowledge of Galen and the Greek. They also added their own discoveries. They wrote their thousand pages' books in *Arabic*. They also translated scientific works from Greek, Syriac (the language of eastern Christian scholars), Pahlavi (the scholarly language of pre-Islamic Iran), and Sanskrit into Arabic. Scholars from different parts of the world were gathered in '*Bait ul Hikma*' بيت الحكمہ at Baghdad which was established in 830 AD. The most notable Islamic scholar in the history of medicine was al-Razi. Al-Razi, known to the Europeans as Rhazes (may be spelt Rhases, Rasis, Rasi or ar-Razi) (850–923 AD), was at the forefront of Islamic research into medicine. A prolific writer, he produced over 200 books. Rhazes was also famous for his work on refining the scientific method and promoting experimentation and observation. His most famous achievement, when asked where to select a location to build a hospital in Baghdad, was to hang meat in locations around the city, and select the spot where the meat rotted the least. Al-Razi wrote extensively on the crucial relationship between doctor and patient, believing that they should develop a relationship built upon trust and, as the doctor had a duty to help the patient, the patient had the duty to follow the doctor's advice. Al-Razi wrote extensively about human physiology and understood how the brain and nervous system operated muscles.[6]

After Renaissance (French= 'Rebirth' —the period of new growth of interest and activity in the areas of art, literature, and ideas in Europe during the 15th and 16th centuries)[7], when Greek was no longer widely

understood, both *Greek* and *Arabic* works were translated into Latin, and the era of medical Latin began. Medical Latin continued to be ordinary Latin with the admixture of numerous Greek and Latin medical terms. In Britain, William Heberden's Commentarii was probably the last notable medical work to be written in Latin. It appeared in 1802 and Dr Johnson referred to the author as ultimus Romanorum (the last of the Romans). In other countries medical Latin survived a little longer: in Denmark, hospital doctors wrote patients' notes in Latin until 1853. Then followed the era of the national medical languages, such as medical English, medical *French*, medical *German*, medical *Italian* and many others. A few of these, especially French, German and English, replaced Latin as vehicles for international communication, but most of the others were only used nationally.[3] Today, all the most influential medical journals are written in *English*, and English has become the language of choice at international conferences. We have entered the era of medical English. Whereas in former times new medical terms were derived from classical Greek or Latin roots, now they are often, partly or wholly, composed of words borrowed from ordinary English, e.g., bypass operation, clearance, base excess, screening, scanning and doctors from non-English-speaking countries now have the choice between importing these English terms directly and translating them into their own language. English acronyms such as AIDS, CT, MRI and PCR are widely accepted and have almost become a noun in their own right.

Greek was the chief language of medicine until around 300 AD when Roman scientists began replacing it with Latin. As the Roman Empire grew, Latin supplanted Greek as the pre-eminent medical language and remained so until the early 1800s. Since the 1950s, English has been the principal language of the medical profession. The global language of medicine in the 21st century is English. It is used by contemporary physicians in the same manner that Greek and Latin was used in earlier times.[8]

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ORIGINAL ARTICLE
NORMATIVE REFERENCE STANDARD FOR CORE MUSCULAR ENDURANCE OF ADOLESCENT 12–16 YEARS FROM SOUTH PUNJAB, PAKISTAN: A CROSS-SECTIONAL STUDY

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Background: Core muscular endurance has been a part of the investigation for several decades in clinical and non-clinical settings. Current research had three objectives as; firstly, the prevalence estimation of muscular endurance; secondly, to provide the normative reference standards of plank test by Lambda (λ), Mu (μ) and Sigma (σ) 'LMS' technique and lastly, to provide the status of healthy and un-healthy zones of aged 12 to 16 years old adolescents of South Punjab for the very first time. **Methods:** This study is a cross-sectional descriptive research design with a total of 2970 students aged 12–16 contributing almost equally in numbers from 60 public high schools. Muscular endurance was estimated from Plank to Fatigue (PTF) test. LMS method/technique was deployed to acquire reference norms for muscular endurance by the plank test. **Results:** The plank endurance test calculated age and gender-specific smoothed percentile curves (5th, 20th, 40th, 50th, 60th, 80th, and 95th). Overall, results reflected that boys were found higher in average plank values than their counterparts; meanwhile, the international comparison showed that current outcomes are similar to the preceding studies. The result showed that 60.1% of the population is falling in the poor, medium and very poor categories. **Conclusion:** The present study exhibited the earliest age-gender specific plank endurance test normative reference values, percentile curves and health-benefit zones of 12 to 16 years old adolescents of South Punjab Pakistan.
Keywords: Human health, Core muscular endurance, Normative reference standard, Percentile curves, Health-related physical fitness

INTRODUCTION

Researchers, scientists, coaches, athletes, and practitioners have intensively scrutinized core muscle endurance in recent years. The abdominal "core" provides all appendicular body movements and stabilizes the lower limb, upper limb, abdominal structure, hips, and spine.[1] Without any muscle attachment bare spine cannot bear the compressive load.[2,3] According to Santos MS (2019) definition of Core muscular endurance has been derived as the ability of the core or trunk muscles to produce and maintain force against a specific position for a maximum period of time.[4] Particularly, core muscular (trunk and hip muscles) endurance is essential for exercise and sports training because the core plays a vital role in transferring power to the appendicular skeleton. Core endurance or core stability is often tested in several capacities, for instance, pre-screening for risk of injury assessment, exercise or rehabilitation prescription or prevalence estimation health-related physical.[5,6] Musculature traversing and subsidizing these regions is related with a core comprising internal obliques, external obliques, transverse abdominis and rectus abdominis. Moreover, the latissimus dorsi, pectorals, hamstrings, quadriceps, iliopsoas, trapezius, hip rotators, and glutei structure core muscles.[7] Every segment of the human body is

vital in accordance with its functioning and capacity, but the core has the most imperative position because not only does exercise or intense sports require core strength, but one's daily living is impossible without core stability.

Currently, measuring abdominal endurance and strength or assessing core stability isokinetic testing is considered the most advanced and standardized way to obtain reliable and accurate outcomes.[8] Though, isokinetic testing is expensive as well as entails large machines, specific physical environment (labs) and specialized technicians, which is good in clinical settings but very impractical for the non-clinical settings.[9]

Muscular assessment is usually conducted in two ways: one is muscular endurance, and the other is muscular strength.[10] Muscular endurance is determined by the ability of a muscle to sustain a certain level of force consistently for a maximum period of time, whereas muscle strength is explained as the maximum force generated by a muscle or group of muscles against a stimulus at once a short period of time.[10]

Additionally, literature has already recommended that operationally muscular endurance is more important in aiding core musculature than muscle strength, so it is more obvious to stay focused on

muscular endurance.[11] In the past, to enhance the strength level of an athlete, to acknowledge the abdominal endurance or performance assessment, non-clinically sit-ups and curl-ups have been utilized by researchers, trainers and coaches. However, literature has also indicated that sit-ups and curl-ups might predict muscular strength or power, but for muscular endurance, these tests are less indicative.[11] Restrained feet require hip flexor to get activated while performing sit-ups which ultimately assist in sit-up motion. There are quite a lot of apprehensions regarding sit-up test gesticulation, which involves activation of hip flexor and the alternative lumbar flexion patterns with hyper lordosis, which causes extra pressure on lumber region discs. Additionally, it is also hypothesized that sit-up movement increases the injury risk.[11] While regarding administration, cost and time effectiveness sit-up and curl-up, both tests are not easy to administrate and effective in terms of money and time. These tests include a high level of supervision and training for the administrators as well as test-takers to maintain reliability and validity.[12] Though both sit-ups and curl-ups are widely used among physical educators and coaches; there is still inadequate data or reference values published on these tests.

Therefore, aiming to assess the muscular endurance literature has a penalty of data on early described drawbacks of the isokinetic testing or core endurance assessment from sit-ups and curl-ups.[13] Regrettably, the concerns (administrative training, cost-effectiveness, time-effective, physical space effective, risk of injury, reliability and validity) as well as criticism regarding sit-ups and curl-ups mentioned and described previously there is a dire need to find out other reliable tests which could address the issues positively to assess the core muscular endurance specifically.[11] Conclusively, going through the literature, the plank test is not as difficult to administrate, a reliable tool to assess core muscular endurance specifically, cost and time effective, and has less risk of injury among all available tools.[14] So, the current study adopted an unlimited time plank or plank to fatigue (PTF) test to measure the core muscular endurance. To achieve the study's objective, a plank test was applied on the 12 to 16 years old adolescents of South Punjab to assess core muscular endurance and further construction of age-gender specific normative reference standards by LMS technique school-aged adolescents.

METHODOLOGY

A cross-sectional study design was conducted to obtain the study's objectives, followed by stratified random sampling. South Punjab was selected as strata, out of which three strums were created as Multan, Bahawalpur, DG Khan. The main strata (South Punjab) hold 485 public high schools, out of which 12% (60 schools approx.) were selected randomly. The further selection of the schools was made among each strum equally as 20 public high schools from Multan, Bahawalpur and DG Khan.

The preliminary study of plank testing on school-going adolescents aged 12 to 16 from South Punjab represented the study as the sample. As suggested by the current literature study used the same equation for the acquisition of sampling size.[15] According to sample size calculator equation with values of P= 0.4 (40%), Q= 0.6 (1-P), Z-Score= 1.96 at 5% significance level, e= 0.0175 level of precision and D= 1 (D is the design effect) resulted 3010 sample size to achieve the research objectives. Hence, 3012 male and female adolescents were included with equal representation. Meanwhile, the 3012 adolescents were measured at that time, but forty-one participants were excluded due to outliers.[16]

The research design and methodology were prior approved by the research ethics committee of the faculty of sports science, school of kinesiology, Shanghai University of Sport (SUS), Shanghai, China. Written and oral consent was obtained before testing from administration, parents, and participants; hence, the participants were allowed to withdraw any time from the study. It was confirmed that all the participants were physically and mentally healthy with no previous record of having injury; additionally, no monetary compensation will be provided. Only the volunteered participants were included in the process of research. Trained research assistants from Bahauddin Zakariya University, South Punjab, Pakistan, were assistants for data collection under the supervision of a senior member of the team.

The anthropometric characteristics, i.e., height, weight, and BMI, were calculated by following international protocols of the Center for Disease Control, CDC, USA, 2012.[17] A stopwatch and a standardized gym mat were utilized as equipment necessary for the test conduction. Plank is an isometric exercise test conducted to assess core muscular endurance. Plank test protocol requires the participants to hold or maintain the static horizontal position as long as the participant can without being fatigued or injured easily. The prone static position was followed while measuring the core endurance as the participant was lying down on the yoga mat with both feet together with curled toes touching the mat. At the same time, the upper body was attached to the floor by elbows and forearm, mainly maintaining the distance between both arms just the same as the distance between shoulders, and hands separate against the floor mat and holding this position for as long as the participant can make it possible. Participants were further asked to maintain eye contact towards the mat floor, maintaining a neutral spine forming a perpendicular line to the parallel

smooth, straight under the shoulders. The plank position should be like a straight line from ankle to head. Every participant was provided with a chance of a 5 to 10 sec practice trial. Upon participant's discretion, the examiner provided a full set of instructions throughout the test and measurement procedure to better understand adolescents. An adequate time for rest was given to the participants between the practice trial and the actual test. The test was terminated, and time was recorded when the participant seemed fatigued or unable to uphold appropriate position or participant informed any illness or examiner observed any sign of illness.[18]

The current investigation applied quantitative research design and analysis techniques. For normality check of the data, Kolmogorov-Smirnov test was applied whereas, for uncovering the age-gender specific mean differences Mann-Whitney U-test a non-parametric test was adopted. Researcher for outlier's identification in anthropometric variables and plank test agreed ±5 Z-scores cut-off points.[19] Finally, 2970 sample sizes instead of 3012 adolescents were further analyzed after removing outliers. Acquisition of said purpose for descriptive and inferential statistics, percentages, frequencies, and mean and standard deviation of variables were formulated. The LMS method was applied and generated in R statistical software version 3.0.2.[20] To obtain the normative reference values and Lambda (λ), Mu (μ) and Sigma (σ) LMS curves of selected anthropometric and health-related physical fitness indicator core muscular endurance (plank to fatigue test) for the adolescent's population; for the sake of statistical analysis researcher adopted SPSS-21.

RESULTS

Table-1 represents the gender-specific average comparison of anthropometric characteristics. Results revealed that boys' mean values of anthropometric were relatively higher than girls. The average height (cm), weight (kg), body mass index (BMI), plank to fatigue test (PFT) of boys and girls was 160.50±11.50 and 158.57±9.34, 45.02±9.78 and 41.00±7.89, 17.30±2.41 and 16.29±2.82, 80.72±73.48 and 69.07±58.16 respectively. While making a comparison between gender and anthropometric characteristics, outcomes were significant ($p<0.05$) with the exception of age.

Table-2 and Figure-1 represent the normative reference values and percentile curves for the plank to fatigue (PTF) adolescents of South Punjab. The median (P50th) centiles for plank, the rise of 1.54 seconds in boys and 22.22 sec. in girls, was observed from 12 to 16 years of age. At the same time, the annual raise of plank seconds throughout the 12 to 16 years was detected 1 sec in boys and 3–8 sec in girls approximately. Overall the gender-specific comparison showed that the plank

values of boys were higher than their counterparts except for 15 and 16 years old girls.

Figure-2 shows the comparison of p50th centiles for both genders with available research evidence found in the USA.[21] while comparison showed that 12–14 years old adolescents showed rising trend of plank values similar to the US boys and girls who also showed rising curves with some exception in US girls.[21]

Figure-3 represents the age and gender-specific plank estimate for the South Punjab school aged 12 to 16. The estimate presented the percentage of adolescents lying in each of the five categories ascribed as 21.72% were 'very poor', 17.34% were 'poor', 20.98% were 'medium', 17.47% were 'good' and 22.49% were 'very good'. Whereas, 'very poor' category represented 21.1% of boys and 22.3% of girls; 'poor' category represented 18% of boys and 16.7% of girls; 'medium' category represented that 19.4% of boys and 22.5% of girls; 'good' category represented that 21.3% of boys and 13.7% of girls; 'very good' category represented that 20% of boys and 25% of girls. At the age of 16, both girls' and boys' maximum percentages were found in the 'very poor' category as 37.67% and 24.75%, respectively. The maximum percentage in the 'poor' category was found as 22.48% boys at 15 years of age and 28.33% girls at 16 years of age. The maximum percentage in the 'medium' category was found as 25.76% boys at 16 years of age and 28.04% girls at 14 years of age. At 13 years, both girls' and boys' maximum percentages were found in the 'good' category as 25.42% and 28.04%, respectively. The maximum percentage in the 'very good' category was found as 31% boys at 14 years of age and 48% girls at 12 years of age.

Table-1: Anthropometric descriptive analysis gender specific

Component	Total (n=2970)	Boys (n=1477)	Girls (n=1493)	p
Age (Year)	14.00±1.41	14.01±1.41	14.00±1.42	0.927
Height (Cm)	159.53±10.51	160.50±11.50	158.57±9.34	<0.001
Weight (Kg)	43.00±9.10	45.02±9.78	41.00±7.89	<0.001
BMI (Kg/m²)	16.80±2.67	17.30±2.41	16.29±2.82	<0.001
PE (Sec)	74.87±66.46	80.72±73.48	69.07±58.16	<0.05

Note: The data were presented as Mean±SD.
BMI: Body mass index; PE (Sec): Plank Exercise (Seconds)

Table-2: Plank Exercise (seconds) percentile by age and gender in adolescents from South Punjab

Age	n	M	P 5	P 20	P 40	P 50	P 60	P 80	P 95
					Boys				
12	291	55.96	10.09	23.99	43.67	55.96	70.25	112.9	203.2
13	295	56.35	10.58	24.34	43.83	56.35	70.75	113.8	218.3
14	298	56.73	11.32	25.86	44.84	56.73	70.80	116.3	220.9
15	298	57.11	15.43	28.74	46.17	57.11	70.85	117.7	231.6
16	295	57.50	15.94	29.45	46.79	57.50	73.60	132.2	288.1
					Girls				
12	299	43.76	9.49	21.85	36.82	43.76	50.81	68.76	97.3
13	298	45.40	11.41	24.41	37.24	45.40	55.40	84.09	138.7
14	296	50.59	13.21	25.16	40.81	50.59	62.77	102.8	201.5
15	300	57.72	18.83	31.36	47.51	57.72	70.67	115.6	244.6
16	300	65.98	20.30	34.93	53.94	65.98	81.23	133.8	280.5

Note: n= Number of the participant; M= Median
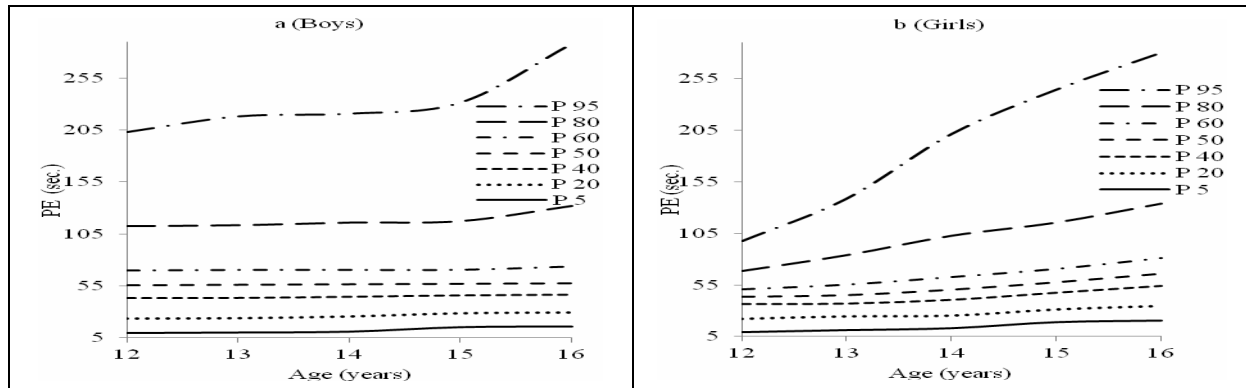
Figure-1: Plank Exercise (seconds) Smoothed percentile curves for South Punjab
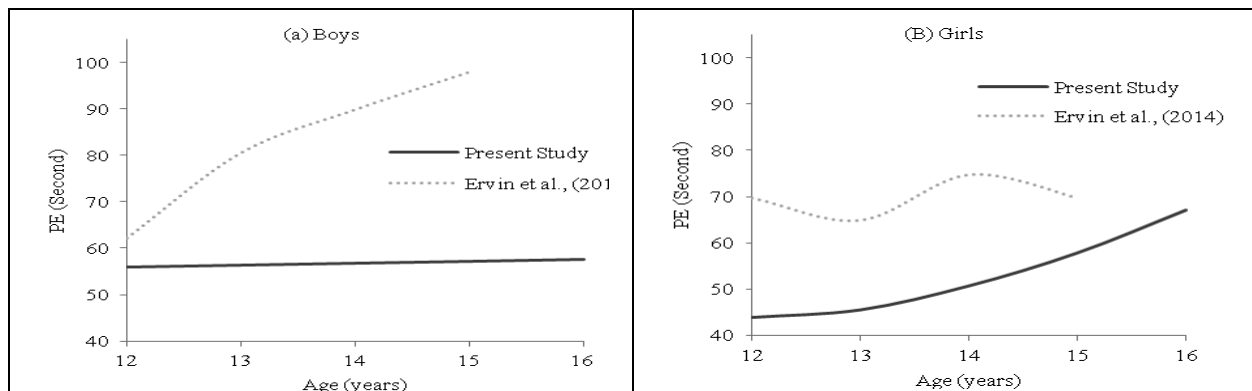
Figure-2: PE Comparison of P50 between present studies and other published studies
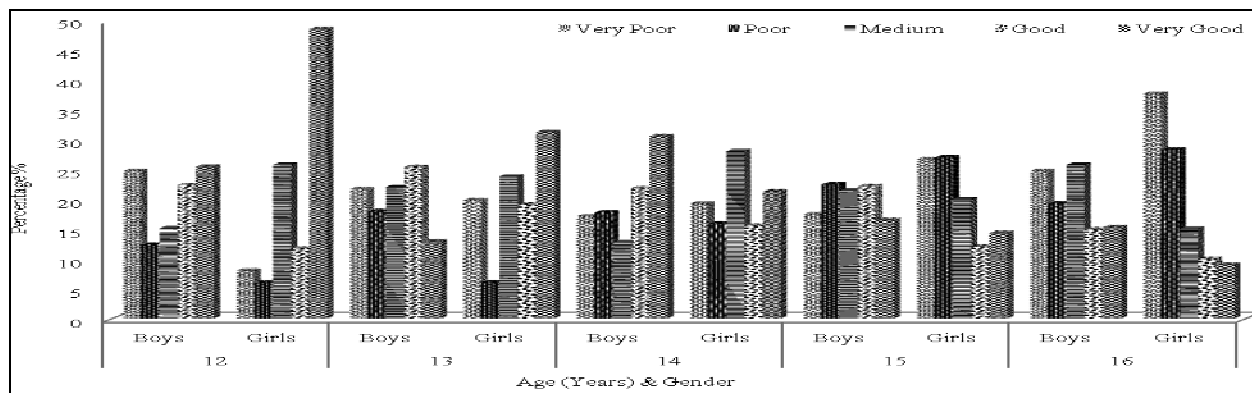
Figure-3: Age and gender-specific PE estimate

DISCUSSION

The current study is the pioneer study in Pakistan, representing the South Punjab school aged 12 to 16 years adolescents providing the average values of anthropometric characteristics and the normative reference values of core muscular endurance by plank to fatigue (PTF) test. The researchers all over accept that age, height, gender and weight standing are vital predictors of physical of adolescents and other age groups. Furthermore, anthropometric indicators provide help in the process of selecting and applying the proper sports training program and designing rehabilitation exercises. These predictors have a diverse impact on performance assessment depending on which test is performed. It is observed that performance predictors become robust with the increase in age until late adulthood, and it correlates with the current findings, which showed that core muscular endurance among adolescents also seemed mounting from age 12 to 16 among both boys and girls with few exceptions.[22]

Table-1 showed that boys dominated anthropometric measurements and core muscular endurance settings as having more plank seconds than girls. Age and gender differences tend to be connected with the physical transformation of bodies among adolescents during puberty. Scientists had ascribed that

the said differences in endurance are due to the change in anthropometric indicators and the volume of muscle mass during puberty.[23] Gender differences also play a significant role in strength and endurance among adolescent boys and girls even while selecting the physical activity, sport and exercise. Current findings are similar to previous studies as boys tend to have higher anthropometric values than girls.[24,25]

While discussing adolescents, as the weight of the adolescent's increases, they are found having problems in moving their bodies or performing tests accurately.[21] Research indicated that aerobic capacities, including core muscular endurance, were non-significantly decreasing with increased BMI among adolescents.[22] Another study concluded that obese and overweight children performed worse than the other normal children in sports.[26] The current research offered the average values of anthropometric variables, i.e., height, weight and BMI. It is found that BMI was higher in boys than in girls of South Punjab adolescents and other regions of Pakistan where the anthropometric measures were higher in boys than girls.[27] BMI is assumed as an important performance predictor in measuring core muscle endurance. While isometric plank testing or other isotonic exercises, obese adolescents are worse than normal adolescents due to excess volume of fats and body sizes which restricts their range of motion.[28]

The average plank test of boys and girls was 80.72 ± 73.48 (seconds) and 69.07 ± 58.16 (seconds), respectively. A consistent rising trend was found between the plank test and increased age (Table-2, Figure-1). Differences were found between both genders' performances compared with single years of age (Table-2). Notably, Figure-1 showed that during 14 to 15 years of age, both populations have slightly raised in the plank values. There might be several reasons for this variation that need further research specifically; the effect of physiological, psychological and socioeconomic determinants should be detected in this age group.[29] Overall, as literature predicted the plank values increased as the age increased among adolescents in South Punjab (Figure-1). The comparison with the only available P50[th] reference values on plank showed similarities in results. Figure-2 showed that boys dominated over girls with a higher centile of plank test percentiles. Though boys had higher values relatively than girls, it is worth noting that girls showed a higher trend of gaining core muscular endurance comparatively. As boys showed annual raise in plank approximately one second, girls tend to rise three to eight seconds annually, which is a remarkable finding.

While comparing 12 to 16 years old children's core muscular endurance with respect to age and gender showed significant results ($p<0.05$) among adolescents. The comparison of median centiles showed that US boys have a rising trend as the boys grow older from 12 to 15 years of age, whereas, girl's trend has variation. The results of the current study are similar to the US study.[21] The boys and girls of 12 to 16 years of South Punjab, Pakistan had lower percentile values than the USA adolescents.[21] The average plank test (sec) of boys and girls was 80.72 and 69.07, respectively. Whereas USA boys performed 91 seconds and girls performed 77 seconds.[30] However, the graphical trend showed that despite having lower values of South Punjabi girls than the US, the South Punjabi girls have a consistent rising trend than their counterparts.

Researchers have already used normative percentiles separated into a number of sections to classify health-related fitness levels among adolescents.[30, 31] Researcher for the acquisition of Plank test categories divided normative percentiles into 'Very Poor' >20th centile, 'Poor' 20–40th centile, 'Medium' 40–60th centile, 'Good' 60–80th centile and 'Very Good' <80th centile.[31] In the core muscular endurance testing, 41.3% of boys and 38.7% of girls were found in good and very good category (health benefit zone) whereas, 58.7% of boys and 61.3% of girls fall in 'poor', 'very poor' and 'medium' categories (unhealthy zones need for improvement). The majority of boys and girls need to improve their core muscular endurance to perform better in sports and daily life activities. Figure-3 showed that 21.1% of boys and 22.3% of girls are falling in a very poor category which is at risk in relation to core muscular endurance indicates, they might adopt several bad physical and health conditions. For the sake of better health of adolescents, current study outcomes might provide better insight and understanding regarding adolescents' existing core muscular endurance to embark on additional strategies for improving core muscular endurance and overall fitness and well-being in the future.

CONCLUSION

This study provided scientific evidence of normative reference standards to assess health-related physical fitness core muscular endurance of 12–16 years old adolescents. It provided an adaptable procedure that helps parents, peers, clinicians, researchers and physical educators to screen and formulate policies to escalate physical activity levels to avoid obesity and increase their core muscular endurance levels among adolescents.

ETHICAL APPROVAL AND CONSENT

The research ethics committee approved the study of the faculty of sports science, school of kinesiology, Shanghai University of Sport (SUS), Shanghai, China.

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ORIGINAL ARTICLE

COMPARISON OF T-WAVE ALTERNANS IN PATIENTS WITH ISCHEMIC AND NON-ISCHEMIC CARDIOMYOPATHY

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Background: Early detection of T-wave alternans in patients with cardiomyopathy can help in risk stratification of ventricular arrhythmias leading to sudden cardiac death. The present study was designed to compare T-wave alternans in patients with ischemic and non-ischemic cardiomyopathy. **Methods:** This cross-sectional comparative study was carried out at Department of Cardiac Electrophysiology, Armed Forces Institute of Cardiology, Rawalpindi during 2019. Thirty patients with ischemic cardiomyopathy along with equal number of non-ischemic cardiomyopathy of matched age and gender were recruited through non-probability purposive sampling. Patients with diabetes mellitus, cerebrovascular accident, heart failure, bundle branch block, systemic arterial hypertension and ongoing anti-arrhythmic therapy were excluded from the study. DMS 300-4L Holters were used to obtain ambulatory ECG recordings. Cardio Scan Premier 12 Lux software was used for analysis of T-wave alternans. **Results:** A total of 60 subjects were studied. The mean value of T-wave alternans was 52.73±30.76 µV and 57.47±36.54 µV for patients with ischemic and non-ischemic cardiomyopathy respectively. The difference between mean values was statistically insignificant (p=0.59). T-wave alternans was present in 8 (26.7%) patients with ischemic cardiomyopathy, while 5 (16.7%) patients with non-ischemic cardiomyopathy showed positive T-wave alternans and the difference was statistically insignificant (p=0.35). **Conclusion:** The mean value of T-wave alternans and frequency of patients with positive T-wave alternans is not significantly different in ischemic and non-ischemic cardiomyopathy.

Keywords: T-Wave Alternans, Ischemia, Ischemic cardiomyopathy, Non-ischemic cardiomyopathy

INTRODUCTION

T-wave alternans refers to a change in the shape and amplitude of T-wave on alternate beat.[1] It represents an increased heterogeneity of ventricular repolarization on a beat-to-beat basis which may provide a substrate for re-entry. T-wave alternans has emerged as a robust tool for arrhythmia risk stratification in patients with cardiac diseases. It gives an insight about the mechanism of arrhythmogenesis leading to sudden cardiac death.[2] The mechanisms involved in T-wave alternans are instabilities in membrane voltage and disruptions in intracellular calcium cycling. They affect a number of ionic currents in ventricular myocytes and duration of action potential.[3] The alternation in the duration of action potential reflected by T-wave alternans occurs at two distinct sites and at opposite phases of action potential. This in turn promotes marked gradients of repolarization and a substrate for re-entry leading to ventricular arrhythmias.[4]

Cardiomyopathy is an anatomic and pathologic diagnosis related with structural or electrical dysfunction of the heart. It is the disease of myocardium, usually with disproportionate ventricular hypertrophy or dilatation. Cardiomyopathy can be classified into ischemic and non-ischemic type. Ischemic cardiomyopathy describes significantly impaired left ventricular function that results from coronary artery disease. Ischemic cardiomyopathy is the most common cause of heart failure.[5] Non-Ischemic cardiomyopathy includes all causes of decreased heart function other than those caused by coronary artery disease. The most common causes of non-ischemic cardiomyopathy are viral myocarditis, drug reactions, inflammation or autoimmune reactions, amyloid and sarcoid infiltration. Ventricular arrhythmias are common in patients with cardiomyopathy, ranging from asymptomatic ventricular premature beats to sustained ventricular tachycardia or ventricular fibrillation, which can lead to sudden cardiac death.[6,7]

Almost all cardiomyopathies have a genetic basis that causes myofibrillar disarray. This disarrangement results in histological changes providing an ideal substrate for re-entry and arrhythmias.[8] Many studies have been carried out to discover the relationship between cardiomyopathy and T-wave alternans.[9,10] Early detection of T-wave alternans in patients with cardiomyopathy can help in risk stratification of ventricular arrhythmias leading to sudden cardiac death.[11] There is a lot of concern in the field of cardiac electrophysiology to find out non-invasive markers for detection of arrhythmogenic sudden cardiac death. T-wave alternans is a relatively newer marker which has been investigated for its association with the genesis of ventricular arrhythmias.[12,13]

Unfortunately, because of lack of awareness and resources, no local study has been conducted to our knowledge. Present study was planned to evaluate T-wave alternans in patients with two different types of cardiomyopathy. The objective of this study was to compare T-wave alternans in patients of ischemic and non-ischemic cardiomyopathy. Results of the study will not only identify patients at high risk of developing ventricular arrhythmias but will also provide an understanding about the probable pathophysiologic mechanism of disrupted electrical activity within the myocardium of these patients. The patients so identified at high risk of ventricular arrhythmias can be subjected to additional investigations for further refinement of arrhythmia risk and appropriate therapeutic measures to avoid sudden cardiac death.

PATIENTS AND METHODS

This cross-sectional comparative study was conducted at the Department of Cardiac Electrophysiology, Armed Forces Institute of Cardiology (AFIC), in collaboration with Army Medical College (AMC), Rawalpindi. An official approval was obtained prior to commencement of the study from Institutional Review Board of AFIC and Ethical Review Committee of AMC, Rawalpindi.

Sample size was calculated using Raosoft sample size calculator using 95% confidence interval. Thirty patients with ischemic cardiomyopathy along with thirty patients with non-ischemic cardiomyopathy were recruited through non-probability purposive sampling. Cases diagnosed as both types of cardiomyopathy by the cardiologist at outpatient department of AFIC were selected for the study. Written informed consent was taken from all the patients included in the study. History and general physical examination of all the cases and controls were carried out and the individuals having known cardiac diseases or diabetes mellitus were excluded. The selected participants were subjected to standard ECG and echocardiography to rule out bundle branch block, heart failure, hypertension and any other structural heart disease. Patients with ongoing anti-arrhythmic therapy were also excluded.

All sixty patients were Holtered with DMS 300-4L from DM Systems Company Ltd. at Electrophysiology Department of AFIC for monitoring in order to detect T-wave alternans. Ambulatory ECG data was transferred to the computer and edited for improper beats (ectopic and artefacts) with the help of DMS Cardioscan software Premier 12 Lux version. Time domain analysis was used for T-wave alternans analysis. T-wave alternans values were analyzed in all channels. It was defined as the highest T-wave alternans value in any channel. T-wave alternans ≥ 60 µV was considered positive.[3]

Data were analysed on SPSS-23. Independent samples t-test was used to compare mean values of T-wave alternans between ischemic and non-ischemic cardiomyopathic patients. Chi-square test was used to compare the frequency of individuals with positive and negative T-wave alternans between the groups keeping alpha at 0.05 and confidence level at 95%.

RESULTS

Among the patients with ischemic cardiomyopathy, there were 23 (76.7%) males and 7 (23.3%) females with mean age of 51.27 ± 12.65 years. There were 20 (66.7%) male and 10 (33.3%) female patients with non-ischemic cardiomyopathy with the mean age of 51.23 ± 16.28 years.

The mean values of T-wave alternans was 52.73 ± 30.76 µV and 57.47 ± 36.54 µV for patients with ischemic and non-ischemic cardiomyopathy respectively and the difference was statistically insignificant (p=0.59) (Table-1).

Frequency of individuals with and without T-wave alternans was also compared between patients of ischemic and non-ischemic cardiomyopathy (Table-2). T-wave alternans was present in 8 (26.7%) patients with ischemic cardiomyopathy. While 5 (16.7%) patients with non-ischemic cardiomyopathy showed positive T-wave alternans and the difference was statistically insignificant (p=0.35).

Table-1: Comparison of mean values of T-wave alternans between ischemic and non-ischemic cardiomyopathy

Group	T-wave alternans (µV)	p
Ischemic Cardiomyopathy	52.73 ± 30.76	0.59
Non-ischemic Cardiomyopathy	57.47 ± 36.54	

Table-2: comparison of individuals with and without T-wave alternans between ischemic and non-ischemic cardiomyopathy

Group	T-wave alternans		p
	Present	Absent	
Ischemic Cardiomyopathy	8 (26.7%)	22 (73.3%)	0.35
Non-ischemic Cardiomyopathy	5 (16.7%)	25 (83.3%)	

DISCUSSION

Cardiomyopathy is a disease of vast aetiology including genetic factors, secondary to effects of ischemia, and other cardiovascular diseases. Most common types of cardiomyopathies are ischemic and non-ischemic cardiomyopathy.[14] In our study the patients were divided into these two categories and T-wave alternans were recorded in both the groups. Mean values and frequency of patients with positive T-wave alternans were slightly higher in patients with ischemic than non-ischemic cardiomyopathy but the difference was statistically insignificant (p=0.59, p=0.35). Our results suggest the probable cause of development of T-wave alternans in both types of cardiomyopathy. The cause

may be present in the primal but not the ischemia affected infracted myocardium of patients with cardiomyopathy thereby suggesting that ischemia has little effect on development of T-wave alternans in patients with cardiomyopathy. Gold et al[15] studied T-wave alternans and other non-invasive tools in patients with ischemic and non-ischemic cardiovascular diseases and suggested that ischemia plays diminutive role in pathophysiology of T-wave alternans in some diseases. This may highlight the importance of evaluation of T-wave alternans as arrhythmia risk stratifier in both types of cardiomyopathy.

Klingenheben et al[16] conducted a study in Germany recruiting 204 patients with ischemic and non-ischemic cardiomyopathy and quantitatively assessed T-wave alternans. In their study the mean value of T-wave alternans was significantly higher in patients with non-ischemic cardiomyopathy as compared to ischemic cardiomyopathy. Our results are in contrary to their findings which may be due to a smaller number of subjects involved in our study or due to difference in recording and analysis methods. They recruited 204 patients while we checked T-wave alternans in 60 patients only. We used modified moving average method for recording T-wave alternans while they analyzed T-wave alternans using spectral method. Another difference may be due to dissimilarity in aetiology of non-ischemic cardiomyopathy in European countries. There, some of the common causes of non-ischemic cardiomyopathy are substance abuse (i.e., cocaine or alcohol), connective tissue disorders, and infection with the human immunodeficiency virus (HIV)[17], these being less common among our population. Difference in aetiology may have affected the mean values, possibly reflecting more extensive myocardial damage and a higher arrhythmia propensity in non-ischemic cardiomyopathy patients.

Many studies endorse the findings of our study emphasizing the significance of T-wave alternans as a promising arrhythmia risk assessment tool in both types of cardiomyopathies.[18] Chow et al[19] conducted a study evaluating 768 patients with ischemic cardiomyopathy and found significantly higher prevalence of arrhythmias in patients with ischemic cardiomyopathy. Four hundred and forty-six patients with non-ischemic cardiomyopathy were recruited in a study conducted by Salerno et al[20] in Italy assessing T-wave alternans. A fourfold increased risk of arrhythmia was calculated in non-ischemic cardiomyopathic patients with positive T-wave alternans as compared to patients with negative T-wave alternans.

T-wave alternans is closely related to arrhythmia events as observed by some studies.[21,22] Bloomfield et al[23] detected 29 positive T-wave alternans cases out of 290 and after five years follow up 20 of them had ventricular tachycardia events. Similar findings were reported by Gold et al[15] as their 22 out of 31 cases with positive T-wave alternans experienced ventricular fibrillation/sudden cardiac death. We were unable to follow the patients with positive T-wave alternans for arrhythmic events, but with ample evidence from literature, can relate the presence of positive T-wave alternans with vulnerability to fatal arrhythmias.

Cardiomyopathy mostly occurs in old age patients.[15] Preferably, age-matched participants must have been taken in the study but due to limited duration it was arduous to find age matched patients.

CONCLUSION

There was no significant difference in mean values and frequency of T-wave alternans in patients with ischemic and non-ischemic cardiomyopathy. It can therefore be concluded that the development of T-wave and vulnerability of these cardiomyopathic patients to ventricular arrhythmias is not related to ischemia as it is equally exhibited in non-ischemic cardiomyopathic patients as well.

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Contribution of Authors:
HA: Main research and writing
MI: Article writing
SN: Result and review of literature
MAK: Supervision and intellectual input

ORIGINAL ARTICLE
INCIDENCE OF VISCERAL LEISHMANIASIS AMONG PANCYTOPENIC PAEDIATRIC PATIENTS

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Background: Visceral leishmaniasis (VL) is a parasitic infection caused by *Leishmania donovani*. The sting of a sand-fly transmits Leishmania to humans. VL can lead to morbidity and even death if there is a delay in diagnosis. The objective of this study was to find out patients with visceral leishmaniasis presenting as pancytopenia in paediatric population. **Methods:** This single centre-cross sectional study was conducted for eight months in Paediatric Department, Ayub Medical Complex Hospital, Abbottabad. After taking informed written consent from all patients fulfilling the inclusion criteria, history was taken, detailed clinical examination done and bone marrow biopsy performed to check the Leishmania Donovani (LD) in the marrow sample. The findings were recorded and analysed. **Results:** Among 159 children presenting with pancytopenia, VL was confirmed in bone marrow biopsy report of 21 (13.2%) children, 100 children (62.9%) were males and 59 (37.1%) were females with the mean age of 5.58±3.44 years. **Conclusion:** VL is not an uncommon cause of pancytopenia in the paediatric population and should always be considered in the aetiology of pancytopenia in children especially those belonging to risky areas and if travel history is positive for the Mediterranean area. As the condition is treatable and has many complications that may lead to death if diagnosis is delayed, so workup and management done in time can actually save life of patient.

Keywords: Paediatric, pancytopenia, visceral leishmaniasis, bone marrow biopsy, *Leishmania donovani*

INTRODUCTION

Pancytopenia is consequence of many haematological conditions with an extensive differential diagnosis. The aetiology of bicytopenia and pancytopenia varies widely in children, ranging from transient marrow viral suppression to marrow infiltration by life-threatening malignancy. Prompt workup to find aetiology is required to avoid complications.[1] Visceral leishmaniasis (VL) is a parasitic infection caused by *Leishmania donovani*. The sting of a sand-fly transmits Leishmania to humans. Unspecific symptoms such as fever, lack of appetite, weight loss, and lymphadenopathy can appear after weeks to months, and are often followed by more specific findings such as pancytopenia and hepatosplenomegaly.[2]

Leishmania may cause cutaneous leishmaniasis, mucocutaneous leishmaniasis, and visceral leishmaniasis, among other clinical syndromes. In the absence of therapy, VL is the most acute type of leishmaniasis that can be lethal.[3] Transmission through the bite of an infected rodent, organ transplant, accidental inoculation among laboratory staff, congenital transmission, and blood transfusion are among rare causes.[4,5] Pancytopenia is caused by VL in 11.7% of cases.[6]

This study was conducted at Ayub Teaching Hospital, Abbottabad with the aim to report the clinical and epidemiological aspects of VL in our area and to highlight its consideration among differential diagnosis of pancytopenic paediatric patients.

MATERIAL AND METHODS

This cross-sectional study was conducted after ethical approval by the Institutional Review Committee of Ayub Teaching Hospital, Abbottabad. Based on consecutive sampling technique, children less than 12 years of age of either gender, presenting with pancytopenia who fulfilled the inclusion criteria and admitted in Paediatrics Department, Ayub Medical College and Hospital Complex, Abbottabad were included in this study. Patient diagnosed with cancers or using anti-cancers/radiation therapy and immuno-compromised were excluded. Pancytopenia was established when haemoglobin <10 g/dL, TCL <4,000/uL and platelets 150,000/uL. Informed consent was taken from the patient or his/her the family. Bone marrow examination was done and *Leishmania donovan* (LD) bodies were checked. LD bodies were counted under 100× oil immersion objective in bone marrow aspiration. Children were labelled as VL if LD bodies were present. The effect modifiers and biasness was controlled by strictly following the inclusion and exclusion criteria.

Data was compiled and analysed through SPSS-22. Frequency and percentage was computed for qualitative variables like gender, VL (Yes/No). Mean±SD was calculated for quantitative variables, i.e.,

age, weight, height, haemoglobin (Hb), total leukocyte count (TLC) and platelets. Stratification was done on gender, age, weight, height, haemoglobin, total leukocyte count and platelets to see the effect of these modifiers on outcome using Chi-square test, and $p \leq 0.05$ was considered as statistically significant.

RESULTS

A total of 159 children of pancytopenia were included in this study. The mean age of the patients was 5.58±3.446 years. The distribution of age is presented in Figure-1.

The mean height of the patients was 34.37±19.690 Cm, and mean weight was 20.42±11.893 Kg. Among total, 100 children (62.9%) were males and 59 (37.1%) were females. Mean haemoglobin level was 9.70±1.08 g/dl, mean total leukocyte count was 2615.2201±795.757/mm^3, mean platelet count was 87572.27±28230.394/µL, and VL was seen in 21 (13.2%) children.

The frequencies of age groups, gender, height, weight, haemoglobin level, total leukocyte count and platelets were calculated according to VL. In our study, VL was significantly associated with age but not significantly associated with gender, height, weight, haemoglobin level, total leukocyte count, and platelets (p=0.033, 00.701, 00.709, 00.872, 0.833, 00.264, and 00.365 respectively). The results are presented in Table-2, 3, and 4).
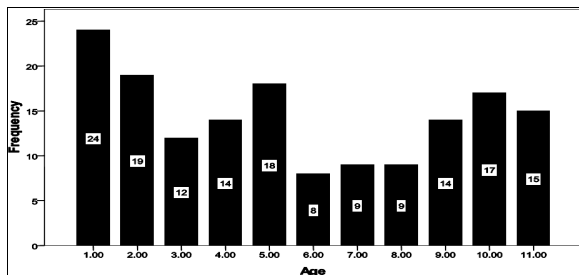
Figure-1: Frequency distribution of age

Table-1: Descriptive statistics of age, height, weight, haemoglobin level, total leukocyte count, and platelets (n=159)

Variable	Mean±SD
Age (Years)	5.58±3.44
Height (Cm)	34.37±19.69
Weight (Kg)	20.42±11.89
Haemoglobin level (g/dL)	9.70±1.08
Total leukocyte count (per mm^3)	2615.22±795.76
Platelets (per µL)	87572.27±28230.39

Table-2: Frequency distribution of gender and VL (n=159)

	Frequency	Percentage
Gender		
Male	100	62.9
Female	59	37.1
Visceral leishmaniasis		
Yes	21	13.2
No	138	86.8

Table-3: Visceral leishmaniasis according to age, gender, height, and weight

	Visceral leishmaniasis n (%)			
Variable	Yes	No	Total	*p*
Age (Years)				
1–5	6 (3.1)	81 (50.95)	87 (54.05)	
6–11	15 (10.1)	57 (35.84)	72 (45.95)	0.033
Total	21 (13.2)	138 (86.8)	159 (100)	
Gender				
Male	14 (8.8)	86 (54.1)	100 (62.9)	
Female	7 (4.4)	52 (32.7)	59 (37.1)	0.701
Total	21 (13.2)	138 (86.8)	159 (100)	
Height (Cm)				
7–43	8 (5.4)	93 (58.8)	101 (64.2)	
44–78	13 (7.8)	45 (28)	58 (35.8)	0.709
Total	21 (13.2)	138 (86.8)	159 (100)	
Weight (Kg)				
5–28	13 (7.8)	93 (58.8)	106 (66.6)	
29–50	8 (5.4)	45 (28.0)	53 (33.4)	0.872
Total	21 (13.2)	138 (86.8)	159 (100)	

Table-4: Visceral leishmaniasis according to haemoglobin level (g/dl), Total leukocyte count (per mm^3), Platelets (per µL) (n=159)

	Visceral leishmaniasis n (%)			
Variable	Yes	No	Total	*p*
Haemoglobin level (g/dl)				
7.5–9.5	9 (5.6)	70 (42)	79 (47.6)	
9.6–11.5	12 (7.6)	68 (44.8)	80 (52.4)	0.833
Total	21 (13.2)	138 (86.8)	159 (100)	
Total leukocyte count (per mm^3)				
1500–2750	12 (7.3)	90 (57)	102 (64.3)	
2751–3905	9 (5.9)	48 (29.8)	57 (35.7)	0.264
Total	21 (13.2)	138 (86.8)	159 (100)	
Platelets (per µL)				
45000–95000	15 (9.2)	83 (50.5)	98 (59.7)	
96000–145000	6 (4.0)	55 (36.3)	61 (40.3)	0.365
Total	21 (13.2)	138 (86.8)	159 (100)	

DISCUSSION

Acquired cytopenias are common in children and are typically caused by infectious agents.[2] Visceral leishmaniasis can affect one or more blood cell lines, resulting in pancytopenia.[7,8] Previous studies on the incidence of Leishmania infection in children with post infectious cytopenia or pancytopenia in endemic areas are limited.[9–11] In developing countries, especially those in the Mediterranean region, VL has been described as one of the most common causes of post infectious pancytopenia in children[12–14] with viral infections, particularly parvovirus B19[15], and bacterial infections being the other major causes[11]. Substitution of natural marrow components by parasitized histiocytes, as well as erythrophagocytosis and haemophagocytosis, are pathogenic mechanisms of pancytopenia in VL patients.[16,17]

In our study VL was a cause of pancytopenia in 21 (13.2%) children. Agrawal *et al*[18] observed bicytopenia in 40% of VL cases, whereas they observed pancytopenia in 25% cases. In Singh *et al*[6] study VL was cause of pancytopenia in 11.7% cases.

Gupta *et al*[10] studied 105 children with pancytopenia, aged 1.5–18 years. They reported that infections, of which *Kala Azar* was the most common, were the third most common cause of pancytopenia. The most frequent presenting complaints in their cohort were fever and gradual pallor, which were present in 81.4% of the cases, accompanied by bleeding presentations in 72.9% of the cases.

VL can be life-threatening if its diagnosis and treatment is delayed. Treatment responses to VL vary by region and recommended treatment also differs. In one study[19] involving children with pancytopenia, a fast therapeutic reaction was reported. In a study from Southern Greece[9] haematological recovery occurred after treatment for a median of 12 days. Similar response was also reported by Verma *et al*[20]. Early diagnosis and prompt intervention may save many lives.

STUDY LIMITATION

It was a single centre study with a smaller sample size. Further studies with larger sample sizes are required.

CONCLUSION

Visceral Leishmaniasis is quite high among patients with pancytopenia. Children with pancytopenia should also be investigated for Visceral Leishmaniasis when belonging to high risk and with a travel history to Mediterranean area. Since it is treatable and has many complications that may lead to death of patient, workup done in time can save life of patient.

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ORIGINAL ARTICLE
PREVALENCE OF ANAEMIA IN PREGNANT WOMEN

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Background: Anaemia is defined as a medical condition in which the red blood count or haemoglobin is lower than the normal levels. Deficiency of haemoglobin can be due to lack of vitamin B_{12}, folic acid and iron, extreme blood loss or nutrient-deficit diet. Anaemia during pregnancy is harmful both to the mother and foetus. It can cause pre-mature delivery and birth defects. Iron supplementation is often recommended to pregnant women. The aim of study was to find out the prevalence of anaemia in pregnant women. **Methods:** The study was carried out using quantitative methods. A sample of 300 participants was taken using purposive sampling technique. Socio-demographic data was collected from participants after informed consent. Haemoglobin levels were recorded for each participant using Blood Complete Picture already available with them. **Results:** There exists high prevalence (74.6%) of anaemia in pregnant women. The prevalence is much higher (80.3%) in the 3rd trimester of pregnancy. Low intake of meat was observed in 73% of the anaemic women. Low intake of other iron rich food was also observed in more than 50% of the participants. Only 93 participants had their MCV reports with them (53.7% among them had low MCV). This high rate of prevalence is associated with iron-deficient diet and unawareness about seriousness of the problem. **Conclusion:** There is high prevalence of anaemia among pregnant women associated with iron-deficient diet and lack of awareness. Introducing screening tests for anaemia, raising public awareness, and educating females about importance of nutrition diet are recommended.
Keywords: Anaemia, iron deficiency, pregnancy, status, prevalence, Kashmir, women

INTRODUCTION

Pregnancy is the time of critical physiological changes which are accompanied by several complications. The most common of the complications during pregnancy is anaemia. Anaemia is defined as a medical condition in which the red blood count or haemoglobin is lower than the normal levels.[1]

Haemoglobin-the iron containing protein, is the essential component of blood. It is present in the red blood cells and its major function is to transport oxygen from lungs to tissues which is accomplished by the binding of oxygen molecules with haemoglobin molecule (formation of oxy-haemoglobin). It is also a rich source of iron in blood.[2]

It is essential that the adequate level of haemoglobin be present in human body for normal functioning. Since, haemoglobin is a transporter of oxygen and a source of iron in blood, so its deficiency can lead to serious physiological problems, the most common of which is anaemia. Normal/average levels of haemoglobin are different for males and females. Normal level of haemoglobin in males is 14–18 g/dL or 8.7–11.2 mmol/L. The normal level of haemoglobin in females is 12–16 g/dL or 7.4–9.9 mmol/L. In pregnant women, the average level of haemoglobin should be >11 gm/dl.[3] According to the WHO, if the level of haemoglobin falls below 11 gm/dl in pregnant women, then she is said to be anemic.[4]

Many factors affect the level of haemoglobin in body, like extreme blood loss, low production of RBCs or destruction of the RBCs due to some other cause. Other most important factors in anaemia are the vitamin B_{12}, folic acid and iron.

Vitamin B_{12}, also called as Cobalamin is a very important micronutrient, also known as the energy nutrient as it is involved in the energy production for the body and also helps in the production of DNA and cell division in bone marrow. So, during pregnancy if Vitamin B_{12} deficiency occurs due to insufficient Vitamin B_{12} intake or problems in absorption, there is increased risk of anemia.[5]

Folic acid or B9 is one of the B complex required for the production of red blood cells. It is a water-soluble nutrient, which is not stored in body in large amounts. It is not synthesized by human body and is required through external sources. In case of folate-deficient diet; there is a risk of folate deficiency which may also serve as a risk factor for anaemia. This complication is also termed as 'folate-deficiency anaemia' or megaloblastic anaemia (red blood cells grow large). Folic acid deficiency during pregnancy is also harmful for foetus development (foetus brain and spinal cord development) leading to birth defects called neural tube defects.[6]

Iron deficiency is the major and most common form of anaemia. Among pregnant women,

iron-deficient diet is the common factor leading to anaemia.[7] According to a study, iron deficiency anaemia and folate deficiency megaloblastic anaemia are the common types of anaemia present in pregnant women. Studies have concluded that iron supplementation is necessary for improving level of iron/haemoglobin during pregnancy.[8]

Anaemia during pregnancy leads to many complications. According to some studies, anaemia during pregnancy can lead to problems in cognitive development, preterm delivery, low birth weight and low neonatal health. It is also found that iron deficiency/anaemia during pregnancy reduces the iron stores in the first year of life in infants. It also increases a risk of iron deficiency anaemia in infants.[9,10]

According to a research conducted in Pakistan, prevalence of anaemia was found to be 90.5% out of which a mild-moderate level of anaemia was observed in a high percentage of pregnant women (20–26 weeks pregnant). This low level of haemoglobin was also associated with low consumption of red meat and eggs, thus showing a lack of nutrition as common factor associated with anemia.[11]

Another study conducted in Pakistan revealed that a very high percentage of pregnant women in Railway Hospital of Multan had anaemia. The poor dietary habits account for this iron deficiency in pregnant women. The study emphasized on the need of a widespread approach regarding education and management of anaemia in pregnant women.[12]

Among the various nutritional disorders, iron deficiency anaemia is most common and pregnant women constitute a high-risk population for this problem.[13] Both in the developing and developed countries, there is a high prevalence of anaemia in pregnant women. This fact also got verified by a survey in India which showed that about 84.9% of the pregnant women were anaemic, among which 13.1% were severely anaemic while 60.1% were suffering from moderate anemia.[14]

Many other studies have also indicated that anaemia prevails commonly in pregnant women. Most of the studies have emphasized the need for iron supplementation for pregnant women.[15,16]

It is significant to study the status of anaemic pregnant women attending hospitals because it provides information about the needs of patients related to their diet and use of necessary supplements during pregnancy. The objective of this study was to find out the prevalence of anaemia in pregnant women through haemoglobin and MCV levels.

MATERIAL AND METHODS

This was a quantitative study carried out in the Obs/Gyn Outpatient Departments of Abbas Institute of Medical Sciences (AIMS) and Sheikh Khalifa Bin Zayed Hospital (SKBZH)/CMH Muzaffarabad after obtaining permission from institutional ethical review committee. Participants were selected using purposive sampling method. Using Sample size prevalence survey calculator, the sample size was calculated to be 289 which was rounded to 300.[17] Participants were selected on the basis of following inclusion criteria:

A total of 300 pregnant women presenting in the Obs/Gyne OPDs of AIMS and Sheikh Zaid Hospital consecutively were included after taking informed consent. Non-pregnant women and those suffering from any chronic systemic disorder or deficiency diseases were excluded.

The study was carried out for 2 months from July 15 to Sep 15 2019. Data were recorded on a special designed proforma. The haemoglobin level was recorded using Blood CP (Blood Complete Picture) available with the participants. They were interviewed using a closed ended questionnaire (self-constructed) which examined the demographics of the subject (socio-economic status, age, education, marital status, number of children etc.) as well as their diet patterns (intake of vegetables, egg, milk, meat) and medical reports regarding pregnancy.

The severity of anaemia was determined on the basis of WHO grading of anaemia as:[4]

Grade 1: Mild= 10–12 gm/dl
Grade 2: Moderate= 7–10 gm/dl
Grade 3: Severe= less than 7 gm/dl

RESULTS

The results indicated that prevalence of anaemia in pregnant women presenting in Gynaecology OPD of AIMS and SKBZ/CMH was 74.6%. Among the anaemic patients, 68.3% had mild anaemia, 29.9% had moderate, and 1.78% had severe anaemia (Table-1).

Out of 300 participants, MCV was available from only 93 participants. Among those 93 participants, 53.7% had low levels of MCV, 41.9% had normal MCV level and 4 had high levels of MCV (Table-2).

Among the anaemic participants, 3.1% were in their 1st trimester of pregnancy, 16.5% were in their 2nd trimester of pregnancy, while 80.3% were in 3rd trimester of pregnancy (Table-3).

The results also indicate that most of the anaemic women (about 73%) had less than 200 gm of meat intake per week (Table-4). More than 50% of the participants consumed iron rich food in less than 3 days a week (Table-5).

Table-1: Prevalence of anaemia (n=300)

Anaemic participants	n (%) (n=224)
Mild (10–12 g/dl)	153 (68.3%)
Moderate (7–10 g/dl)	67 (29.9%)
Severe (less than 7 g/dl)	4 (1.78%)
Prevalence	**74.6%**

Table-2: MCV (Mean Cell Volume) in participants (n=300)

MCV level (n=93)	Low (<80 fl)	Normal (80–95 fl)	High (>95 fl)
No. of participants	50	39	4
Percentage	53.7%	41.9%	4.3%

Table-3: Prevalence of anaemia in different trimesters (n=300), [n (%)]

Anaemia	1st Trimester	2nd Trimester	3rd Trimester
Mild (10–12 g/dl)	5	25	123
Moderate (7–10 g/dl)	2	11	54
Severe (<7 g/dl)	0	1	3
Total anaemic	7 (3.1)	37 (16.5)	180 (80.3)

Table-4: Meat intake per week (n=300)

Meat Intake	Number	Percentage
No meat intake	49	21.8
<100 gm	55	24.5
100–200 gm	60	26.7
200–300 gm	36	16
300–400 gm	24	10.7

Table-5: Intake of other iron rich food (leafy vegetables, fruits, eggs, milk etc) per week

Days Per Week	Number	% out of 300
1 day	92	31
2 days	73	24
3 days	48	16
4 days	31	10
5 days	21	7
6 days	17	6
7 days	18	6
Total	300	100

DISCUSSION

The results of this study were consistent with the literature findings. The prevalence of anaemia was very high (74.6%) among the pregnant women. This high prevalence of anaemia is an indicator of low maternal and neonatal health. Anaemia is the risk factor for several other complications during pregnancy[8–12], it can be stated that the women of this area are at greater risk for several complications. Development of foetus is dependent upon the health of the mother. In case of maternal anaemia, there are chances of many developmental problems in the foetus.

The MCV (mean corpuscular volume) is an indicator of size, and indirectly of Hb content of RBCs. It was found that majority of the participants did not have their MCV checked out. Only 93 participants out of 300 had their MCV checked, and among them 53.7% had low MCV indicating iron deficiency anaemia. These patients seem to have visited the healthcare attendant(s) (Lady Health Worker/*Dai*) in a far-flung area where appropriate health facilities and equipment is not available. The fact that majority of participants were having an iron deficiency anaemia is explained by poor nutrition intake. Iron deficient diet is the leading factor towards iron deficiency anaemia.[7,8] The diet pattern of the participants showed that majority of the anaemic

pregnant women took less than 200 gms of meat per week. Moreover, the consumption of other iron rich food like leafy vegetables, eggs, milk etc. was also very low and less than 1 in 5 women consumed iron rich food more than 5 times a week. Such nutritional deficiency is the major cause of high prevalence of anaemia. The lack of awareness and low nutrition intake can be explained by factors like socio-economic status. Most of the participants belonged to middle or lower socio-economic status. Due to economic crises, burden of family and lack of education, women stay ignorant about the importance of nutritional diet and problems which could arise from its deficiency. They also have less access to iron rich food or supplements which leads to anemia.[18]

Another important finding of the study was the different prevalence rates of anaemia in the three trimesters of pregnancy. In the last trimester of pregnancy, there is haemodilution due to plasma volume expansion, so there is some degree of physiological anaemia as well. There is also an increased requirement of iron (2–3 folds) and folate (10–20 folds) for the growing foetus during this time. The women require extra intake of iron and folate to cope up with the growing demand[19] especially in the last trimester of pregnancy. It was observed that most (80.3%) of the anaemic participants were in their last trimester of pregnancy. This highlights that the last trimester of pregnancy is a very critical time period so, special care is needed in maintaining healthy diet and nutrition to meet the demands of pregnancy and to prevent iron and folate deficiency.

The results have highlighted the fact that most of the time, anaemia goes unchecked and unreported (especially mild cases of anaemia) because of unawareness. People do not report the symptoms until they become extremely severe. This high prevalence of anaemia in pregnant women especially during the 3rd trimester of pregnancy can be due to high salt-water retention, and is associated with poor diet and careless attitude towards health. A comprehensive screening process has to be developed for checking anaemia in pregnant women and to treat it before it gets severe.

CONCLUSION

There exists a high prevalence of anaemia/iron deficiency among pregnant women. The major causal factors found are nutrient-deficient diet and lack of awareness about seriousness of the disorder.

LIMITATIONS

It was a single station study representative of the local area only. The study can be extended to other stations and locations to include a variety of subjects from different socioeconomic and demographic origins. The hospitals/healthcare facilities were not adequate which affected the data collection process.

RECOMMENDATIONS

The high prevalence can be controlled by setting up cost-effective screening tests (Hb level) to diagnose the disorder at a mild stage and by providing awareness to the general public for understanding the complications of this disease. The Lady Health Workers should visit the pregnant women in their early pregnancy and provide necessary awareness about their health issues during pregnancy. Media can also be used to provide awareness about preventing and controlling anaemia.

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ORIGINAL ARTICLE

COMPARISON OF D-DIMER LEVELS IN PREECLAMPSIA AND NORMAL PREGNANCY

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Background: Preeclampsia is associated with deposition of fibrin in microvasculature and maternal organ dysfunction. D-dimer is used as a marker of degradation and production of fibrin in the body. The objective of this study was to determine the mean plasma D-dimer levels among pregnant women and to compare mean plasma D-dimer levels between preeclamptic patients and women without preeclampsia. **Methods:** A cross sectional study was designed and conducted in the Obs/Gyn Department, Hayatabad Medical Complex, Peshawar. A total of 154 pregnant women fulfilling inclusion criteria were included. Non-probability consecutive sampling was used for collection of samples. Mean±SD was calculated for quantitative variables while qualitative variables were presented in the form of frequency and percentage. Independent sample *t*-test was used to compare mean plasma D-dimer levels between preeclampsia and normal patients. Stratification was done on the basis of age, gestational age, BMI, and booking status. Post-stratification *t*-test was applied and $p \leq 0.05$ was considered significant. **Results:** The mean age of patients was 27.73±2.68 years (Range: 18–34 years). The mean gestational age was 37.43±1.15 weeks (Range: 35–40 weeks). Preeclamptics were 58 (37.7%) while 96 (62.3%) were normotensive. The mean plasma D-dimer levels in total cases was 0.50±0.41 ng/ml (Range: 0.115–1.156). The mean plasma D-dimer level in preeclamptics and normal cases was 1.02±0.07 and 0.18±0.04 (ng/ml) respectively ($p<0.00$). **Conclusion:** Mean plasma D-dimer level was higher in preeclamptic patients as compared to normotensive pregnant women showing a significant correlation between preeclampsia and plasma D-dimer level in third trimester of pregnancy.
Keywords: Plasma D-dimer, preeclampsia, hypertensive disorders, pregnancy

INTRODUCTION

D-dimer (DD) is a small portion of protein formed by fibrinolysis and significantly increases during pregnancy.[1,2] It's concentrations in maternal blood increases throughout gestational period without any thromboembolic complication.[3] A proper increase in blood coagulation is important for a pregnant female to reduce the risk of postpartum haemorrhage.[4] D-dimer is one of the most valued indicators to diagnose and manage thrombotic states with venous thromboembolism, and atrial fibrillation.[5]

Globally, hypertensive disorders of pregnancy (HDP) complicate around 10% of pregnancies and are the second largest cause of maternal mortality but aetiologies and pathogenesis are not yet fully understood.[6] HDP may lead to adverse events for foetus and mother, i.e., low birth weight, maternal stroke, mortalities, and neonatal intensive care needs.[7] Preeclampsia (PE) is the most shared pregnancy complication of HDP, affecting 5–8% of pregnancies.[8] It is diagnosed by arterial pressure of >140/90 mmHg measured on two occasions at more than 4 hours interval with higher than 300 mg of urinary protein in 24 hours after 20th week of gestation. About 5–7% of primigravida and 1–3% of multigravida get complicated by preeclampsia.[9] World Health Organization (WHO) estimates 50,000–75,000 annual deaths associated by complications of preeclampsia making it the leading cause of death during pregnancy.[10]

The higher levels of D-dimer indicate presence of high level of thrombus in blood.[11] This explains the mechanism of damage in preeclampsia where dissolution of fibrin clot plays a vital role in endothelial damage.[12] Therefore, more precautions must be implemented in high risk pregnancies to prevent development of preeclampsia and its complications.[13] Recently, plasma D-dimer level is labelled as a biomarker for fibrinolytic and haematologic changes during preeclampsia. The studies conducted on various population has shown variations in its values therefore this study was designed to compare mean plasma D-dimer levels in preeclampsia and controls in our population.

MATERIAL AND METHODS

This cross-sectional study was conducted in Obs/Gyn Department of Hayatabad Medical Complex Peshawar, after approval from Institutional Ethical Committee, from 1st December 2018 to 31st May 2019. The sample size was calculated by using WHO sample size calculator. Sample size of pregnant women calculated was 154 (58 cases and 96 controls). Non-probability consecutive sampling was used for collection of samples.

There were 91 (59.1%) booked and 63 (40.9%) un-booked cases. The primigravidas of third trimester aged 20–40 years visiting Obs/Gyn Department for routine check-up or for hypertension were enrolled after their informed written consent. They underwent screening for preeclampsia and were grouped into primigravida with preeclampsia and normal pregnant females. Women with the history of coagulopathy, liver disease, or any inflammatory disorders were excluded.

About 3 ml of venous blood was collected in a container containing 3.2% tri-sodium citrate. The platelets poor plasma (PPP) was immediately prepared through centrifuge for 15 minutes at 3,000 rpm at room temperature. The plasma D-dimer levels were measured using (MISPA-i2 reagent, Switzerland).

The data were recorded on a pre-designed proforma and analysed using SPSS-16. Mean±SD were calculated for quantitative variables like, age, gestational age, plasma D-dimer level, weight, height, and BMI. Qualitative variables like education, rural, urban, booking status, status of blood pressure were presented as frequencies and percentages. Independent sample t-test was used to compare mean plasma D-dimer levels between preeclamptics and normal patients. Stratification was done on the basis of age, gestational age, BMI, and booking status. Post stratification t-test was applied, and $p \leq 0.05$ was considered as significant.

RESULTS

The mean age of cases was 27.73±2.68 (Range: 18–34) years. Mean height, mean weight, and BMI were 1.64±0.05 m, 67.71±9.86 Kg, and 25.02±3.19 Kg/m^2 respectively. Mean gestational age was 37.43±1.15 (Range: 35–40) weeks. Mean plasma D-dimer level was 0.59±0.41 (0.12–1.16) ng/ml. (Table-1).

Preeclamptic cases were 58 (37.7%), and 96 (62.3%) were normotensive. Mean plasma D-dimer level in preeclamptics was 1.02±0.07 and 0.18±0.04 ng/ml in normal cases ($p<0.00$) (Table-2).

When data were stratified for age, period of gestation, BMI, and booking status, the mean plasma D-dimer levels in females with preeclampsia were statistically higher compared to normal females ($p<0.05$) (Table-3).

Table-1: Age, height, weight, BMI, gestational age, and D-dimers in total cases

Parameters	Min	Max	Mean±SD
Age (Years)	20	34	27.73±2.68
Height (m)	1.55	1.75	1.64±0.05
Weight (Kg)	55	86	67.71±9.86
BMI (Kg/m^2)	20	31	25.02±3.19
Gestational age (Weeks)	35	40	37.43±1.15
D-dimers (ng/ml)	0.115	1.156	0.50±0.41

Table-2: Comparison of D-dimers in preeclamptics and normal females

Blood pressure Status	n	Plasma D-dimers level (ng/ml)		
		Mean	SD	p
Preeclamptics	58	1.02	0.07	<0.00
Normal females	96	0.18	0.04	

Table-3: Stratification of plasma D-dimers level in women with and without preeclampsia with respect to age, gestational age, BMI, and booking status

Parameters		Groups	Plasma D-dimers		p
			n	Mean±SD	
Age (years)	20–28	Preeclampsia	35	1.02±0.07	<0.00
		Normal	49	0.18±0.04	
	29–40	Preeclampsia	23	1.03±0.06	<0.00
		Normal	47	0.18±0.05	
Gestational age (weeks)	<37	Preeclampsia	7	1.02±0.04	<0.00
		Normal	15	0.18±0.05	
	≥37	Preeclampsia	51	1.03±0.07	<0.00
		Normal	81	0.18±0.04	
BMI	Non-Obese	Preeclampsia	50	1.02±0.07	<0.00
		Normal	92	0.18±0.04	
	Obese	Preeclampsia	8	1.03±0.06	<0.00
		Normal	4	0.18±0.05	
Booking status	Un-booked	Preeclampsia	36	1.03±0.06	<0.00
		Normal	27	0.17±0.05	
	Booked	Preeclampsia	22	1.01±0.08	<0.00
		Normal	69	0.18±0.04	

DISCUSSION

The results of present study revealed that the mean plasma D-dimer level in preeclamptics were statistically higher than normotensive females ($p<0.00$). This is in accordance with the study conducted by Khawaja et al. in 2019 who documented significant positive association between preeclampsia and high D-dimer levels.[15] Similarly, a study conducted by Bozkurt et al. in 2015 also concluded that the levels of D-dimers in preeclamptics ($p=0.03$) and eclamptics ($p=0.02$) were significantly higher than the controls.[16]

An African study conducted in Sudan in 2017 also documented similar findings stating a statistically significant rise in plasma fibrinogen ($p=0.00$) and D-dimer levels in preeclamptics compared to normotensive pregnant females ($p=0.00$).[14] However, Catarino et al. after studying a small sample size concluded that there is no significant difference in mean D-dimer level between preeclamptics and normotensive pregnant females.[17] Contrarily, Kim et al, reported a significantly higher D-dimer in hypertensive pregnant females ($p<0.01$).[2]

Recently, Fazal S et al, concluded that pregnant females having a tendency of developing gestational hypertensive complications have higher levels of D-dimer.[18] A study conducted by Lefkou et al. also assessed biomarkers of hypercoagulability in females suffering from mild and severe preeclampsia. Only females with severe preeclampsia showed increase in D-dimer levels and consumption of natural coagulation inhibitors. This has also reflected the

tendency of prolonged PT and aPTT in severe preeclamptics.[19] Similarly, Baboolall *et al*, in a retrospective observational cohort study reported obvious relationship between D-dimers concentration and severe preeclampsia.[1] A cross sectional study carried out in Dhaka Medical College Hospital, showed that the risk of having plasma D-dimer >0.5 µg/ml was 9 times more in preeclamptic women than normal pregnant women.[20]

However a comparative cross sectional study conducted at King Edward Medical University Lahore, found fibrinogen, Platelet Distribution Width (PDW), platelet count and D-dimers changed in preeclamptics compared to normal pregnant females but were not statistically significant (p>0.05).[21]

CONCLUSION

The mean plasma D-dimer level was higher in preeclamptic patients as compared to normotensive pregnant women in our study population showing a significant correlation between preeclampsia and plasma D-dimer level in 3rd trimester of pregnancy. Monitoring D-dimer level can be used as a tool for early diagnosis and decision on management of complications of pregnancy induced hypertension.

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ORIGINAL ARTICLE

COMPARISON OF PORT SITE WOUND INFECTION WITH AND WITHOUT ENDOGLOVES TECHNIQUES FOR RETRIEVAL OF GALLBLADDER AFTER LAPAROSCOPIC CHOLECYSTECTOMY

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Background: Laparoscopic cholecystectomy decreases postoperative pain, the need for postoperative analgesia, and hospital stay from 1 week to less than 24 hours. Endogloves are frequently used in laparoscopic cholecystectomy. The study was conducted to compare the frequency of port site wound infection with and without endogloves techniques of retrieval of gallbladder (GB) in pouch after laparoscopic cholecystectomy for chronic calculus cholecystitis. **Methods:** This comparative analytical study was done at Department of General Surgery, CMH, Muzaffarabad, Azad Kashmir from March 2018 to March 2020, and included 260 patients in the study. Patients with symptomatic cholelithiasis but without acute onset were selected for the study. The patients were divided into two groups; one group underwent conventional laparoscopic cholecystectomy with endogloves technique and the other group without endogloves. All operations were done by the same surgical team and all patients got same preoperative and postoperative antibiotics. Port site wound infection was looked for. Data was recorded and analysed using SPSS-20. **Results:** The mean age of the patients in group undergoing laproscopic cholecystectomy with endogloves was 48.09±15.40 years and in group undergoing laproscopic cholecystectomy without endogloves it was 47.51±16.48 years. Male to female ratio was 1.06:1. The post-op wound infection was found in 11 (4.23%) patients (2 from with-endoglove group, 9 from without-endoglove group). Statistically significant differences were found between the groups ($p<0.05$). **Conclusion:** The use of endoglove technique of retrieval of gallbladder in pouch after laparoscopic cholecystectomy for chronic calculus cholecystitis potentially reduces port site wound infection compared to retrieval without endogloves.

Keywords: Laparoscopic cholecystectomy, endoglove, gallbladder

INTRODUCTION

Gallstones constitute a significant health problem in both developing and developed societies affecting 10–15% of the adult population and 3–6% of adult Pakistani population.[1] Cholecystectomy is the treatment of choice for symptomatic gallstones because it removes the organ that contributes to formation of gallstones and the complications ensuing from them. Laparoscopic cholecystectomy is now the gold standard procedure for symptomatic gallstones and is the commonest operation performed laparoscopically worldwide. Gallbladder (GB) perforation and spillage are the commonest (25%) complications encountered during dissection and removal of gallbladder.[2] In order to prevent above complications, gallbladder specimen is retrieved in an endobag. An inflamed or distended gallbladder packed with stones always creates a problem during its retrieval.[3]

Retrieval of gallbladder is an important terminal event of laparoscopic cholecystectomy and is reported as one of the factors affecting postoperative port site pain.[4] Gallbladder is extracted commonly either from the epigastric or umbilical port exactly spot on. Both the ports have been recommended for retrieval of gallbladder in laparoscopic cholecystectomy.[3]

There have been increasing reports of infectious complications due to un-retrieved stones and spillage of bile. Such complications mask not only the advantages of minimal access surgery but also increase the economic burden on the patient.[5] Work load on the staff is also increased and the reputation of hospital and attending surgeon bears the brunt.[6]

In routine, endogloves are not used, especially in poor resource setting. Without endogloves, the chances of port site infection increase. The purpose of this study was to evaluate the technique that is associated with a decreased post-laparoscopic cholecystectomy port site wound infection in our setup.

PATIENTS AND METHODS

A total of 260 cases were enrolled in the study through Department of General Surgery, who were admitted for elective cholecystectomy. Informed consent was obtained from all participating patients. Demographic details were recoded. Patients were randomly divided in two groups using lottery method.

In group I, patients underwent conventional laparoscopic cholecystectomy with four port technique. In these patients, the gallbladder was retrieved through

umbilical port exactly spot on using a sterile surgical hand glove (size 6½ or 7) endobag. The 10 mm umbilical port (fascial defect) was closed using vicryl '1' with a J-shaped needle, while three 5 mm ports were closed using prolene 2/0.

In group II: patients underwent conventional laparoscopic cholecystectomy with four port technique. In these patients, the gallbladder was retrieved through umbilical port exactly spot on without sterile surgical hand glove endobag. All surgeries were done by consultant general surgeon or chief resident (Resident Year IV) under general anaesthesia with four ports technique. In both groups, if GB was found distended or contained large stones, it was opened at the time of retrieval and bile was suctioned (and/or stone was retrieved) under vision. Then patients were followed-up for 7 days. Postoperative port site wound infection was assessed after 7 days of the procedure and findings recorded. Data was analysed using SPSS-20. Chi-square test was applied to compare postoperative port site wound infection in both groups, and $p \leq 0.05$ was considered statistically significant.

RESULTS

The mean age of the patients with endogloves group was 48.09±15.402 years and in patients without endogloves it was 47.51±16.48 years. Male patients were 134, out of which 65 were in with-endogloves group and 69 were in without-gloves group. The female patients were 126, out of whom 65 were from with-endogloves group and 61 were from without-endogloves group. Mean BMI in endogloves group was 26.20±4.74 Kg/m^2 and in without-endogloves group it was 26.26±4.84 Kg/m^2. The mean duration of the patients' symptoms in with-endogloves group was 3.16±1.46 months and in without-endogloves group it was 3.05±1.37 months. (Table-1).

The post-op wound infection was found in 11 cases, out of which 2 were from with-endogloves group and 9 were from without-endogloves group. Statistically significant differences were found between the groups for post-op wound infection (p=0.031). (Table-2).

Table-1: Baseline characteristics of patients

Characteristics	With endogloves	Without endogloves
Number of cases	130	130
Age (Years)	48.09±15.40	47.51±16.48
Male	65	69
Female	65	61
BMI (Kg/m^2)	26.20±4.74	26.26±4.48
Duration of symptoms (Months)	3.16±1.46	3.05±1.37

Table-2: Comparison of post-op wound infection in both groups [n (%)]

Post-op wound infection	With endogloves	Without endogloves	Total
Yes	2 (0.77)	9 (3.46)	11 (4.23)
No	128 (49.2)	121 (46.54)	249 (95.76)
Total	130	130	260

DISCUSSION

While treating a case of chronic calculus cholesystitis, surgery is always the final resort and laparoscopic cholecystectomy is considered better than open cholecystectomy due to short hospital stay, early recovery, less postoperative pain, good cosmetic results and early return to work. The rate of port site infection after laparoscopic cholecystectomy is lower than that of open cholecystectomy because laparoscopic procedures are minimally invasive and have less impact on the immune system than the open one.[7–8] Laparoscopic cholecystectomy is associated with spillage of gallstones in 5–40% cases.[9,10]

In a study by Narayanswamy and Prajwal[9] it was observed that using an endobag for retrieval of gallbladder resulted in less port site infection compared to retrieval without endobag. But it has its own disadvantages; to deliver a gallbladder in an endobag needs for extending the incision of facial layer and time of whole procedure also increases while using an endobag. We observed that it not necessary to increase the size of facial layer incision in every case, sometime we can retrieve gallbladder in endoglove by only finger dilatation of already present incision. In case the incision in facial layer is extended, it can be repaired with vicryl 1/0 meticulously to prevent incisional hernia.[3]

In our study the post-op wound infection was found in 11 (4.23%) patients in whom 2 were from with-endogloves group and 9 were from without-endogloves group. Statistically significant differences were found between both groups for post-op wound infection. A study conducted by Rehman UH *et al*[11] concluded that the frequency of port site wound infection in group where endoglove was used was 0.4% whereas in group where endoglove was not used was 5.5% showing that the frequency of port site wound infection was less with the use of endoglove removal of gallbladder during laparoscopic cholecystectomy. Moreover, port site wound infection was insignificantly associated with categories of age, gender and duration of cholelithiasis. Almost similar results were observed in our study which showed no significance of age or gender on wound infection but wound infection was less in group of patients in whom endogloves were used in comparison to patients in whom endogloves were not used for retrieval of gallbladder.

In a randomized control trial conducted by Kothapalli *et al*[12] it was concluded that the wound site infection in case of gallbladder retrieval using powdered sterile gloves is less in comparison to retrieval without it, but the time duration of retrieval increases using a glove. We too had similar observations that when a glove is used it definitely increases the time of the surgery. On the other hand when compared with morbidity of the patient due to wound infection and

financial burden on patients while treating a wound infection, it seems better to spend some more minutes to remove gallbladder in an endoglove. It also reduces the intraperitoneal septic complications. Intraperitoneal spillage of bile and gallstones, during dissection of gallbladder from liver bed and its retrieval without endobag are documented complications.[13,14]

Peponis et al[13] and Singh et al[15] stated that best way to avoid complications of spilled gallstones and port site contamination is to use endobag. Endobag facilitates collection of operative specimens and spilled gallstones, and minimizes the chances of contamination of the abdominal cavity and the retrieval port site, hence decreases the port site wound infection.[7,16] In our study almost similar results were observed that removing gallbladder in an endoglove decreases the chances of intraperitoneal spillage of bile and also decreases wound site infection. There was no significant impact of age or gender on wound infection in both groups of patients.

CONCLUSION

Endoglove technique of retrieval of gallbladder in pouch after laparoscopic cholecystectomy for chronic calculus cholecystitis potentially reduces port site wound infection compared to that without endogloves.

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ORIGINAL ARTICLE
LEVELS OF INTERLEUKIN-7 IN UNTREATED AND INTERFERON TREATED PATIENTS OF HEPATITIS-C: A CROSS-SECTIONAL STUDY

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Background: Hepatitis C is chronic viral disease that affects liver cells. Interleukin-7 (IL-7) is an important marker in adaptive immunity as it helps immunity to fight various infections as it acts as a growth and survival factor for T lymphocytes. It improves adaptive immunity towards viral infections like hepatitis C itself. In Hepatitis C patient, its role remains debatable. In our research, we studied the levels of serum IL-7 in Control group, interferon (IFN) treated and untreated groups of Hepatitis C in order to assess the effect of IFN treatment on the indicators used. Objective of this study was to measure and compare the levels of IL-7 in treated and untreated patients of Hepatitis C. **Methods:** After written, informed consent, 26 subjects in each group (Control, Untreated HC patients and Interferon Treated HC patients) of both sexes were recruited from all districts of Hazara Division. The PCR was done for viral load. Serum IL-7 level was measured using ELISA. Data obtained were analysed using SPSS-20 and was compared within three groups. **Results:** Mean serum levels of IL-7 measured in in treated group were higher as compared to untreated groups. No statistically significant difference was observed in the serum levels of IL-7 between any of the three groups studied. **Conclusions:** The study showed no significant differences on comparison in IL-7 levels within control, untreated and interferon treated groups. However, with a higher number of samples may define he role of IL-7 in HC.
Keywords: Hepatitis C; Interferon; Interleukin-7; patients; Comparison
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INTRODUCTION

Hepatitis C (HC) is a chronic disorder of health that affects approximately 184 million worldwide.[1] Pakistani population has a prevalence rate of about 5%.[2] Hepatitis C virus (HVC) has got four main genotypes that are 1–4 and more than 50 sub genotypes. Out of these genotypes, 3a is most common in Pakistan.[3]

INF is a cytokine that is normally produced during any infection inside the body by white blood cells. INF along with antiviral agent Ribavirin was the main stay in the treatment of HC but has been replaced by sofosbuvir recently.[4] HCV infection is diagnosed by using Enzyme linked immunoassay (ELISA) followed by quantitative and qualitative PCR. Quantitative PCR gives an estimated viral load or number of viruses in the blood of the affected person. IFN treated patient should ideally have undetected viral load at the end of treatment. ETR is the amount of viral load at the end of treatment and SVR is the viral load 24 weeks after treatment. ETR and SVR are the markers for the success of IFN treatment in any HC patient.[5] Nearly 80% of the HC infections end up in to chronic stage and only 20 per cent infections are spontaneously resolved with or without treatment in the acute stage.[6]

IL-7 is a cytokine that helps in the proliferation and survival of T lymphocytes in the blood thus may contribute to the eradication of viral infection by cell mediated immunity. IL-7 is the factor that may improve or help clearance of virus by promoting helper and cytotoxic T lymphocytes survival, function and maintaining their number.[7,8] Cytotoxic T lymphocytes are the main antiviral immune cells in human body. These are effective and higher in number at early stage of the disease however they lose their potential function and decrease in number as the disease progress. IL-7 plays a vital role in adoptive immunity and augments immunity to viral infections and tumors.[7]

IL-7 immunotherapy will be of substantial benefit in the treatment of HIV/HCV co-infection and should enhance the likelihood of HCV eradication in poorly responding patients.[9] HCV had got better response to interferon treatment that may be due to higher IL-7 levels.[10] This rationale led the current study and aimed to find and compare the level of IL-7 in treated and untreated patients of the HCV was measured and compared.

SUBJECTS AND METHODS

This cross-sectional study, based on a total of 80 adult subjects from Hazara division of Khyber Pakhtunkhwa province, was approved by the Ethical Committee and Research Board of the University of Health Science, Lahore. During 2017–18, Consecutive random sampling was used for recruiting the patients into the

study and informed written consent (Urdu language) was explained and signed by all subjects.

The study subjects were divided into the following three groups. Group I: Controls with normal LFT's and negative PCR for HCV (n=28). Group II: Untreated patients of HC that had no prior INF treatment (n=26). Group III: INF treated cases of HC who have recently completed their treatment (n=26)

Inclusion criteria was the untreated cases, diagnosed cases of HCV based on ELISA/PCR, adults of both sexes of 18–60 years, and fresh cases of HCV were included. Similarly, inclusion criteria for treated cases were diagnosed cases of HCV based on ELISA/PCR, adults of both sexes of 18–60 years of age and treated cases of HCV who completed interferon treatment in the last week. Patients who were obese, had BMI>25, diabetics, had Hepatitis A and/or B positive, and any other disease known to interfere with HCV were excluded from the study.

Sampling was done at Hepatitis Centre at DHQ Hospital, Abbottabad and the samples were carried to University of Health Sciences, Lahore with ice packs maintaining a cold chain. After 10–12 hours of overnight fast, 5 ml of blood was drawn from a superficial vein with aseptic techniques. Fasting blood glucose was checked with a glucometer (Xceed, Abbott®). Blood was secured in serum separation tubes (SST) vacutainers (yellow top) for extraction of serum. Serum was extracted by centrifuging the blood for 10 minutes at 3,000 rpm. The serum was put in aliquotes and stored at -80 °C till analysis. All other tests were conducted on serum. Serum IL-7 levels were measured using commercially available ELISA kits (Glory Science Co., USA®).

The data were analyzed on SPSS-20. Mean±SD was given for quantitative variables (age, IL-7 levels in serum). Frequencies and percentages were given for qualitative variables (gender, sample group). The data was given either as Mean±SD for normally distributed variables, or median interquartile range (IQR) for non-normally distributed variables.

The comparisons of various variables for association or significance were done to see any significant difference in their means/medians. In case of normally distributed data, single factor ANOVA was applied followed by Tukey's post hoc test for the three groups. Independent t-test was used for comparing means of two groups, and $p \leq 0.05$ was considered statistically significant.

RESULTS

Out of total 80 samples, 31 (38.8%) were male and remaining 49 (61.3%) were female. Median (IQR) age of male in control group was 31.50 (24.25–34.23) years, and 28.50 (20.00–38.75) years for female.

Level of IL-7 levels of control, untreated and treated group were 44.63±22.11, 35.11±14.68 and 37.87±17.66 respectively, whereas Median (IQR) was 39.95 (31.62–53.49) for controls, 32.80 (22.86–46.19) for untreated and 40.54 (20.05–51.91) showing no significant difference (Kruskal-Wallis test; p= 0.214) (Table-1).

IL-7 level of males in control group was 40.63±18.95 pg/mL and female 47.12±24.11 pg/mL showing no significant difference (Independent sample t-test; p=0.478). Serum IL-7 levels of males in untreated group were 32.50±17.03 pg/mL and for female it was 36.98±13.09 pg/mL showing no significant differences (Independent sample t-test; p=0.437 (Table-2).

There were no significant differences between levels of IL-7 of controls and treated/ untreated patients (Mann-Whitney U Test; p=0.624, and p=0.113 respectively). Also, no significant differences of IL-7 levels were found between treated and untreated cases (Independent sample t-test; p=0.528). Post hoc comparison of age for control and treated/untreated cases showed significant differences (Mann-Whitney U Test; p<0.001). There was non-significant negative correlation found between age and IL-7 in control group (Spearman Rho correlation; -0.226; Mann-Whitney U Test; p=0.267).

Table-1: Comparison of IL-7 between controls, treated and untreated groups by Kruskal-Wallis test (pg/mL)

Parameter	Controls (N=26)	Untreated Cases (N=26)	Treated Cases (N=28)	p
Mean±SD	44.63±22.11	35.11±14.68	37.87±17.66	0.342
Median (IQR)	39.95 (31.62–53.49)	32.80 (22.86–46.19)	40.54 (20.05–51.91)	

Table-2: Comparison of IL-7 among controls, untreated and treated groups [Mean±SD, Mean (IQR)]

Parameters	Gender	N	Controls (N=26)	p	N	Untreated Cases (N=26)	p	N	Treated Cases (N=28)	p
IL-7 (pg/mL)	Male	12	40.63±18.95 38.71 (27.65–53.49)	0.478	12	32.50±17.03 29.70 (17.43–42.89)	0.437	12	42.83±18.81 42.78 (27.17–57.56)	0.266
	Female	14	47.12±24.11 39.95 (32.80–68.88)		14	36.98±13.09 35.47 (25.33–7.45)		16	34.76±16.75 36.78 (20.05–51)	

DISCUSSION

According to best of our knowledge, there is no such study conducted previously anywhere in HCV patients for levels of IL-7 in untreated and treated HCV cases

to observe the effect of interferon treatment. In current study, there was notable difference in mean levels of IL-7 in controls untreated cases and treated cases but it was not statistically significant (p<0.342).

Lundstrom *et al*[12], reported serum IL-7 levels of 2–8 pg/mL in healthy individuals and as high as 60 pg/mL in lymphopenia. The level of IL-7 in our healthy control group were 44±22.11 pg/mL, while in untreated and treated groups the levels of IL-7 were 35.11±14.68 and 37.87±17.66 pg/mL respectively.

A non-significant correlation of IL-7 with age was found in controls and untreated subjects. This observation points to the fact that there is a decrease in immunity of the body with increasing age as decreasing IL-7 levels fail to maintain the number of T lymphocytes required for the immunity of the body. IL-7 contributes to both innate and acquired immunity by increasing proliferation and inhibiting apoptosis of T lymphocytes. According to Bolotin *et al*, IL-7 levels were lower in adults (2.82 pg/mL) as compared to children (4.8 pg/mL; *p*<0.05).[11]

CONCLUSION

No significant differences on comparison in IL-7 levels within controls, untreated and interferon treated groups and could not provide any mounting evidence as far as the function of IL-7 in Hepatitis C is concerned.

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ORIGINAL ARTICLE
FREQUENCY AND ANTIBIOTIC SENSITIVITY OF MOST COMMON ORGANISMS CAUSING URINARY TRACT INFECTIONS IN CHILDREN

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Background: Urinary tract infections (UTIs) are common and significant clinical condition in children. This study was conducted to determine the frequency of most common organisms causing urinary tract infections (UTI) and their sensitivity to various antibiotics in children admitted in a tertiary care health facility. **Methods:** In this retrospective study, 225 patients of both genders, aged 1–15 years and diagnosed as a case of UTI of any duration were included. Relevant data was collected from hospital electronic patients' record section. The data was analysed using SPSS-16. **Results:** There were 38% male and 62% female patients in the study. Mean age of the patients was 6 ± 1.26 years. Mean duration of symptoms was 3 ± 2.21 days. *Escherichia coli* was found in 45%, *Klebsiella pneumoniae* in 20%, *Staphylococcus aureus* in 18%, *Pseudomonas aeruginosa* in 11%, *Proteus mirabilis* in 3%, and *Enterococcus* in 3% of patients. All organisms were sensitive to most of the commonly used antimicrobial agents. **Conclusion:** The most common organism causing UTIs in our setup was *Escherichia coli*, followed by *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus mirabilis*, and these pathogens were sensitive to Ceftriaxone, Amikacin, Tetracycline, Ciprofloxacin, Augmentin, Ceftazidime and Nitrofurantoin. Resistance to these antibioticd was low in our setup.
Keywords: Micro-organisms, Children, Antibiotics, Urinary tract infection, Sensitivity, Resistance

INTRODUCTION

Urinary tract infection (UTI) is a combination of upper (infection of kidneys known as pyelonephritis) and lowers (infection of urinary bladder known as cystitis) urinary tract.[1] Urinary tract infection is defined as the growth of a single pathogen of $\geq 10^5$ colony forming units/ml of clean catch midstream urine.[2] UTIs are among the very prevalent infections which are diagnosed in infants and children presenting in outpatient department or admitted in the ward.[3] Throughout the world nearly 150 million cases of UTIs are diagnosed each year[4] and it causes about 6 billion US dollar cost yearly to the world economy.[5]

UTIs are a specially common infections in infant and children throughout world[6,7] but its frequency, sign and symptoms and the etiologic agents can varies depending upon the age and sex of the patients.[8] Urinary tract infections are usually asymptomatic. In young infants, symptoms include fever, vomiting, lethargy, nausea, decreased feeding and decreased urinary output.[9] in neonates the sign and symptoms of UTIs can be very non-specific as compared to other age groups.[10] The collective incidence of UTI shown by studies in children of up to 6 years of age is 3–7% in girls while its 1–2% in boys.[9]

According to the previous studies the commonest pathogen is *Escherichia coli* (*E. coli*), which is responsible for approximately 85% of UTIs.[11]

According to one study, *E. coli* was the most common pathogen responsible for urinary tract infections (74.6%), followed by *Klebsiella spp.* (11.7%)[12], *Staphylococcus saprophyticus* (6.4%), and *Proteus mirabilis* (7.02%).[13] A retrospective analysis showed Citrobacter isolates were found to be third most common organism causing UTI.[14]

E. coli is the most common pathogens causing UTIs throughout childhood from day 1 to 16 years age group followed by *Klebsiella* and *Pseudomonas aeroginosa*.[12] Results of a study on antimicrobial susceptibility analysis of *E. coli*, to commonly used antibiotics as the most common cause of UTI, showed amikacin sensitivity in 97.8% of cases, gentamicin in 97%, ciprofloxacin 94%, nitrofurantoin 87.1%, nalidixic acid 93.7%, trimethoprim-sulfamethoxazole 48.2%, cephalexin 76%, and ampicillin in 6.9% cases.[13] However, there are several recent studies which suggest that there is an emerging pattern of resistance toward the empirical antibiotics[15,16] particularly to ampicillin[17,18].

This study aimed at finding out bacterial etiologic agents responsible for urinary tract infection and assessing their pattern of *in vitro* susceptibility to most commonly used antimicrobial agents.

MATERIAL AND METHODS

This was a retrospective study conducted at Paediatric Department of King Abdullah Teaching Hospital

Mansehra from 15 June to 15 August 2019 after approval from Hospital Ethical Committee. Sample size with WHO sample size calculator came out to be 225 patients. Non-probability convenience sampling method was used for sample collection. Patients from both genders with age range 1–15 years, and diagnosed cases of urinary tract infection of any duration were included in this study. Patients aged more than 15 years, or having renal stones on ultrasonography were excluded. Relevant data was collected from hospital electronic patients' record section. The results were recorded on a proforma.

Data was analysed on SPSS-16. Mean±SD was calculated for age, duration, and type of urinary tract infection. Frequency and percentage were computed for qualitative variables like gender, identified organisms and antibiotics sensitivity.

RESULTS

In 225 patients 79 (35%) children were aged 1–5 years, 108 (48%) were 6–10 years, and 38 (17%) children were 11–15 years old. Mean age was 6±1.26 years. Out of 225 children, 85 (38%) were male and 140 (62%) were female. Duration of symptoms among 255 patients showed that 72 (32%) children had symptoms for 2 day, 90 (40%) had symptoms for 3 days and 63 (28%) children had symptoms for 4 days. Mean duration of symptoms was 3±2.21 days. Percentages of common microorganisms causing UTI in children is given in Table-1. The antibiotic sensitivity of these common uropathogens is shown in Table-2.

Table-1: Frequencies and percentages of common bacteria causing UTIs

Common Bacteria	Frequency	Percentage
Escherichia coli	101	45
Klebsiella pneumoniae	45	20
Staphylococcus epidermidis	40	18
Pseudomonas aeruginosa	25	11
Proteus mirabilis	7	3
Enterococcus	7	3
Total	225	100

Table-2: Antibiotic sensitivity of isolated pathogens causing UTIs [n (%)]

Organism Identified	Sensitivity/ Resistance	Antibiotics						
		Ceftriaxone	Amikacin	Tetracycline	Ciprofloxacin	Augmentin	Ceftazidime	Nitrofurantoin
E. coli	S	91 (90)	86 (85.1)	89 (88.1)	83 (82.1)	89 (88.1)	84 (83.1)	85 (84.1)
(n=101)	R	10 (9.9)	15 (14.8)	12 (11.8)	18 (17.8)	12 (11.8)	17 (16.8)	16 (15.8)
Staphylococcus	S	33 (82.5))	32 (80)	33 (82.5)	34 (85)	31 (77.5)	23 (57.5)	34 (85)
(n=40)	R	7 (17.5)	8 (20)	7 (17.5)	6 (15)	9 (22.5)	7 (17.5)	6 (15)
Pseudomonas	S	20 (80)	21 (84)	20 (8)	22 (88)	23 (92)	22 (88)	21 (84)
(n=25)	R	5 (20)	4 (16)	5 (20)	3 (12)	2 (8)	3 (12)	4 (16)
Klebsiella	S	40 (89)	41 (91)	40 (89)	39 (87)	41 (91)	40 (89)	41 (91)
(n=45)	R	5 (11)	4 (9)	5 (11)	6 (13)	4 (9)	5 (11)	4 (9)
Proteus	S	5 (71)	5 (71)	5 (71)	4 (57)	5 (71)	6 (86)	4 (57)
(n=7)	R	2 (28)	2 (28)	2 (28)	3 (42)	2 (28)	1 (14)	3 (42)
Enterococcus	S	5 (71)	5 (71)	5 (71)	4 (57)	5 (71)	6 (86)	4 (57)
(n=7)	R	2 (28)	2 (28)	2 (28)	3 (43)	2 (28)	1 (14)	3 (43)

DISCUSSION

In children, UTIs are a significant cause of morbidity and mortality. UTI is a combination of upper (infection of kidneys known as pyelonephritis) and lower (infection of urinary bladder known as cystitis) urinary tract.[1] Urinary tract infection is amongst the most common bacterial infection of childhood. Urinary tract infections are usually asymptomatic. Normally UTIs occur at a relatively higher frequency in girls than in boy. The estimated incidence of UTI in children by 6 years of age is 3–7% in girls and 1–2% in boys.[9]

In a study in Iran, *E. coli* was shown to be the most common cause of UTIs while *Klebsiella*, was the second most common cause which goes in agreement with our study. The reason behind *E Coli* being the commonest organism causing UTI in children may be due to faecal contamination in children which leads to *E Coli* UTI.[19] In a study by Pouladfar *et al*, *E. coli* was the most (51.5%) commonly reported uropathogens causing UTI followed by *Klebsiella* (16.8%) and *Enterococcus spp.* (9.9%).[20]

In a study in Nepal, *E. coli* was stated to be the commonest of uropathogens (53%) causing UTIs in children, while other bacteria were *Enterococcus faecalis* (22%), *Klebsiella pneumoniae* (7%) and *Staphylococcus aureus* (7%).[21] In this same study *E. coli* was extremely resistant to ampicillin, ceftriaxone and ofloxacin. Amikacin and nitrofurantoin were the maximally effective drugs for gram-negative rods while linezolid and vancomycin were active against gram-positive cocci.[21]

In a Malaysian study[22], *E. coli* was the leading (41.6%) pathogen, followed by *Klebsiella spp.* (21.2%), and *Enterococcus spp.* (11.0%). In that study *E. coli* was extremely resistant to ampicillin but sensitive to cefuroxime and gentamicin. *Klebsiella spp.* and *Enterococcus spp.* were also resistant to ampicillin. The difference between the results of this study and our study may be due to difference in ages of study populations or ethnic differences.

In a study by Sidra tul Muntaha *et al*[23], *E. coli* was the most common pathogen (72.26%) while *Klebsiella pneumoniae* (14.84%) was the second most common organism followed by *Staphylococcus saprophyticus* (10.32%); in 2.58% cases others pathogens were detected. All these uropathogens were sensitive to amoxicillin-clavulanic acid and trimethoprim-sulfamethoxazole. Mirsoleymani *et al*[24] in Iran revealed a high *E. coli* resistance rate to antibiotics which is contrary to our study.[24]

CONCLUSION

The most common organism causing UTIs in our setup was *Escherichia coli*, followed by *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*. These pathogens were mostly sensitive to ceftriaxone, amikacin, tetracycline, ciprofloxacin, augmentin, ceftazidime and nitrofurantoin.

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ORIGINAL ARTICLE
EFFECT OF VITAMIN D SUPPLEMENTATION ON LIPID PROFILE IN PATIENTS OF ACUTE CORONARY SYNDROME

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Background: Association studies have proposed a link between vitamin D levels and lipid profile. Deranged lipid profile is hallmark of acute coronary syndrome (ACS) patients. The present study was aimed to determine the role of vitamin D supplementation in improving the lipid profile of ACS patients. **Methods:** A total of 40 patients diagnosed with ACS were included in the study. They were divided into control (n=20) and experimental group (n=20). Experimental group received vitamin D supplementation as a single dose of 200,000 IU orally. Baseline vitamin D levels and lipid profile were done. Post sampling was done after an interval of 2 months for the same parameters. **Results:** The serum levels of cholesterol were decreased significantly in the experimental group ($p=0.05$) while TG levels showed trend towards a decrease ($p=0.084$). Serum HDL was increased ($p=0.03$), while serum LDL ($p=0.04$) and cholesterol ratio ($p=0.012$) were decreased significantly. **Conclusion:** Vitamin D supplementation improved lipid profile in ACS patients, and can be used as adjunct therapy in ACS patients.

Keywords: Vitamin D, Cholesterol, TG, HDL, LDL, Cholesterol ratio, Acute Coronary Syndrome

INTRODUCTION

Coronary artery disease (CAD) is one of the leading cause of morbidity and mortality in the world. According to World Health Organization, CAD causes 17.1 million deaths per year worldwide.[1] The most significant expression of CAD is Acute Coronary Syndrome (ACS). The underlying pathophysiological mechanism for acute coronary syndrome is coronary atherosclerosis.[2] Atherosclerosis is considered as a chronic inflammatory vascular disease which causes hardening of the arteries as a result of plaque formation.[3] It can subsequently lead to erosion, fissure or rupture of the plaque leading to thrombosis. The conventional risk factors for coronary atherosclerosis include hypertension, cigarette smoking, hypercholesterolemia, diabetes, obesity and genetic predisposition.[4] Research has proved atherosclerosis as an inflammatory vascular disease that is initiated after accumulation of lipids within the arterial walls. Hypercholesterolemia and other risk factors for atherosclerosis induce changes in the endothelium that leads to increased endothelial wall permeability and allows accumulation and aggregation of LDL-cholesterol within the arterial wall.[5] Besides the more common and traditional risk factors for development of acute coronary syndrome, studies are conducted to discover new risk factors towards which future therapeutic targets can be directed. Among these, vitamin D deficiency is a new emerging risk factor for development of acute coronary syndrome. Vitamin D deficiency has been demonstrated in patients suffering from acute myocardial infarction.[6] Observational studies have proposed a link between dyslipidemia and vitamin D deficiency.[7] A study by Kim MR *et al* showed that low vitamin D levels can progress to dyslipidemia and obesity.[8] A study by Ying Wang *et al* showed that serum concentrations of vitamin D are associated with serum lipids and atherogenic index of plasma (AIP).[9]

The pathogenesis of acute coronary syndrome involves the process of atherosclerosis which accelerates because of deranged lipid levels in serum. The studies linking the vitamin D deficient state with deranged lipid profile have revealed controversial results. Vitamin D deficiency has been proved by various researches to affect lipid profile. Correction of vitamin D levels among ACS patients may reduce the incidence of future adverse cardiovascular events. Data on supplementation impact on these parameters does not provide conclusive results. The incidence of ACS is rising despite vast srudies and therapeutic advancement. The present study is aimed towards highlighting the impact of vitamin D supplementation on serum lipid profile parameters in ACS patients.

MATERIAL AND METHODS

This non-randomized experimental study was conducted in the Department of Physiology and Multidisciplinary Laboratory, Islamic International Medical College, Rawalpindi in collaboration with Armed Forces Institute of Cardiology (AFIC). The study was approved by the Ethical Review Committee of Islamic International Medical College, Riphah International University, Islamabad along with approval from the Ethical Review Board of AFIC.

All patients were recruited from AFIC. They were approached from PCI units, wards, and CCU of the respective hospital. Informed written consent was obtained from patients who were willing to participate in the study. Complete history was recorded including complete personal profile along with present history of presenting complaints, history of the percutaneous coronary intervention procedure, past history, family history, assessments of risk factors for myocardial infarction and vitamin D deficiency. General physical examination was done including blood pressure, pulse rate, temperature and respiratory rate.

A total of 50 patients were interviewed for the study out of which 40 consented for the study. All these patients were diagnosed with ACS and had undergone percutaneous coronary intervention procedure. All patients below 70 years of age, not on any vitamin supplementation for the last 6 months and not having any renal impairment were included in the study. These patients were divided into control group (n=20) and experimental group (n=20).

Overnight fasting blood samples were taken from cubital fossa, and 4 ml blood was withdrawn using aseptic techniques. Samples were collected in serum vials with proper numbering and precautions keeping it upright and storage under cool temperature. Samples were centrifuged at 1,500 rpm for 5 minutes. The supernatant serum was collected through micropipettes in ependorf tubes with proper numbering on caps and were kept at -80 °C till analysis.

The experimental group (n=20) received vitamin D as single oral dose of 200,000 IU. Nothing was administered to the control group. The patients were reassessed on their follow up visits. After 4 months blood sampling was performed for same parameters. Pre and post sampling was done for serum lipid profile including total cholesterol, TG, HDL, LDL and TC/HDL ratio. The parameters including total cholesterol, TG, HDL were analysed in the laboratory using Microlab 300 analyser (ELITech Group). The concentration of LDL was determined by using a formula:

$$LDL = Total\ cholesterol - HDL - TG/5$$

The cholesterol ratio was calculated by dividing total cholesterol and HDL levels of the patients.

$$Cholesterol\ ratio = Total\ cholesterol/HDL[10]$$

Statistical analysis of the data was done on SPSS-21. Results of data were expressed as Mean±SD and percentages. Independent sample t-test was used to compare effect of vitamin D intervention between control group and intervention group, and $p \leq 0.05$ was considered statistically significant.

RESULTS

The baseline characteristics are presented in Table-1 which shows no significant differences between the two groups.

Table-1: Baseline characteristics of study participants

Variable	Control group (n=20)	Experimental group (n=20)	p
Age (Yrs) (Mean±SD)	53.70±11.50	54.10±8.03	0.799
Weight (Kg) (Mean±SD)	79.90±15.57	85.50±14.32	0.345
Smoking status			
Smoker	8 (40%)	12 (60%)	0.224
Non-smoker	12 (60%)	8 (40%)	
Diabetes			
Diabetic	7 (35%)	7 (35%)	1.000
Non-diabetic	13 (65%)	13 (65%)	
Hypertension			
Hypertensive	9 (45%)	11 (55%)	0.602
Normotensive	11 (55%)	9 (45%)	
Family history			
Positive	14 (70%)	13 (65%)	1.000
Negative	6 (30%)	7 (35%)	

In the control group the serum vitamin D levels were decreased significantly from 16.10±5.90 ng/dl to 10.20±3.99 ng/dl. In the experimental group, the serum vitamin D levels were increased significantly from 18.27±8.98 ng/dl to 30.74±18.40 ng/dl. The total cholesterol levels were increased in the post group from 163.65±52.9 mg/dl to 177.88±48.6 mg/dl. The experimental group showed a trend towards decrease from mean value of 214.95±60.61 mg/dl to a value of 174.05±44.75 mg/dl. The independent t-test comparing the effect of intervention among the post values of both the groups showed $p=0.05$ which was statistically significant. The mean serum TG levels in the control group was increased from 152.9±74.76 mg/dl to 157.75±67.07 mg/dl. The mean value in the experimental group showed a trend towards decease from mean value of 124.6±55.73 mg/dl to mean value of 94.6±54.34 mg/dl. The independent t-test comparing the post values of both groups showed insignificant results ($p=0.084$). The mean values of HDL changed from 37.95±20.6 mg/dl in the control group to the mean of 40.95±26.3 mg/dl. In the experimental group the mean value was increased to 66.6±21.25 mg/dl from mean value of 49.45±16.73 mg/dl. The independent t-test showed significant results ($p=0.03$). The mean of pre-control group for LDL came out to be 125.12±61.83 mg/dl which was increased to 135.42±55.54 mg/dl in the post-control group. The mean value of the pre-vitamin D group showed a trend towards decrease from 144.5±55.71 mg/dl to mean value of 88.53±45.43 mg/dl in the post-vitamin D group. The impact of intervention was compared by independent t-test. The two groups showed significant differences ($p=0.04$).

The calculated ratio was 6.06±5.21 in the control group initially which was high. On the other hand, the experimental group showed a pre-mean of

5.5±5.13 which was also high. In the control group the ratio increased to a mean value of 7.02±3.2 while the experimental group showed a significant decrease (p=0.012) with a mean value of 2.91±2.1. (Table-2).

Table-2: Serum levels of Vitamin D, Total Cholesterol, TG, HDL, LDL and Cholesterol ratio among control and experimental groups

Parameters	Control group		Experimental group		p
	Pre-levels	Post-levels	Pre-levels	Post levels	
Vitamin D (ng/ml)	16.10±5.9	10.20±3.99	18.27±8.98	30.74±18.40	0.000
Total Cholesterol (mg/dl)	163.65±52.9	177.88±48.6	214.95±60.61	174.05±44.75	0.05
TG (mg/dl)	152.9±74.76	157.75±67.07	124.6±55.73	94.6±54.34	0.084
HDL (mg/dl)	37.95±20.6	40.95±26.3	46.45±16.73	66.6±21.25	0.03
LDL (mg/dl)	95.12±61.83	135.42±55.54	141.5±50.71	88.53±45.43	0.04
Total Cholesterol/HDL ratio	6.06±5.21	7.02±3.2	5.51±5.13	2.91±2.1	0.012

TG= triglycerides, HDL= high density lipoproteins, LDL= low density lipoproteins

DISCUSSION

The present study has shown improvement in serum lipid profile after supplementation with vitamin D, with the exception of TG. Serum total cholesterol was decreased significantly in the experimental group. This finding is in accordance with a study in which type 2 diabetic patients were treated with a weekly dose of 16,000 IU vitamin D for 8 weeks and this correction significantly decreased total cholesterol levels and also produced a statistically non-significant reduction in LDL and TG.[11] Our results are in contradiction to some other studies which have reported that correction for deficiency of vitamin D levels may not be able to translate into meaningful alterations in the lipid profiles.[12] The possible explanation for these null results may be that vitamin D levels more than 28–32 ng/ml are needed for extra-skeletal benefit of supplementation.[13]

The randomized control trials presenting impact of vitamin D intervention on serum lipid profile have revealed controversial results.[14,15] These studies are divergent in terms of dose of supplementation used, duration of study, disease state of the body, and increment of vitamin D levels after supplementation. Vitamin D may have direct effects on lipid metabolism or it may affect the lipid profile indirectly. Direct effects according to some studies, involve maintaining adequate levels of apolipoprotein A-I which is an important component of HDL cholesterol.[16] HDL cholesterol has also been positively correlated with vitamin D levels. The indirect effects of vitamin D on lipid metabolism can be attributed to its effects on calcium and phosphate metabolism. Vitamin D can enhance insulin sensitivity and its release affecting lipid metabolism through hormonal regulation.[16]

Vitamin D has been involved in reducing expression of adipocyte uncoupling protein-2 and regulates lipid metabolism by inhibiting lipogenesis and inducing lipolysis. The reduction of proliferation of T-helper cells also reduces generation of important cytokines involved in regulation of fat metabolism.[11]

The present study showed insignificant reductions in levels of TG in experimental group. In some of the previous studies vitamin D supplementation had proved beneficial effects on serum lipid profile parameters including total cholesterol and TG.[14] Vitamin D was able to reduce the serum levels in patients with hypercholesterolemia. The insignificant results of serum triglycerides are similar to study conducted by Ponda MP *et al*[12] which was highly attractive cost-effective approach. These results showed an unfavourable lipid profile with vitamin D deficient state. On improving vitamin D levels non-significant changes in the LDL and TG levels have been reported.[17]

Serum HDL in the present study showed significantly increased level in Experimental Group (p=0.03). The intervention of vitamin D was able to raise sufficiently the HDL levels to 66.6 mg/dl. Previous studies have demonstrated a positive association between serum vitamin D levels and HDL in which improved vitamin D levels were linked with sufficient HDL level.[18] HDL-C was also found to be significantly high in vitamin D sufficient group while reduced in vitamin D deficient group in a study performed by Alkhatatbeh *et al*[19]. On the other hand some studies also have revealed controversial results. A randomized control trial by Ponda MP *et al*[20] showed that short term repletion of vitamin D may not be able to improve serum lipid levels including HDL levels.

Serum LDL levels in our study were reduced significantly. In a study performed on children suffering from non-alcoholic fatty liver (NAFL), the association of vitamin D deficiency with LDL was found to be negative and significant.[21] These results are contrary to the results of previous trials which proved an increase in the LDL level on supplementation with vitamin D.[14,22] Association of vitamin D levels with the serum lipid profile discussed in the observational studies do not show a causal relationship. A study by Kane L *et al*[23] has explained that the vitamin D if given along with atorvastatin, a lipid lowering drug, in vitamin D insufficient subjects, can lead to significant reductions of total cholesterol and LDL levels. This might be the case in the present study that patients were on statin drugs, and vitamin D might have augmented the lipid lowering effect.

Serum lipids can be influenced by various factors like diet, and exercise etc. The randomized control trials have revealed controversial results.[14,15] All these were heterogeneous in terms of vitamin D dose

used, duration of treatment, baseline vitamin D and lipid profile, and characteristics of study population. Many studies were not able to indicate baseline vitamin D levels and percentage increment of vitamin D with supplementation. The form of vitamin D used whether D_2 or D_3 is also important. Vitamin D_2 biologically has reduced activity as compared to the more active form, vitamin D_3. These factors can influence the results.

The present study showed significant results in improving the cholesterol ratio. The cholesterol ratio was decreased significantly from 7.02 ± 5.99 to 2.91 ± 1.33. According to American Heart Association the ideal ratio is 3.5, while the cardiovascular risk increases with increase in ratio of >5. TC/HDL ratio seems to be a strong indicator and powerful predictor of acute coronary events.[10] Vitamin D may be involved by multiple mechanisms improving lipid profile. It may serve as lipid lowering agent in patients of ACS specially who are on statin drugs. Its use as an adjunctive therapy may be considered in future research.

CONCLUSION

Vitamin D supplementation showed promising results in improving the lipid profile parameters resulting in significant reductions in total cholesterol, low density lipoproteins and cholesterol ratio while significant rise in high density lipoproteins levels when given along with statin drugs. Triglyceride levels were non-significantly reduced. Giving vitamin D as an adjunct therapy in the form of single high dose can help improve these parameters in patients of ACS.

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ORIGINAL ARTICLE
SERUM LEAD LEVELS IN FEMALE AGRICULTURAL FARM-WORKERS FROM BAHAWALPUR, PAKISTAN

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Background: Lead (Pb) is a heavy metal present in our environment in soil, drinking water, air, petroleum products, insecticides and pesticides, and is excreted in body secretions like breast milk, saliva, semen, and sweat. We studied serum lead levels in lactating female farm workers of Bahawalpur who are exposed to various chemicals that contain lead arsenate. **Methods:** This study was conducted in the Department of Physiology, University of Health Sciences, Lahore after approval from the Ethical Review Committee. After informed consent, blood samples of 2.5 ml each from a total of 91 lactating mothers were collected. Serum lead levels were determined by Inductively Coupled Plasma Spectrophotometry (ICP). **Results:** High lead levels were detected in 90 out of 91 (98.90%) women. Mean serum lead levels were 61.05±29.59 µg/L. **Conclusion:** The serum lead level was considerably higher than the WHO allowable level in our study population.
Keywords: serum, lead, farm workers, female

INTRODUCTION

Toxic heavy metals are ever persistent pollutants in the environment and can cause serious health hazards. Human contact with toxic metals and other environmental contaminants has increased significantly over the last few years because of industrialization, urbanization and other anthropogenic activities like food adulteration and refinement, and use of food additives.[1] In the third world and less developed countries, an additional source of metal contamination is the problem with cleanliness and removal of garbage and contamination of drinking water with sewer lines, use of cheaper cooking utensils and household metal containers used for food storage.[1]

Lead is a recognized neurotoxic heavy metal.[2] It exists as an elemental form in nature. Levels of lead in soil range between 50 and 400 ppm. However, WHO acceptable lead levels in drinking water is 10 µg/L or parts per billion (ppb).[3] Considerable rise in lead levels in the environment are due to mining, smelting and refining activities. Effective preventive measures are adopted to decrease adverse effects in primeval rural areas.[4]

Lead gets entry into the body mainly through inhalational and gastrointestinal routes.[5] The main route of lead exposure is inhalation of lead compounds in different occupational workers. Larger than 2.5 µm particles deposit in nasopharyngeal and tracheobronchial ciliated epithelium which can be transported to the oesophagus and are swallowed. Oral route is major route of exposure for the general population. Organic lead is well absorbed through the skin also.[6]

For young children <10 µg/dl of blood lead is considered safe. However, currently, there is no confirmed safe concentration of lead in blood. Adverse health effects can occur even at lower concentrations.[7]

Maximum amount of inhaled lead is absorbed. Absorption of lead through gastrointestinal tract is higher in children (40–50%) compared to adults (3–10%). Food present in GIT decreases absorption of lead. Lead accumulates first in blood and then makes its way to the bone. In children 73%, and in adults 94% of lead is present in bone. Half-life for inorganic lead in blood is 30 days and in bones it is 30 years.[8] The main routes of excretion of lead are urine and feces.[9] Lead is bio-accumulated in bones and resides in the body for a long period of time. Calcium from bone is released along with lead during pregnancy and lactation, causing higher lead levels in the blood.[10]

Levels of lead in human blood or serum have been studied around the globe. Farm workers of Southern Punjab, Pakistan are exposed to soil over which pesticides, insecticides and weed killers are sprayed. These chemicals contain lead in the form of lead arsenate[11] and that may cause higher levels of lead in the serum. Because of the importance of lead in human life, and as it is not studied in Southern Punjab, Pakistan, we designed this study for the rural population of Bahawalpur, Pakistan.

MATERIAL AND METHODS

This descriptive study was conducted in the Department of Physiology and Cell Biology, University of Health Sciences (UHS), Lahore in collaboration with the Chemistry Department, University of the Punjab, Lahore. The study was approved by the Ethical Review

Committee of University of Health Sciences, Lahore. Sample size was determined as 90 using the formula:

$$n = \frac{\sigma^2(Z_{1-\alpha/2} + Z_{1-\beta})^2}{(\mu_1 - \mu_2)^2}$$

A total number of 91 lactating female farm workers working in the farms for at least 1 year in the last 3 years were included in the study. They were permanent residents of rural areas adjoining Bahawalpur City. Participants taking drugs that can chelate metals like Ethylenediaminetetraacetic acid (EDTA) and Dimercaptosuccinic acid (DMSA) etc., tobacco smokers and those taking any drug that can increase body metabolism were excluded from the study. After taking informed written consent, information was entered on a specially designed proforma.

Blood sample of 2.5 ml each was taken under aseptic conditions and serum was separated by centrifugation and stored till final analysis through Inductively Coupled Plasma Spectroscopy (ICP) on Optima 2100-DV, Perkin Elmer. For serum sample preparation for ICP analysis, 1 ml of serum was taken in a lead free polypropylene tube and it was diluted with distilled deionized water to make it up to 5 ml.

Standard solutions of lead with concentrations of 25, 50, 75 and 100 ppb were prepared. Prepared standard solutions and diluted serum samples were subjected to ICP-OES (Optical Emission Spectrometry) at wavelength of 220.353 nm for detection of serum lead. The minimum detection limit of ICP machine used was 0.10 ppb. The readings of serum samples were multiplied with dilution factors to get the final serum levels. The data was entered into and analyzed on SPSS-20. Normal distributions of data were checked with Shapiro-Wilk's statistics. Serum lead levels were compared with the recommended WHO serum lead levels.

RESULTS

The mean age of all participants was 25.55±4.91 years. Majority (79.10%) of them were illiterate. All of these women had been working at the agricultural farms for a mean duration of 5.79±2.95 years. Serum lead levels were estimated in all serum samples of the participants (n=91). Higher than WHO allowable lead levels were detected in 90 (98.90%) serum samples and the mean serum lead levels were 61.05±29.59 µg/L. (Table-1).

Table-1: Estimated levels of lead in serum of female agricultural farm-workers compared with WHO permissible values (n=91)

Participants having high lead in serum	Serum lead levels (Mean±SD)	WHO permissible serum lead levels[6]
90 (98.90%)	61.05±29.59* µg/L	<10 µg/L

*p<0.001

DISCUSSION

Some early investigators from various parts of United States of America (USA) reported different levels of serum lead, i.e., as low as 1.4 µg/L to as high as 119 µg/L.[12–14] Kovar *et al*[15] using 28 patients from Central London Maternity Hospital reported blood lead levels of 0.49 µg/L. Ong *et al*[16] reported lead concentrations 0.73 µg/L in blood in a multi-racial sample of women from Malaysia. They had a sample size of 114. These women were not occupationally exposed to lead. On the other hand, Li *et al*[17] reported lead levels of 132 µg/L in cord blood from Chinese women. The sample size in that study was 165 and women from Shanghai were occupationally non-exposed to lead. The Chinese found gradual rise in blood lead level during 1980 to 1996. After introduction of lead-free gasoline for use in vehicles the blood lead level dropped gradually after 2016. Kulkybaev *et al*[18] showed that the levels of lead were as low as 0.51 µg/L in the blood of a small sample from Russian natives. Khan *et al*[19] determined higher levels of lead in exposed population (industrial workers and traffic police constables) which was higher than their control group. Thus, it is clear that the blood lead levels in people excessively exposed to lead are higher than the WHO allowable levels.

In our study, the mean serum lead levels among the female farm workers were found as 61.05±29.59 µg/L. The samples taken were from that of an environmentally exposed population. Table-2 shows levels of serum lead in different parts of the world.

Table-2: Levels of lead in women's blood from different countries

Country	Reported Blood Pb levels
Pakistan (This study)	61.05±29.59 µg/L
USA[12–14]	1.4–119 µg/L*
UK[15]	0.49 µg/L*
Malaysia[16]	0.73 µg/L*
China[17]	132 µg/L*
Russia[18]	0.51 µg/L*

*Reported levels converted to µg/L

Mean serum lead levels in occupationally exposed female rural population of Bahawalpur was 61.05 ppb, which is far higher than the WHO permissible limits. This fact is of great concern as higher levels of serum lead in lactating mothers may affect the children of these workers and may cause heavy metal overload and poisoning in these breast-fed babies. It necessitates a specific probe into the adverse developmental effects of lead in children of this vulnerable population.

CONCLUSION

The serum lead level in female agricultural farm workers in Bahawalpur area is considerably higher than the established WHO permissible limits of <10 µg/L.

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MAR: Conception of work
MIK: Interpretation of data
MWKS: Drafting of work
TI: Analysis of data
ST: Critical review
MA: Critical review

ORIGINAL ARTICLE
SPUTUM CULTURE AND POLYMERASE CHAIN REACTION TESTS FOR IDENTIFICATION OF BACTERIAL PATHOGENS IN EXACERBATION OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Background: Chronic Obstructive Pulmonary Disease (COPD) is a growing pulmonary disorder comprised of chronic bronchitis and emphysema. This condition is characterized by breathlessness, decrease immunity, and recurrent respiratory tract infections (RTI). The objective of this study was identification of predominant bacterial pathogens in respiratory tract, responsible for acute exacerbation of chronic obstructive pulmonary disease (COPD) patients using highly sensitive real-time Polymerase Chain Reaction (PCR) and conventional methods. **Methods:** The retrospective study was conducted in tertiary care hospitals of Karachi, 120 diagnosed regular follow-ups of chronic obstructive pulmonary disease (COPD) patients with informed and written consent aged between 40–55 years from both sexes were included in the study. All samples were analyzed through sputum culturing and PCR assays. **Results:** Sixty stable state samples and 60 aggravated condition samples were collected from subjects on treatment for the chronic obstructive pulmonary disease (COPD). Stable state samples were negative for Chlamydophila pneumonia, and Mycoplasma pneumonia. However, samples showed the presence of *Moraxella*, *Streptococcus*, *Nocardia*, *Lactobacilli*, and *Legionella*. In aggravated state samples *Haemophilus influenza*, *Staphylococcus aureus*, *Staphylococcus pneumonia*, *E. coli*, *Lactobacillus*, *Bifidobacteria*, and *Nocardia* were observed. **Conclusion:** The current findings suggest that there is a unique relationship between bacterial pathogens and the recurrence of the severity of symptoms in COPD. To understand the diversity of atypical and typical microbiota it is important to utilize sensitive advanced techniques for routine investigations of the COPD population.
Keywords: Chronic Obstructive Pulmonary Disease, COPD, Haemophilus, Moraxella, Nocardia, Legionella, Bifidobacter

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD)[1] is a chronic respiratory disease characterized by progressive, debilitating respiratory conditions, including emphysema and chronic bronchitis, characterized primarily by dyspnoea, lung airflow limitations, cough, sputum production, and other symptoms.

Chronic obstructive pulmonary disease is a major cause behind a heavy health and economic burden around the world.[2] According to the Global Burden of Diseases Study (GBD) 2017 reports[3], COPD resulted in 3.2 million deaths. It accounted for 81.6 million disability-adjusted life years (DALYs).[3]

Exacerbation of chronic obstructive pulmonary disease is mainly due to increased respiratory tract inflammation. Patients suffering from acute COPD exacerbation have impaired health status, a higher risk of lower airway bacterial colonization, and accelerated lung function decline.[4] It was observed that more than half of COPD patients were infective, and bacteria were isolated from 70% of their samples.[5]

Among various bacterial species involved in infectious exacerbations, the most common bacteria are *Haemophilus influenza*, followed by *Streptococcus pneumoniae* and *Moraxella catarrhalis*.[6] However, atypical pathogens like *Mycoplasma pneumonia, Chlamydia pneumonia,* and *Legionella pneumophila* may not play important role in acute exacerbation of COPD.[7]

Conventional methods have proved to be instrumental in identifying that bacterial pathogens play a pivotal role in the recurrence of COPD symptoms. Sputum culture has identified bacteria, which account for 30–50% of COPD exacerbations.[8] However, due to its limited ability, we have also used sensitive real-time Polymerase Chain Reaction (PCR) assays. PCR can detect tiny fragments and small fragments of nucleic acids of all pathogens, and it is not dependent upon the capability of the target microbe.

This study is based on analysis of respiratory tract samples by comprehensive real-time polymerase chain reaction to detect bacterial species together with conventional methods, including sputum culture. It focuses on the evidence that the recurrence of the severity of symptoms is due to the presence of bacterial etiological agents.

METHODOLOGY

This retrospective, randomized double-blind study was aimed to analyze 120 clinically diagnosed COPD patients enlisted from tertiary care hospitals of Karachi having age ≥40 from 21 Feb to 17 Aug 2018. Sample of Sputum was collected from the outpatient department and from inpatients who were admitted in wards due to severity of illness at Sindh Government Lyari General Hospital. Written and informed consent from COPD patients was taken.

Data was collected from each patient and recorded on a pre-tested questionnaire. The questionnaire included duration of hospitalization, smoking habit, fever, antibiotic usage, and duration of symptoms before admission. Variables included for the study were age, sex, signs, and symptoms of the patient.

Subjects were divided into two equal groups. Group-A consisted of 60 randomly selected COPD subjects with acute severity of symptoms. The sputum samples of this group were marked as aggravated state sputum. Group-B consisted of 60 COPD subjects with no acute severity of symptoms their sputum samples were marked as stable state sputum.

After rinsing their mouths, the COPD patients were instructed to collect early-morning, deeply coughed sputum samples into a sterile wide-mouth container with a screw cap. All the sputum samples were incubated at 37 °C for 15 min with a volume of 0.1% dithiothreitol was added. Then each sample was divided into two equal portions: one part was utilized for gram staining and culturing sputum samples, while the other was subjected to two different PCR techniques. Each portion had $<10^5$ epithelial cells/mL which is equal to <1 epithelial cell per high-power field.

The collected sample was subjected for culture and DNA extraction within 4–5 hours. Two hundred (200) µL sputum sample was treated using NucliSens® easyMAG™ (bioMérieux, USA) according to the manufacturer's instructions. Five (5) µL sputum samples were utilized as a PCR template. DNA was stored at -20 °C until PCR was done.

For identification of *E. coli*, *Lactobacillus*, *Bifidobacteria*, *Legionella*, and *Nocardia* two different assays were utilized directed on explicit zones of 5S *r*RNA and macrophage infectivity potentiator (*mip*) gene. PCR-probe assay for *E. coli*, *Lactobacillus*, *Bifidobacteria*, *Nocardia*, and *Legionella*, are based on the primers, and Leg5S detected in real-time using a TaqMan probe, Netherlands. For identification of *Haemophilus influenza*, *Staphylococcus aureus*, and *Staphylococcus pneumonia* an assay was performed using 2 and 4 outer membrane protein, variable nucleotide sequence. This PCR was based on the sequences of the *mip* gene, P1 adhesion gene assay. Sixty-six base pairs amplicons were extracted using forward primers 5′-TGG TAA CTG CCC CAC AAG C-3′ and 5′-GGT CAA TCT GGC GTG GAT CT-3′. Fluorescent labelled 6-carboxyfluorescein Taqman probe 5′-TCCCCC GTT GAA AAA GTG AGT GGG T-3′ was used for Real-time PCR.

All the samples were processed for DNA extraction using both Qiagen and microLYSIS techniques. This was done to detect every possibly available bacterial DNA present in the samples and avoid missing microbiota.

This research was conducted using open epic software. Statistical analysis was done using SPSS-20. The data of categorical variables were presented as counts and percentages. Descriptive frequencies were used for analyzing all categorical data.

All clinically diagnosed cases of COPD and acutely exacerbated COPD formed the subject of the study group. Patients with previous diagnosis of any active lung disease, on treatment with oral steroids, pulmonary disease of allergic origin, and patients on antibiotics before hospitalization were not included in the study.

RESULTS

One hundred and twenty (120) samples were collected from COPD patients from the outpatient and inpatient Departments of Sindh Government Lyari General Hospital. Diagnosed patients of age ≥40 years with COPD were divided into two groups based on the severity of symptoms. Group-A included 60 randomly selected COPD subjects with acute severity of symptoms. Group-B included 60 randomly selected COPD subjects with no acute severity of symptoms (Table-1).

Table-2 compares bacterial detection capability of culture assay and PCR technique. The detection capacity of simple culture techniques was less than that of PCR. In Group-A (Aggravated State Sputum) the efficacy of culture techniques were 18/60 (30%) in comparison with PCR having a detection capability of 45/60 (75%). In Group-B (Stable State Sputum) the detection capacity of the culture technique was 27/60 (45%) and PCR was able to detect 40/60 (66%) cases. On comparing results of both groups the PCR method had better detection capacity as compared to conventional culture techniques.

DNA was extracted in triplicates, from each pure culture using the Qiagen and the microLysis techniques. 16S qPCR assays were performed to enumerate the genome/ml readings from the extracted DNA. CFU/ml counts were performed. Results depicted that microLYSIS technique towards the detection of atypical bacteria is slightly better than Qiagen. However, the study was unable to identify any major differences in detection of bacteria between both techniques. (Table-3).

Figure-1 represents the gender-wise group distribution on its X-axis, while it shows the log (CFU/ml and Genome/ml) on its Y-axis. The different colour bars show the type of bacteria being identified in each group.

In group A the male gender showed a high presence of *Nocardia* along with *Homophiles*, *E. coli*, *Moraxella*, *Bifidobacteria*, and *Lactobacillus* (highest to lowest order). In group A the female gender showed *Nocardia* along with, *Moraxella*, *Homophiles*, *E. coli*, *Lactobacillus*, *Streptococcus*, and *Bifidobacteria* (highest to lowest order).

In group B the male gender showed a high presence of streptococcus along with *Homophiles*, *Moraxella*, *E. coli*, *Nocardia*, *Bifidobacteria*, and *Lactobacillus* (highest to lowest order). In group B the female gender showed *Homophiles* in highest percentage along with *E. coli*, *Moraxella*, *Streptococcus*, *Nocardia*, *Bifidobacteria*, and *Lactobacillus* (highest to lowest order).

Table-1: General characteristics of study population

Gender	Age Group	Average Age	Occupation	Residence	Past personal habits/ Occupational history	Therapeutic History
Group-A Aggravated State Sputum (n=60)	Males (n=35)	55	Factory workers/ Skilled Labourers	Karachi	Smoker+Occupational	Non Compliant/Symptomatic treatment
	Females (n=25)	50	House wives	Karachi	Gutka+Huqqa+Biomass Fuel stove	Non Compliant/Symptomatic treatment
Group-B Stable State Sputum (n=60)	Males (n=31)	50	Factory workers/ Skilled Labourers	Karachi	Gutka+Huqqa	On regular treatment+follow up
	Females (n=29)	42	House wives	Karachi	Gutka+Huqqa+Biomass Fuel stove	On regular treatment+follow up

Table-2: Comparison between Conventional microbiological results and Molecular microbiological results

Groups	CULTURE (n=120)	PCR (n=120)
Group-A Aggravated State Sputum (n=60)	18/60 (30%)	45/60 (75%)
Group-B Stable state sputum (n=60)	27/60 (45%)	40/60 (66%)

Table-3: Comparison between QIAGEN and Microlysis DNA bacterial detection

Pure cultures	QIAGEN	Microlysis
Haemophilus	2.144E+8	2.490E+9
Moraxella	1.899E+8	2.011E+9
Nocardia	1.759E+8	2.595E+9
E. coli	1.251E+8	2.112E+9
Lactobacilli	2.679E+8	3.214E+8
Streptococcus	3.203E+8	2.114E+8
Bifidobacteria	2.858E+8	2.254E+7

E=Exponent

Figure-1: Percentile of atypical bacteria detected in both groups
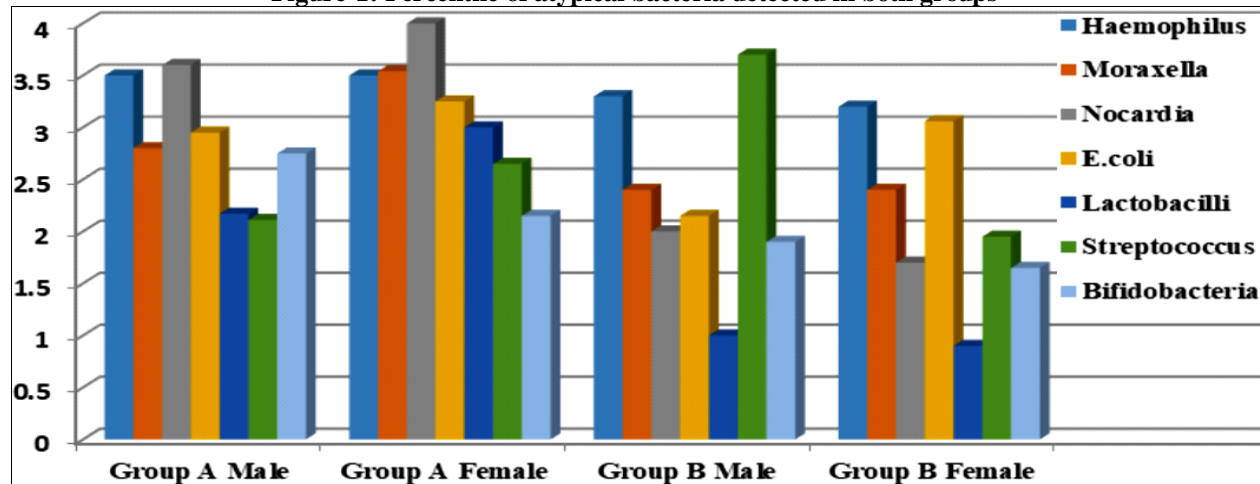
X-axis: Group Division gender-wise, Y-axis: log (CFU/ml and Genome/ml)

DISCUSSION

Chronic obstructive pulmonary disease exacerbation is one of the major causes of morbidity in chronic respiratory diseases patients.[3] It has a strong economic burden and also strongly influences health-related quality of life (HRQL).[9] This study was conducted to delineate the most common typical and atypical bacterial agents involved in COPD exacerbations.

Previous reports have shown the association between acute COPD exacerbation and the presence of bacterial pathogens by using only a single procedure, either conventional microbiological assays (sputum culture) or Polymerase chain reaction (PCR), while the use of a combination of techniques was extremely occasional. The current study was conducted using the combination of both the culture and the PCR assays.

When conventional culture techniques were compared with real-time PCR it was observed that the accuracy of real-time PCR was slightly superior to the conventional techniques. These findings were quite similar to the findings of Shimizu K et al[8]. Only a few differences were observed in the capacity of both techniques.

Culturing sputum samples is considered the first-line investigation, for exploring the pathogenesis of exacerbations of COPD. They are cost-effective and provide the pathogens to be studied further. However, when used alone, sputum culture technique has many limitations.[10] Researchers like Aydemir et al[11], Halder et al[12], and Perotin et al[13] had also used PCR methods along with culture techniques to compensate for the shortcomings of conventional methods.

Talking about PCR assays, on comparing its different DNA extraction techniques, the most commonly used techniques were Qiagen and microLYSIS. It was observed that both techniques have almost the same efficacy in the identification of microbiota. However, the capacity of the microLYSIS technique towards the detection of atypical bacteria is slightly better than Qiagen. The focus of this study was to examine the predominant bacterial (typical or/and atypical) pathogens associated with exacerbation of COPD.

It was an astonishing observation that *Nocardia* became the predominant pathogen log/ml in both sexes of the group suffering from acute exacerbation of symptoms. It is one of the most transmitted bacteria by inhalation or aspiration.[14] It particularly affected immunocompromised patients. In recent research by Garcia-Bellmunt L et al[15] an increase in *Nocardia* infection cases has been reported among patients with COPD. Though the factors behinds its association with exacerbation of COPD is unknown, it was observed that corticosteroid therapy was prevalent amongst individuals diagnosed with *Nocardia* infection.[16]

Another important finding in Group-A is *Haemophilus*, *Moraxella*, and *E. coli* in high percentage log/ml in the acute exacerbation group. According to Sethi S[17], three predominant bacterial species were isolated from patients experiencing an exacerbation of COPD. Those three pathogens were *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Streptococcus pneumoniae*. These three species were also among the most common pathogens responsible for other respiratory mucosal infections. *H. Influenzae* has also been reported to be the most common isolate.[18]

Monso et al[19] concluded that the potential bacterial pathogens cultured from sputum during COPD exacerbations were less frequently cultured during the period of clinical stability of the COPD patient. In the current study, it is well established finding that in the acute exacerbation phase bacteria for the respiratory tract such as *Moraxella*, *E. coli*, and *Lactobacillus* were identified in the group with recurrence of acute exacerbation of symptoms in both sexes. *Lactobacilli* and *Bifidobacteria* were also identified in more than normal percentage log/ml in the acute exacerbation group. Research by Sethi et al identified differences in culture densities of *Haemophilus influenza, Moraxella catarrhalis*, and *Streptococcus pneumonia* in sputum collected during acute exacerbations compared to samples during clinical stability.[20] *Lactobacilli* association with acute exacerbation of COPD was also observed during the research. This finding is supported by Sze MA et al, who also detected an increase in the *Lactobacillus* genus (i.e., *Firmicutes* community) in severe COPD exacerbation.[21]

The relationship between COPD exacerbation and acute bacterial infection of the respiratory airway is still debatable. However, these findings indicate the probable positive association of these pathogens with recurrent acute exacerbations of symptoms of COPD patients in our subjects.

It has been observed that one of the major issues in the non-uniformity of data is due to regional variation[22,23] and segregated methodology for identification of pathogens. This lack of standardization has lead to the variation of published data concerning COPD. The mechanism which can be postulated is the use of random medication and antibiotics along with the non-quantified dosage of steroid-based drugs. Guidelines for antibiotic therapy for COPD patients have been published by many researchers like Llor C et al[24], but these cannot be applied on a worldwide basis due to geographical variations in antibiotic sensitivity and pathogen types. However, judicious use of antibiotics is crucial to avoid the emergence of multidrug-resistant bacteria.

CONCLUSION

The PCR assays showed more sensitivity toward detecting bacteria in sputum samples than the conventional assays. *Nocardia* emerged as a major pathogen, irrespective of patient's gender. Moreover, the bacteria like *Bifidobacteria, Moraxella, E. coli*, and *Lactobacillus* were identified in the group with the recurrence of acute exacerbation of symptoms. *Haemophilus* was among some majorly found bacteria. Bacteria are the major cause of acute COPD exacerbation. Regional variation and the difference in methodology for identification of pathogens were some of the main reasons behind the non-uniformity of data. The researchers should increase usage of novel molecular techniques along with other conventional detecting methods for better detection of bacterial communities.

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ORIGINAL ARTICLE

RISK FACTORS OF PREMATURE RUPTURE OF MEMBRANES IN AYUB TEACHING HOSPITAL: A CASE CONTROL STUDY

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Background: Premature rupture of membranes (PROM) is one of the important causes of preterm delivery and is associated with increased incidence of neonatal and maternal morbidity and mortality worldwide. The aim of this study was to find out the risk factors associated with PROM in patients admitted to Obstetrics Ward of Ayub Teaching Hospital, Abbottabad. **Methods:** This case control study was conducted on pregnant females (>28 weeks gestation) from 1st November, 2018 to 30th May, 2019. The sample size was 195 patients (130 controls, 65 cases in 2:1 ratio) collected through convenience sampling. Data was analysed using SPSS-16. Odds ratio with confidence interval was used to estimate the strength of association of PROM with its risk factors, and $p \leq 0.05$ was considered significant. **Results:** The mean age of the patients was 25.48 ± 3.65 years ranging between 19–38 years. Most of the patients belonged to the age group 26–35 years. Most women were illiterate, unemployed, and had poor socio-economic background. The most important risk factors of PROM were PROM in previous pregnancies (OR=3.978, CI=1.484–10.666, p=0.012), foul smelling vaginal discharge (OR=2.700, CI=1.197–6.089, p=0.014), polyhydramnios (OR=2.5, CI=1.178–5.404, p=0.015), and vaginal bleeding (OR=2.486, CI=1.117–5.534, p=0.023). **Conclusion:** PROM in previous pregnancies, foul smelling vaginal discharge, vaginal bleeding, and polyhydramnios were found to increase the risk of PROM significantly.

Keywords: PROM, PPROM, pregnancy, risk factors, case control study

INTRODUCTION

The PROM is defined as 'rupture of membranes at any time before the onset of uterine contractions'.[1] It is one of the common complications of pregnancy and occurs in 8–10% of pregnancies approximately.[2] Premature rupture of membranes, especially preterm PROM (PPROM) is one of the important causes of preterm delivery and is associated with increased incidence of neonatal and maternal morbidity and mortality worldwide.[3] PROM can be divided into preterm PROM and term PROM. Preterm PROM occurs after 28 weeks and before 37 weeks of gestation, while term PROM occurs between 37 up to 42 weeks of gestation.[2] Preterm PROM and term PROM can be further divided on the basis of time after the rupture of membrane into: Early PROM (after the rupture of foetal membranes less than 12 hours have passed) and prolonged PROM (twelve or more hours have passed after the rupture of foetal membranes).[4,5]

The prevalence of PROM is about 5–10% while PPROM occurs in 3% of all pregnancies approximately. PROM is responsible for complicating about 3% of all preterm pregnancies, 10% of term pregnancies, 11% of preterm deliveries, and is also responsible for causing 40–75% neonatal deaths in USA.[6–8] Prevalence of PROM in a study from Karachi was 2.7%.[9] A research conducted in 2006 in Abbottabad found that preterm PROM occurred in 9.6% of total pregnancies.[6] To our knowledge no recent data about the prevalence and risk factors of PROM is available at the regional level. The risk factors of PROM are numerous, but localized or systemic infection and inflammation is one of the most important causative factors.[10] The others include low socioeconomic status, inadequate prenatal care, inadequate nutrition during pregnancy, sexually transmitted infections, vaginal bleeding and smoking during pregnancy.[11] Previous preterm delivery and uterine distension (e.g., polyhydramnios) are also well known risk factors. Cerclage and amniocentesis are also risk factors of preterm PROM.[12]

The most common maternal complication of PROM is amniotic cavity infection. Other complications include endometritis, abruptio placentae, retained placenta and haemorrhage, sepsis and death though uncommon can still occur.[7] Preterm PROM is one of the leading causes of preterm birth thus foetal complications such as respiratory distress, infection, necrotizing enterocolitis, brain bleeds, muscle dysfunction, pulmonary hypoplasia and death are associated with prematurity.[7,13]

Most typical sign of PROM is leakage of clear fluid or bleeding from vagina without any contractions. Diagnosis is made through detailed history from the patient and by a sterile speculum examination of vagina and is confirmed when there is

pooling of amniotic fluid in the posterior vaginal fornix or its leakage from the cervical os.[2,14,15] However, 47% cases of PROM present with no visible symptoms.[15] Its management depends on the gestational age as well as other complicating factors although it is still controversial.[16,17] In case of preterm PROM the management mainly involves administration of antibiotics (reducing the risk of perinatal infection and increasing the latency period) and steroids (reducing perinatal morbidity and mortality), while in case of term PROM induction of labour is the course of action.[2,16]

The aim of this study was to determine the important risk factors associated with PROM especially in our setup at Ayub Teaching Hospital, Abbottabad.

MATERIAL AND METHODS

This case control study was carried out from 1st November 2018 to 31st June 2019 on patients admitted to Obstetrics Unit of Ayub Teaching Hospital, Abbottabad after taking ethical approval. Study population comprised of admitted pregnant women with and without PROM beyond 28 weeks of gestation.

Using Open Epi online software Version 3 for sample size estimation for case control studies, the sample size was calculated as 189 rounded to 195, (65 cases and 130 controls). The case to control ratio was 1:2. The assumed 2-sided confidence interval was 95% and power of the study was 80%. For the purpose of hypothetical exposure value in controls for sample size calculation, abnormal discharge from vagina was used with a frequency of 36.8%, and assumed Odds ratio of 2.4 was taken from a previous study from Uganda.[17] Convenience sampling technique was used for sample selection.

Pair matching was done by matching age of the cases with controls. For cases, only those patients were selected who were diagnosed to be a case of PROM including both early and prolonged PROM. For controls, all other pregnant women were selected who were of the same age as cases and who didn't have PROM, and were admitted for reasons other than PROM. Patients with multiple pregnancies, unwilling patients, those who did not know the local language, and patients in labour were excluded. The cases of PROM were diagnosed on the basis of speculum examination and history of leakage of clear fluid from vagina enough to wet the sides of thighs and perineum without any uterine contractions.

A pre-tested structured questionnaire that included bio data as well as various risk factors that predisposes to PROM was developed. Pilot testing of the questionnaire was done on 10 patients admitted in Obstetric Unit before collection of actual data. Informed consent was taken from all the subjects.

Data was analysed on SPSS-16. Mean and standard deviation for continuous variables and frequencies and percentages for categorical variables were determined. Chi-square test was used to determine association between PROM and its risk factors. Odds ratio with 95% confidence interval was used to measure strength of association between PROM and its risk factors. Confidence interval excluding 1 and $p \leq 0.05$ was considered statistically significant.

RESULTS

A total of 195 patients (65 cases and 130 controls) were interviewed. The overall mean age of the patients was 25.48 ± 3.65 years (Range: 19–38 years). The mean age of the cases was 25.47 ± 3.67 years while the mean age of the controls was 25.49 ± 3.66 years. Majority of patients were in the age group 26–35 years, most of them were housewives, illiterate, and belonged to low socio-economic class. Presentation was cephalic in most of the patients (Table-1).

Table-1: Socio-demographic variables (n=195)

Variables	Frequency	Percentage
Age Groups (Years)		
16–25	83	42.6
26–35	108	55.4
36–45	4	2.1
Area of Residence		
Rural	79	40.5
Urban	81	41.5
Semi-urban	35	17.9
Occupational Status		
Professional	4	2.1
Housewife	191	97.9
Socioeconomic status		
Low	115	59.0
Middle	67	34.4
Upper	13	6.7
Literacy level		
Illiterate	83	42.6
Primary	47	24.1
Secondary	47	24.1
Graduate	18	9.2
Presentation of Foetus		
Cephalic	158	81.0
Breech	36	18.5
Others	1	0.5

PROM was found to be strongly associated with history of PROM in previous pregnancy, polyhydramnios, foul smelling discharge from vagina, and vaginal bleeding during current pregnancy. Odds ratio with 95% confidence interval for each risk factor found to be associated with PROM is given in Table-2.

History of intrauterine growth retardation (IUGR), urinary tract infection (UTI), McDonald's stitch in current pregnancy, presence of any systemic disease, previous Caesarean sections, and abortions were not found to be significant risk factors for developing PROM (Table-2).

Table-2: Risk factors for PROM

Risk Factors	Cases (n=65)		Controls (n=135)		Odds ratio	95% CI	p
	n	%	n	%			
Previous history of PROM							
Yes	12	63.2	7	36.8	3.978	1.484–10.666	0.012*
No	53	30.1	123	69.9			
Polyhydramnios in current pregnancy							
Yes	17	51.5	16	48.5	2.5	1.175–5.404	0.015*
No	48	29.6	114	70.4			
Foul smelling discharge during pregnancy							
Yes	15	53.6	13	46.4	2.7	1.197–6.089	0.014*
No	50	29.9	117	70.1			
Bleeding during pregnancy							
Yes	15	51.7	14	48.3	2.486	1.117–5.334	0.023*
No	50	30.1	116	69.9			
Intrauterine Growth Retardation							
Yes	5	50	5	50	2.08	0.58–7.4	0.25
No	60	32.4	125	67.6			
Urinary Tract Infection							
Yes	20	35.1	37	64.9	1.11	0.58–2.1	0.73
No	45	32.6	93	67.4			
McDonald's stitch							
Yes	6	54.5	5	45.5	1.11	0.58–2.1	0.12
No	59	32.1	125	67.9			
Systemic disease							
Yes	6	24	19	76	1.6	0.63–4.4	0.28
No	59	34.7	111	65.3			
Previous Caesarean Section(s)							
Yes	23	28.4	58	71.6	0.68	0.36–1.2	0.21
No	42	36.8	72	63.2			
History of abortion(s)							
Yes	19	33.9	37	66.1	0.96	0.50–1.8	0.91
No	46	33.1	93	66.9			

*significant

DISCUSSION

According to our study previous history of PROM, polyhydramnios, foul smelling vaginal discharge and vaginal bleeding during pregnancy were found to have significant association with PROM. In our study, majority (55.4%) of the women were in the age group 26–35 years. It is in agreement with a study done by Mishra S et al[14]. Dars et al[11] also showed that incidence of PROM was more among patients aged 20–30 years.

We observed a strong association between PROM and history of PROM in previous pregnancies. It is in line with the studies conducted by Al-Hussain TK et al[18] and Lee T et al[19]. It may be due to untreated cervio-vagnial infection by bacterial vaginosis or *Chlamydia*, cervical incompetence, or short cervical length.[4,7,20] Patients with history of foul smelling discharge during pregnancy had 2.7 times increased risk of PROM. This is in line with Assefa NE et al[7] and Choudhary M et al[20]. Organisms causing infections of the genital tract that have been associated with PROM include *Chlamydia trachomatis*, *Gardnerella vaginalis*, *Trichomonas vaginalis*, Candidiasis etc. Abnormal vaginal discharge indicates infection that results in inflammation of the membranes ultimately leading to their rupture.[20–22] Unhygienic practices of majority of the population makes it a significant risk factor in developing countries.[22]

In our study, another important risk factor associated with PROM was polyhydramnios. Our results match with results of the research conducted by Mishra S et al[14], but are contradictory to the work of Assefa NE et al[7]. Over distension of the uterus due to increase in amniotic fluid volume increases the intrauterine pressure and thus increases the risk of PROM.[14,20]

Vaginal bleeding was also found to be a risk factor for PROM increasing the risk by 2.4 times. Chronic abruption of placenta may result in necrosis of decidua which weakens membranes or predisposes to intra-amniotic infection, eventually resulting in membrane rupture.[20] Hossain R et al[23] reported that bleeding specially in second trimester can lead to preterm labour and PROM.

The UTI was considered one of the main risk factors for PROM by Moore RM et al[24]. In our study, however, it was not found to be strongly associated with PROM and the history of UTI was strong both in cases and controls which could be the reason of insignificance. This can be attributed to wide prevalence of UTI in females of reproductive age due to inability to maintain good hygienic conditions.

In this study, McDonald stitch was not found to be a significant risk factor which is contradictory to the study conducted by Choudary M et al[20]. The reason may be fewer number of cerclage in cases and controls in this study.

Previous Caesarean section, IUGR, abortions, and any systemic illness did not have significant effect on PROM in this study. The frequency of Caesarean section was 28.4% in cases and was found not to be significant risk factor of PROM. This rate was 30.5% in a study conducted by Kayiga et al[25] which is quite similar to our results; however, it is low as compared to the work of Pasquier JC et al[26] in which the frequency of Caesarean section was 58.7%.

The frequency of abortion was 32.4% in cases while it was 67.6% in controls. Abortion was also not a significant risk factor for PROM in the study of Choudhary M et al[20]; however, it contradicts the work done in Tigaray[7]. The reason may be comparable sample size of the studies.

History of both systemic illness and trauma were not significant risk factors in a research done by Assefa NE et al[7] which supports our results.

Some limitations of this study were that matching of cases and controls was done with one variable only, i.e., age; and it was conducted in only one tertiary care hospital.

CONCLUSION

The most important risk factors in our setup found to be associated with PROM were previous history of PROM, foul smelling vaginal discharge, polyhydramnios, and vaginal bleeding. With the help of this knowledge, obstetricians will be able to pinpoint high risk patients and thus will be able to provide rapid and effective management. It will also help them to establish prevention protocols so as to decrease the burden of this disease in this area.

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ORIGINAL ARTICLE

COMPARISON OF DRY NEEDLING WITH CONVENTIONAL PHYSIOTHERAPY IN PATIENTS WITH KNEE OSTEOARTHRITIS FOR PAIN AND FUNCTIONAL IMPROVEMENTS

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Background: Knee Osteoarthritis is a degenerative disease that affects many individuals in their forties or later and can lead towards impairment of physical functions and poor quality of life. Our study aimed to compare the effectiveness of dry needling with conventional physical therapy in patients with knee osteoarthritis (OA). **Methods:** It was a non-randomized clinical study conducted after ethical approval at Syed Medical Complex and Amin Welfare and Teaching Hospital Sialkot in 6 months. Sample size was calculated to be 58 using WHO calculator. Purposive sampling technique was used to enrol the participants according to predetermined eligibility criteria. They were allocated into two groups. Group A received conventional physical therapy treatment and group B received dry needling for 3 weeks, 2 sessions per week. Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scale and Numeric Pain Rating Scale (NPRS) were used to measure the outcomes before 1st treatment session and after last session. Paired sample *t*-test and independent sample *t*-test were applied to compare the results, and $p \leq 0.05$ was considered significant. **Results:** Pre-treatment WOMAC score in group A was 50.07±11.835 and in group B it was 45.87±12.512. Post-treatment WOMAC score in group A was 38.87±13.731 and in group B it was 24.33±8.926. The scores improved in both groups, but group B showed significant improvement. **Conclusion:** Both conventional physical therapy and dry needling are effective to manage pain and functional limitation in patients with knee OA. Dry needling proved more effective compare to conventional physical therapy.

Keywords: Knee, Osteoarthritis, Dry needling, Conventional physical therapy, Western Ontario and McMaster Universities Osteoarthritis Index, WOMAC, Numeric Pain Rating Scale, NPRS

INTRODUCTION

Osteoarthritis affects more than 0.5 billion people worldwide, mainly involving women. Population affected during 1990–2019 rose by 48%, rendering OA 15th highest cause of the years lived with disability.[1] The aetiology is poorly understood. Since it is a multi factorial disease, studies have discovered a number of causative factors including obesity, genetic predisposition, bone density, trauma, and occupational injuries.[2] At the initial stage the symptoms include occasional pain and stiffness while advanced stage symptoms of osteoarthritis involve continuous pain and swelling at the localized areas, crepitus and joint locking.[3] Treatment varies according to the condition and preferences of the patient. Non-pharmacologic therapies[4] include conventional physical therapy (ROMs, knee stretching and strengthening exercises, knee isometrics), assistive devices, electrotherapy devices, acupuncture and lifestyle modification.[5] Patients seek to adopt treatment options that are relatively less invasive, cheap and safer.[6]

Dry needling is one of the emerging physical therapy interventions getting popularity in the current era. It is relatively less invasive and cheap intervention with low risk factors.[7] According to Leonid and Simon, dry needling is a new technique which was developed to treat pain caused by the trigger points in the muscles.[8] In this method practitioner insert needle at pain points producing analgesic effect.[9] There are a few studies on acupuncture for knee osteoarthritis. Dry needling (DN) work on the principle of acupuncture, both procedures used special needles but acupuncture have specialized different theory of energy channels and energy flow in the body.[10] DN alters both central and peripheral sensitization, reduces pain of local and remote areas and improves activation of muscles.[11] A moderate quality evidence synthesized in a recent systematic review, on short term effects of DN with periosteal stimulation on pain and functions in subjects with Knee Osteoarthritis (KOA).[12] Many studies have used dry needling of the knee joint without targeting specific trigger points to treat pain and disability in patients with knee osteoarthritis, although point specific has been reported more effective.[13] The effects of trigger point dry needling in the treatment of knee osteoarthritis has been well documented.[14] In a recently reported case report, dry needling was found effective in an elderly lady with chronic lumbar radiculopathy and medial KOA.[15] Dry

needling is an effective treatment under the scope of physiotherapy practice to treat musculoskeletal problems, but anatomy and risk factors of problem consideration are critical. DN is a new technique to treat musculoskeletal pains with low risk factors being less invasive and cheap.[16]

Currently, little evidence is available about the effectiveness of dry needling in patients with KOA. Although many physiotherapists have been practicing DN in their clinical practice, but no primary studies have been reported from Pakistan about its outcome in patients with KOA. The current study evaluates the efficacy of DN as compare to conventional physical therapy treatment in KOA for pain management and improving functional abilities.

METHODOLOGY

It was a non-randomized clinical study conducted at Physical Therapy Departments of Syed Medical Complex, and Amin Welfare and Teaching Hospital Sialkot from November 2018 to April 2019. This study was approved by Riphah Ethics Committee vide Ref No. RCR and AHS/REC MS-OMPT/027 dated 15 Oct 2018.

Taking into account the number of patients with KOA visiting both the hospitals during last 6 months, a sample of 58 patients was calculated through WHO calculator.[16] Confidence interval was 95% with 5% margin of error. The patients with KOA visiting outpatient Physical Therapy Departments were screened for their eligibility. Inclusion criteria were patients aged 40–60 years with KOA meeting the American College of Rheumatology criteria[17] and knee pain for more than 6 months. Patients with osteosarcoma, fracture, or with history of trauma were excluded from the study. All those patients who agreed to participate signed informed consent and were enrolled in the study.

Fifty-eight participants were divided into two groups. Both groups were given similar baseline treatment in the form of heat by moist heat pads. Group A received conventional physical therapy treatment in the form of quadriceps isometric (10×10) and group B received dry needling. Treatment duration was 30 min, 2 sessions per week for 3 weeks. A thorough case history, detailed physical examination and knee regional assessment was taken by physiotherapist before the start of treatment. Numeric Rating Pain Scale (NPRS)[18] and Western Ontario and McMaster Universities Osteoarthritis index (WOMAC)[19] were used to collect the data at baseline and 3 weeks after the treatment.

Group A Protocol: After 10 min of moist hot pack application, the patients were treated with conventional physical therapy, including strengthening exercises of muscles around the knee joint (10 reps in 3 sets) and stretching exercises of calf muscle and hamstrings, soft tissue mobilization, cycling on a static cycle for 5 min and knee Range of Motion (ROM's).

Group B Protocol: After 10 min of moist hot pack application, the patients were treated with dry needles around the knee joint at four points. Needles were left for 10 min at those points (Figure-1).

Shapiro-Wilk test was used to test the normality of data distribution. Descriptive statistics were displayed as mean and standard deviation. Paired sample *t*-test was used to interpret the results and differences within each group, independent sample *t*-test was used to compare the results between the groups, and $p \le 0.05$ was considered statistically significant.

Figure-1: Dry needles applied to patient with KOA

RESULTS

After 3 weeks of intervention 58 participants with KOA completed the study, none were lost to follow-up. There were no significant differences between groups in the baseline values. Mean age of group A was 55.67±10.66 years, height 164.53±9.78 Cm, weight 84.00±13.70 Kg, and BMI was 31.26±13.70. Mean age Group B was 57.13±10.45 years, height 167.20±10.79 Cm, weight 79.87±11.89 Kg, and BMI was 28.76 ±5.07.

Pre-treatment WOMAC score in group A was 50.07±11.835 and in group B was 45.87±12.512. Post treatment WOMAC score in group A was 38.87±13.731 and in group B it was 24.33±8.926. Pre- and post-treatment differences of scores in both groups indicate the reduction of pain and functional limitation in both groups (Table-1). Reduction in WOMAC score in group B was more as compare to group A ($p<0.05$), which indicates that dry needling proved more effective as compare to conventional physical therapy to reduce pain and functional limitation.

Pre-treatment NPRS score in group A was 6.27±1.163 and in group B it was 5.73±1.280. Post-treatment NPRS in group A was 4.67±1.676 and in group B 2.40±1.242. Pre- and post-treatment differences of scores in both groups indicate a reduction in pain in both groups but reduction in numeric rating pain score in group B is more as compared to group A ($p<0.05$) revealing that dry needling proved more effective as compare to conventional physical therapy to reduce pain (Table-2).

Table-1: Paired sample *t*-test for difference in pre- and post-treatment NPRS and WOMAC scores within group A and B

Groups		Mean±SD	*p*
A	Pre-treatment score of NPRS	6.27±1.163	0.001
	Post-treatment score of NPRS	4.67±1.676	
	Difference in score of NPRS	1.60±0.487	
B	Pre-treatment score of NPRS	5.73±1.280	0.000
	Post-treatment score of NPRS	2.40±1.242	
	Difference in score of NPRS	3.33±0.038	
A	Pre-treatment score of WOMAC	50.07±11.835	0.000
	Post-treatment score of WOMAC	38.87±13.731	
	Difference in score of WOMAC	11.20±2.104	
B	Pre-treatment score of WOMAC	45.87±12.512	0.000
	Post-treatment score of WOMAC	24.33±8.926	
	Difference in score of WOMAC	21.54±3.586	

Table-2: Independent sample *t*-test between groups A and B for pre- and post-treatment NPRS and WOMAC scores

Time of assessment	Group	Mean±SD	*p*
Pre-treatment score of NPRS	A	6.27±1.163	0.242
	B	5.73±1.280	
Post-treatment score of NPRS	A	4.67±1.676	0.000
	B	2.40±1.242	
Pre-treatment total score of WOMAC scale	A	50.07±11.835	0.353
	B	45.87±12.512	
Post-treatment total score of WOMAC scale	A	38.87±13.731	0.002
	B	24.33±8.926	

DISCUSSION

Our study aimed to identify the effectiveness of dry needling as compare to conventional physical therapy to treat pain and functional limitation. Majority of the individuals were overweight. Both dry needling and conventional physical therapy had produced significant outcomes to reduce pain and functional limitation.

A recently published systematic review by Khan I *et al*, from Pakistan which included ten studies from 2000 to 2019, reported that dry needling had a positive effect in 50% of the screened studies in managing pain in myofacial trigger points, heel pain and pain due to muscle tightness. There was a negative effect of DN in KOA, atraumatic knee pain, and ROM in subjects with plantar fasciitis.[20] Our results contradict the findings about effects of dry needling in KOA of that review.

Conventional physical therapy includes patient education, strengthening exercises, and aerobic ROMs. It is effective in KOA patients to reduce pain and disability. Aerobic walking and home-based quadriceps strengthening exercises are equally effective for managing pain and reducing disability.[21] In our study, dry needling was found to be a useful adjunct to the conventional PT in patients with KOA.

Saha P *et al*[22] surveyed the practices followed by physiotherapists in India for management of KOA, and found that most of the physiotherapists were using Transcutaneous Electrical Nerve Stimulation (TENS), ultrasonic therapy and dry needling along with patients'

education and strengthening exercises. Although there are no details of results of these interventions, still the experiences of Indian physiotherapists may be considered.

Farazdaghi MR *et al*[23] in a double blind randomized controlled trial on 40 subjects with pain and trigger points around hip or knee joints, found that DN was effective in improving the outcome measures.[23] They used Visual Analogue Scale for pain and sensitivity, Knee Injury and Osteoarthritis Outcome Score, algometer, Y balance test and administered DN for three sessions only on participants of 45–70 years of age, while our population was 40–60 years old and we used NPRS and WOMAC to measure the outcome. We offered 6 treatment sessions in 3 weeks. They also measured balance and sensitivity to pressure which was not included in our study.

The results of our study agree with the findings of a large multicentre clinical trial, in which Dunning *et al*[16] used periosteal dry needles in conjunction with exercise and manual therapy to treat knee OA. The participants could discontinue the medication for pain management and there was significant improvement in the WOMAC scores, the major difference in this study was the use of periosteal DN, a larger sample size and their treatment time was three months as we used only DN (without currents) on a smaller sample and for three weeks only.

In a secondary study conducted in Spain, low to moderate level evidence were found suggesting a positive effect of trigger points dry needling on pain and subsequent disability in subjects with Patello-femoral pain syndrome, but not for knee OA or post-surgical knee pain especially in short term treatment.[24] In our study, dry needling was found effective in managing pain in subjects with KOA.

In a pilot study of a double blind RCT conducted by Sánchez-Romero *et al*[25] compared the effects of DN and sham DN along with a therapeutic exercise program for 12 weeks in subjects with KOA and myofacial trigger points in the muscles of the thigh. They found no improvement in pain intensity in terms of NPRS and functional abilities on WOMAC. The differences with our study are in inclusion criteria and length of treatment period.

The results of a double blind multicentre RCT, explored the effects of DN using a sham group in older adults with KOA on pain and function. Their sample size was almost comparable to our study (n=62), while they offered 6 treatment sessions and followed after one year. They measured outcomes using NPRS, WOMAC, Barthal Index, Time up & go test, global rating of change scale and EuroQoL group 5-dimension self-report, questionnaire for between group difference in pain, function, functional status evaluation, balance assessment, clinical assessment and health related

quality of life respectively. They found no differences in NPRS and WOMAC scores after one year but found reduced consumption of medications in DN group.[26] There were cultural and lifestyle differences in the populations of both the studies, and they used more outcome measures than our study. Although they found no significant differences but our study population reported better outcomes in terms of pain and functional abilities.

Vervullens S *et al* reported that single session of DN used to release the myofacial trigger points in the muscles surrounding knee joint in subjects with KOA may relive pain via nociception processing, but there were no long lasting outcomes.[27] They used visual analogue scale for pain and gait analysis for spatiotemporal assessment of outcomes; their sample size was comparable to us (n=62). We used NPRS and WOMAC only.

The limitations of our study included, small sample size, gender distribution disparity, lack of classification of stage of osteoarthritis and only short-term effects of both the treatments. Further studies may be conducted to explore the long-term effects of dry needling and conventional physical therapy in patients with osteoarthritis of the knee joint. Comparative effects of both techniques at different stages of knee osteoarthritis must also be explored.

CONCLUSION

Both conventional physical therapy and dry needling are effective interventions to reduce the pain and improving functional limitations in patients with KOA. However, dry needling was found comparatively more effective and may be used as an adjunct to other treatment options.

DISCLOSURE

The abstract of study including some of the data was presented as Oral Presentation in 38th Physiotherapy Research Society, UK, annual scientific meeting held on 16th April 2021 virtually, and the proceedings have been published in Journal of Academic Development and Education (JADE), issue 14 autumn 2021, Keele University UK.

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ORIGINAL ARTICLE
JOURNEY OF EDUCATIONAL LEADERS IN BRINGING CURRICULAR REFORM IN MEDICAL EDUCATION IN PAKISTAN

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Background: Reforms in medical school need strong leadership to create an environment for effective adaptation. The aim of this study was to identify the challenges faced and strategies used by educational leaders during their journey of bringing the curricular reform at their respective institutions. **Methods:** This qualitative study was carried out across four institutes in Pakistan. Purposive sampling technique was used, and 14 medical educationists involved in curricular reforms participated. In depth semi structured interviews were conducted to explore the journey of educational leaders, the medical educationists who were involved in guiding the faculty in improving the educational processes in the institutes. Thematic analysis of the interviews was done. **Results:** The major challenges identified can be broadly grouped into two categories: student and faculty related. The underlying common principle in the journey of leaders was to remain aware of the emerging situations and take decisions accordingly. **Conclusion:** As leaders navigating the change, they must be prepared for the uncertainty and unexpected events and adapt themselves to the changing environment, and deal with perseverance.
Keywords: Educational leaders, Curricular Reforms, Change

INTRODUCTION

Reforms in medical education has been an ongoing process since Flexner's time.[1] Flexner voiced his concerns about the process and product of the medical schools in America[2] and since then medical education has been in a state of unrest.

Pakistan has more than a hundred recognized medical schools, yet most of them are following discipline-based curriculum and only a minority have included new pedagogical approaches and reforms in curriculum.[3] The need for change has been noted and accepted to some extent but bringing the change has not been an easy endeavor.[4] Recently in May 2018 Pakistan medical and dental council revised their standards for medical education. Although the standards are only a single part of the recognition process, but this has prompted the medical schools across Pakistan to deliberate over their curriculum.

This study focuses on those medical schools of Pakistan where reforms have already been introduced successfully. Medical educationists are the middle leaders responsible to bring about change[5] and they are largely responsible for translating the vision of their higher-up and transform it into reality till the ground troops. They are the change agents who generate commitment and alignment to the vision and generate practices which make the change possible.

METHODOLOGY

A qualitative study was conducted across four institutions from Feb to Apr 2019. Sampling was purposive (homogenous) and carried out in stages. During first stage the medical colleges who had successfully adopted the integrated system in last 10 years were identified and the key persons involved in reforms were singled out. These individuals had a background in medical education.

Eighteen participants from 6 medical colleges were approached to participate. Fourteen Medical Educationists involved in curricular reforms of four different universities responded and were included in the study. Study setting was Riphah International University, Shifa International University, Fauji Foundation University, Islamabad, and Khyber Medical University, Peshawar.

In-depth interview of the participants was carried out. All audiotaped data was transcribed, and Data analysis was done using Atlas ti version 7.5.7.

RESULTS

Fourteen participants were interviewed, 6 males, and 6 females. All participants were more than 30 years of age, had a minimum of Master's Degree in Medical Education, and more than 10 years of teaching experience.

One-hundred-fifty-one (151) open codes were identified from all interviews during the first round. Later the codes were grouped into 21 subthemes and 5 themes. The themes were challenges, creating climate for change, implementation, evaluation and coping strategies used by educational leaders.

Multiple challenges were highlighted during the course of interviews which were grouped into seven subthemes. Resistance, student problems, changing of leadership, difficulties in designing the curriculum are some of the main problem encountered by the participants.

Initially when reforms were introduced students performed poorly in examinations.

'Their previous methods were Teacher-based system, i.e., the cramming-based system. So, when they reach here and are subjected to discussion and interaction, that is when they face difficulty in adopting this new system' (R6, M)

Resistance to change was one issue identified by all participants. Resistance was encountered mostly from the faculty who would also create negativity with the students.

'This was the same faculty who was agreeing with us on the front but would create negativity with the students.' (R1, F)

During implementation phase the faculty showed resistance in classes and would also convey their apprehensions to the students. This type of covert resistance was difficult to tackle. On the front of things the faculty was on your side.

Not being appreciated for your efforts and not being awarded any incentives for your efforts is another factor which lead to negativity.

'Biggest issue with me was that people don't appreciate what you're doing.' (R9, M)

Lack of planning and focusing on the details beforehand was another issue faced by the middle leaders. The colleges faced the problems as they came along which lead to unnecessary delays.

'It is unfortunate that in Pakistan there's an issue we work on ad hoc basis, we don't believe in full time work.' (R8, M).

Another technical issue the colleges faced was creating learning objectives and aligning the curriculum with the learning objectives. This was more challenging and required competent staff along with repeated sittings of the faculty members.

Infrastructure in medical schools was made for large group learning. After deciding on the curriculum and level of integration, changes were made in infrastructure according to available resources.

The challenges identified cannot be viewed as conclusive as they differ from institute to institute. But these challenges can be taken as guidelines to be kept in mind and addressed while designing curricular reforms.

Multiple strategies were adopted by the faculty in handling the reforms. These strategies have been categorized according to Kurt Lewin model for change. Strategies adopted in the three main phases for change namely unfreezing, institutionalizing the change and refreezing.

During the initial stage of reforms a platform for change was introduced. One institution initially conducted SWOT Analysis and started sensitizing their faculty for the reforms. Faculty development was started and a core team was identified to bring out the reforms.

'SWOT analyses was to be conducted on all the Faculty of this college & all the head of departments' (R1, F)

Faculty development and motivation also has a major part in creating a platform for change.

'70 percent is your planning, but you do need to modify things according to the situations. You need your people in depts... … because implementation at the level of departments is hard to ensure' (R10, F)

One strategy adopted by an institute for the preclinical years was deciding the percentage of basic sciences and clinical. They opted for a 70/30 rule with 70% for basic sciences and 30% for clinical. Then they held multidisciplinary meetings for modules. They tackled one module at a time and decided on the content, the instructional strategy, and assessments. This practice continued till the modules were in print. Secondly, they incorporated a pilot project before the actual process. A pilot study identifies the loopholes and they can be addressed beforehand. Once the curriculum runs it becomes very difficult to make further the changes.

'We had a skeleton implementation; it was chaotic and needed a lot of work.' (R7, F)

A clear road map for implementing reforms should be chalked our beforehand. Having the new curriculum for the complete five years on paper saves undue delays. After the curriculum design is finalized, the second step is altering the infrastructure of the medical school according to available resources.

During the 'Change' phase Faculty development and addressing the problems as they arise go hand in hand. One institute opted for running the completed modules alongside designing of the next modules. This step was not very wise as reflected by the participants as it was very difficult to complete the modules once the curriculum started running.

Professional development of the faculty is utmost important in educating the faculty. Participant highlighted that they have faculty development programs throughout the year. Having everyone involved in the forms creates an environment more favourable for change.

'The faculty who feels they can influence the direction of reforms, they are more likely to participate in the reforms.' (R7, F)

During the last 'Refreezing phase' the new curriculum is in place. At this point in time it has to be re-evaluated to identify the deficiencies and for incorporating the improvements.

One of the flaws in our system was we ran the faculty development plan parallel with the reforms. Faculty development should have been done before, so the faculty was well prepared for the reforms.

To address the minor resistance, first step taken was identification of the potential 'hidden

counterforces'. This was possible only in an environment where open communication is the trend and different views are welcomed. Having your people in different departments also helped to identify resistance. Once the people responsible for creating problems were identified, multiple strategies were adopted. Assigning them responsibility for a task took

care of the minor resistance. Participants opted for harsher measures when the faculty refused to participate. Replacing those faculty members is the viable option but requires support from principals and deans. During the implementation or execution phase if this issue is not addressed timely all previous change endeavours fail.
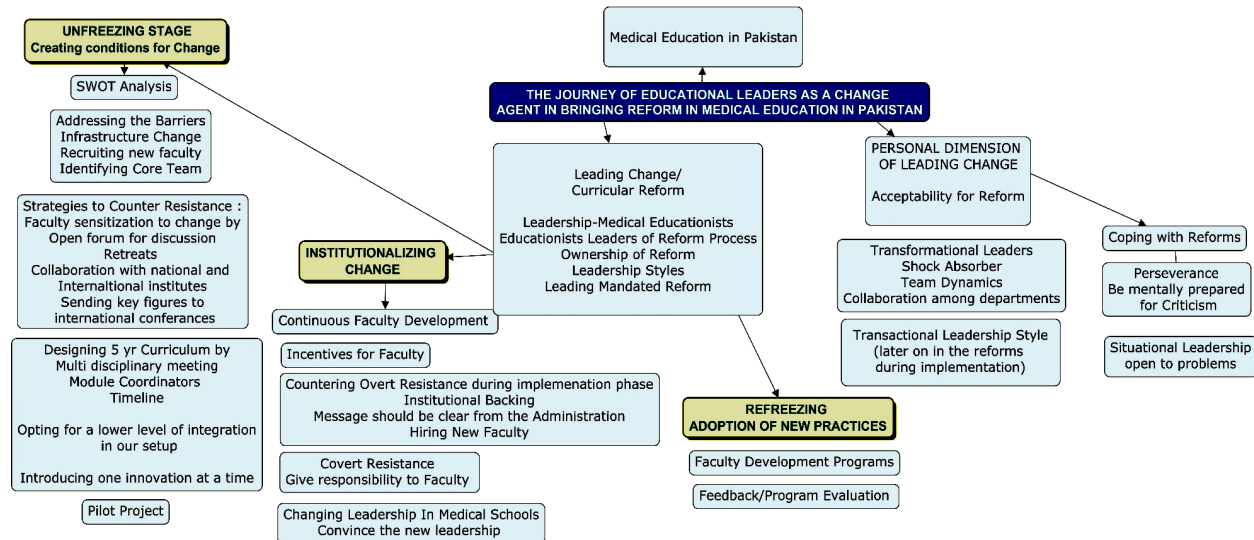

Figure-1: Summary of major events during the phases of Curriculum Change

DISCUSSION

Change is a complex multifaceted phenomena and literature is abundant in the strategies adopted in successful change process but having said that 70 percent of change initiatives are not successful. Leaders and managers adopt a number of strategies and are so engrossed with the recommendations and literature available that they lose their primary focus.[6]

Challenges faced by medical school top hierarchical leaders have been explored on multiple occasions and the need to explore the process from different stakeholders has been highlighted.[7,8] This study focuses on the middle leaders: Medical educationist who believed in the reforms and played a central role in materializing the vision, but the initiator of reforms was the top leaders. It builds on the work of Bland et al and Velthius to have an in-depth understanding how the medical schools across Pakistan were successful in curricular reforms. Bland et al has argued that literature is abundant in new curriculum introductions and their results but the process of how it is achieved needs to be further explored.[9]

The major challenges which this study came across were Resistance, unstable Governance, Politics and Lack of Acknowledgment.

Resistance has been an established challenge in reforms. Whenever people are pushed from their comfort zones it's a natural response to oppose. Fullan has argued that if teacher's vision and knowledge is

increased they can have a very major role in implementation. Change is only superficial without their involvement.[10] Another strategy is eliminating the resisting faculty. One of the medical schools in the study adopted this harsher step but as a result, key faculty members resigned. It further lead to delay and rehiring had to be done. The best way forward as described by a participant is to guide and sensitize the faculty early on. With proper knowledge faculty is motivated to adapt into the new role.[11]

Participants in my study argued that tackling major change at a single point can be difficult but by introducing the in a stepwise fashion and addressing the problems that arise along the way is the best way forward. Initially faculty was in denial in the necessity of the reforms. Later with repeated sensitization they revised their views and became a strong support system, but for faculty who created negativity later on. Ultimately the faculty who was not willing to participate had to be replaced. To jump to this step strong administration is required and the Principals and Deans need to advocate for the changes. If the top leadership does not take drastic steps at this point all change endeavours fail. Marie Jippes from Maastricht conducted interviews of change agents in medical schools of Austria where successful reforms took place despite the national culture as an acknowledged barrier. She concluded that faculty opposition can be countered if change is encouraged at national level.[12]

Medical educationists agreed that another issue they faced was designing the curriculum and steering the change process. The strategies adopted by these medical schools varied. One Medical school opted for the complete 5-year change in curriculum but the medical educationists agreed that the transition phase had been very rough and although the end was kept in mind it was a difficult change. Two medical schools took it one year at a time and introduced the innovative educational strategies in an incremental manner. This gave them the flexibility and time to adapt and also address the issues as they arose but respondents all agreed that curriculum document for all the five years should be made in advance as the medical schools who implemented and planned side by side faced problems after their academic years had already started.

Recurrent change in higher echelons is one of the hurdles which lead to instability in reforms. Every new leader would question the strategy of the predecessor and ultimately valuable resources are wasted. In addition the minor opposition which was under control previously, created problems.

Certain leadership characteristics essential to successful change were highlighted during the course of the study. Namely Perseverance, reflective practices and a shock Absorber.

The success of the reforms depends on how the educational leaders approach on different issues. In change literature two strategies have been discussed simultaneously 'Reflection' and 'Action Practice'. Reflective practices refer more to changing strategies as the circumstances evolve. Action practice is focused on to changing the mindset of people around to fit their purpose. Action approach is effective for simple change but for complex endeavours study leaders advocated to take a broader approach and honour the diversity of people included in the reform process.[9]

Involving multiple medical schools across Pakistan is strength to this study as this can now be applied to medical schools across Pakistan. Although the researcher has no background as a change agent she had an unbiased view while transcribing but having said that if that experience would have been present a better and relatable understanding of the process would have occurred.

Changing medical curriculum in Pakistan needs to be explored form various stakeholders especially the students. Their perspectives can be valuable in understanding and improving the curriculum.

CONCLUSION

Curriculum change in medical schools is a complex, expensive undertaking but it's more cumbersome and expensive if it fails. The purpose of conducting this research is to have insight into the curriculum change so future endeavours are successful. Middle leaders face multiple challenges during the process and adopted multiple strategies to overcome them. Their success stories can be used as a guideline for future curriculum change. The change leaders identified that regardless of the planning curriculum change is still a bumpy road. The leadership qualities which sustained the change process were perseverance and avoiding confrontations.
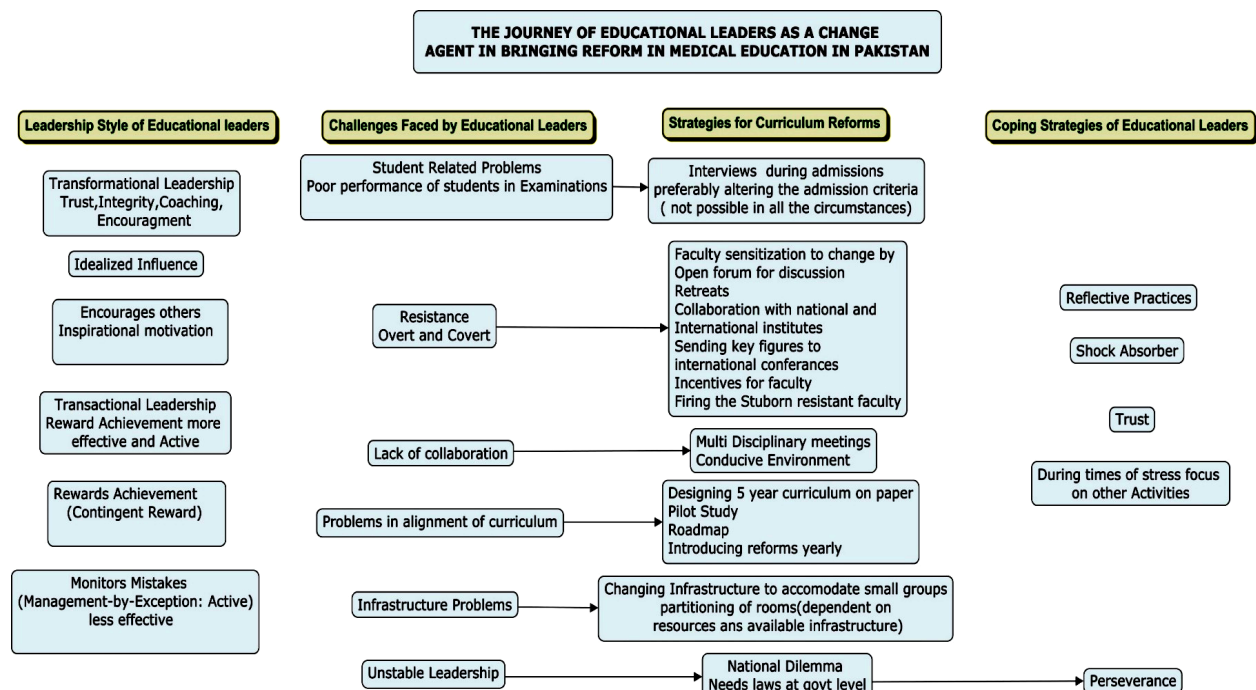

Figure-2: Challenges faced and strategies adopted by Educational Leaders during Curriculum Reforms

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ORIGINAL ARTICLE
AWARENESS AND KNOWLEDGE AMONGST MEDICINE AND ALLIED STUDENTS REGARDING CORONAVIRUS IN A UNIVERSITY IN KARACHI FOLLOWING AN OUTBREAK

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Background: Awareness motivates one to adopt protective measures and enables them to spread the knowledge amongst their peers. The study was conducted to assess and compare the knowledge and awareness among Medical and Allied Students regarding COVID-19 at a University in Karachi, Sindh. **Methods:** This was a cross-sectional survey consisting of 23 close-ended items conducted online, amongst undergraduate Medical and Allied Students. **Results:** The 520 study participants filled out the web-based survey 232 (44.6%) from Medicine and 288 (55.4%) from Allied Sciences. The mean age of the study Participants were 21.18±1.784 years. The majority students were from medical 176 (33.8%) and medical technology field 108 (20.8%), followed by nursing, dental, physiotherapy and other allied health sciences departments. Awareness and knowledge regarding corona virus were found more in allied sciences students than in medical students (*p*=0.0001). **Conclusion:** Awareness and knowledge regarding COVID-19 were better allied sciences students than in medical students.
Keyword: Covid-19, knowledge, awareness, medical, allied sciences, students, pandemic

INTRODUCTION

News about the CoVID-19 outbreak sparked quite the conversation at the beginning of 2020. Its history dates back to 8th Dec 2019, when an array of seemingly idiopathic pneumonia cases resembling viral pneumonia emerged in Wuhan, Hubei, China.[1] On the 30th Jan 2020, the World Health Organization declared the CoVID-19, a global health emergency[2] whereas on 11th Mar, 2020 the disease was declared a pandemic[3]. The term 'human coronavirus' (HCoV) was used for a series of harmless pathogens that cause the common cold in otherwise healthy people, with the first report dating back to 1965.[4] Recently, however, more severe forms of the virus have emerged, causing dangerous infections and even resulting in death.[5] Currently, common findings are cough, fever, and fatigue, with clinical findings of leukopenia, leukocytosis, and lymphopenia. Patients critically ill with the disease present with acute respiratory distress syndrome, arrhythmia, and shock.[6]

During any emergency, the constant stream of information we receive through social media and news channels reaches an all-time high, and very little is done to stop unchecked facts from unleashing themselves onto the public.[7] The novel coronavirus is not different.[8] There seem to be vast differences of opinion amongst the public. Some look at the low fatality rate and the fact that most patients have underlying co-morbidities and think that the virus is 'no big deal' and continue with their lives blissfully, unaware that they might be asymptomatic carriers and infect those to whom the virus may actually be fatal[9], and undermine the importance of social distancing[10], whereas others at the other end of the spectrum panic and proceed to buy the entire stock of their nearest supermarket in order to stay at home for the rest of the year. It is vital especially for the youth pursuing higher studies to educate themselves about the virus so they can educate and thereby ensure the safety of those around them. Unchecked facts and rumours can seriously hinder public health efforts and must be debunked immediately.[11] Increased awareness results in better infection control whereas a lack thereof enables avoidable disease transmission and inaction regarding one's health.[12] This causes problems in detection and treatment.[13] Students with information from the correct resources tend to have more positive attitudes towards those who are infected and less likely to stigmatize victims.[14] Without adequate knowledge or comprehension of the gravity of the situation, students will not understand why complying with the recommended behavioural changes is essential.[15] Awareness of the situation motivates one to adopt protective measures.[16] University students are at the forefront of the next generation and it is vital for them to be able to spread knowledge amongst their peers.[17]

The objective of this study was to assess and compare the knowledge and awareness among medical and allied sciences students regarding the novel coronavirus at a university in Karachi.

MATERIAL AND METHODS

This was a cross-sectional survey and was conducted in medical and allied sciences students from a university in Karachi, from Jun to Nov 2020. Ethical Approval was obtained from the Principals of medicine and allied colleges to conduct the research and the proposal was submitted to Institutional Review Board of the University (Ref: IRB-1686/DUHS/ Approval/2020/). Informed written consent was sought from all participating students and confidentiality was maintained. No harm was done to anyone whosoever in conducting this research.

Sample size was calculated by using Open-Epi software. In a study from India regarding COVID-19 awareness among health care students the correct responses reported were 72.1%.[18] Hence taking 95% confidence level the computed sample size was 315. By adding, 10% expected non-response rate, a total of 347 was suggested. A total of 520 undergraduate students, 176 belonging to MBBS (88 students from a public Medical College and a private Medical College each) and 344 from allied sciences were chosen by convenience sampling.

Since there was a major crisis globally in respect to Covid-19 pandemic, there was lockdown in Karachi, and social distancing was to be maintained, the data was collected online.

A 23 item questionnaire was used from two studies.[19,20] The demographic data was asked in 6 items whereas 17 items were to evaluate knowledge and awareness regarding Covid-19 pandemic, the filling of the questionnaire was piloted on a few students to check the understanding, and on an average it took 8 minutes for each student to complete the form. The demographics included the gender, university, age, and level of education. The primary outcome included about how the student got to know about the virus, its fatality rate, the symptoms, risk factors, if the student thinks it's curable, and safety precautions concerning the virus. All questions used were closed-ended.

Data was analysed using SPSS-20. Descriptive statistics (frequencies and percentages) were used to describe the quantitative and categorical variables. Chi-square tests were used to determine any significant association between the students' responses and independent variables such as gender and educational level of the students. Student's t-test was applied comparing medicine versus allied students' knowledge scores, and $p \leq 0.05$ was considered significant.

RESULTS

The 520 study participants filled out the web-based survey. Mean age of the study participants was 21.18 ± 1.784 years. The majority (176, 33.8%) of students was from medical, and medical technology field (108, 20.8%), followed by nursing, dental, physiotherapy, and other allied health sciences. The demographic characteristics of the study participants are detailed in Table-1.

Table-2 illustrates the knowledge and awareness about novel Covid-19 among the respondents. Majority (73%) of the study participants in both medical and allied departments did not correctly identify when the first outbreak of novel corona virus occurred. A high proportion (96.80%) of participants provided the correct response while the rest (2.9%) did not know the place of origin of corona virus. Majority (72.9%) of the participants had the knowledge regarding incubation period of the corona virus. A high proportion (48.50%) of allied study participants provided the significant correct response as compared to medical students (41.90%), while 7.1% did not have any idea whether COVID-19 is contagious or not (p=0.031).

Only a few participants had knowledge regarding the routes of transmission of Covid-19, (19.8% allied students, compared to 12.1% medical students) (p=0.028). Awareness regarding the fatality rates of Covid-19 was 10.8% among allied students and 8.30% in medical students (p=0.003), majority of the students got their responses incorrect (57.2%). Awareness of covid-19 vaccine was seen more in allied students (46.9%) as compared to medical students (41%) (p=0.004). Awareness regarding the curability against covid-19 disease was seen considerably less overall but medical students (5.6%) had more correct responses as compared to allied students (2.1%) (p=0.001). Majority (45.3%) of the students did not have awareness regarding the severity of corona virus infection. Allied students were more aware about the severity of the disease as compared to medical students.

It was significant that allied students (52.1%) had more knowledge about the symptoms of COVID-19 as compared to medical students (44%) (p=0.007). More allied students (41.3%) had the awareness about the cause of spread of covid-19 as compared to medical students (30.2%). Widely the allied students (55.2%) were aware about the precautions against covid-19 as compared to medical students (44.2%). Most of the allied students (53.70%) were more knowledgeable regarding the spread of Covid-19 as compared to medical students (44.20%). Knowledge of age group involved in severity of Covid-19 and knowledge of treatment regarding Covid-19 patients were not expressed by both the study groups. (Table-2)

The main sources of information was social media (n=466, 66%) followed by news media (n=149, 27.30%). Remaining participants reported that they got the information through friends and other sources. A few students got information from their college resources. Details of sources of information are represented in Figure-1.

Table-1: Demographic characteristics of study participants (n=520)

Characteristics	Participants	Percentages
Gender		
Male	86	16.5
Female	434	83.5
Mean Age (Years)	21.18±1.748	
Categories of student		
National students	483	92.9
International students	37	7.1
Course of study		
Medical	176	33.8
Dental	56	10.8
Nursing	96	18.5
Medical technology	108	20.8
Physiotherapy	44	8.5
Other allied sciences	40	7.7

Table-2: Knowledge and awareness about Corona virus among study participants (n=520)

| Variable	Medical sciences	Allied sciences	*p*
When did the first deadly outbreak of Coronavirus occur?			
Correct	30 (5.80%)	19 (3.70%)	
Incorrect	172 (33.10%)	208 (40.0%)	0.005
Don't Know	30 (5.80%)	61 (11.70)	
Where did the current Coronavirus originate from?			
Correct	225 (43.30%)	278 (53.50%)	
Incorrect	1 (0.20%)	1 (0.20%)	0.925
Don't Know	6 (1.2%)	9 (1.70%)	
Incubation Period			
Correct	179 (34.40%)	200 (38.50%)	
Incorrect	49 (9.40%)	74 (14.20%)	0.054
Don't Know	4 (0.80%)	14 (2.70%)	
Contagious Awareness			
Correct	218 (41.90%)	252 (48.50%)	
Incorrect	5 (1.00%)	8 (1.50%)	0.031
Don't Know	9 (1.70%)	28 (5.40%)	
Routes of Transmission			
Correct	63 (12.1%)	103 (19.8%)	
Incorrect	169 (32.5%)	182 (35.0%)	0.028
Don't Know	0 (0.0%)	3 (0.6%)	
Awareness about fatality rate			
Correct	43 (8.3%)	56 (10.8%)	
Incorrect	149 (28.7%)	148 (28.5%)	0.003
Don't Know	40 (7.7%)	84 (16.2%)	
Awareness about vaccine			
Correct	213 (41.0%)	244 (46.9%)	
Incorrect	9 (1.7%)	8 (1.5%)	0.004
Don't Know	10 (1.9%)	36 (6.9%)	
Awareness about cure			
Correct	29 (5.6%)	11 (2.1%)	
Incorrect	201 (38.7%)	270 (51.9%)	0.001
Don't Know	2 (0.4%)	7 (1.3%)	
How severe is the infection of corona?			
Correct	120 (23.1%)	164 (31.5%)	
Incorrect	112 (21.5%)	124 (23.8%)	0.235
Symptoms of corona			
Correct	229 (44.0%)	271 (52.1%)	
Incorrect	3 (0.6%)	17 (3.3%)	0.007
Awareness about spread of disease			
Correct	157 (30.2%)	215 (41.3%)	
Incorrect	75 (14.4%)	73 (14.0%)	0.08
Awareness about age groups involved regarding disease severity			
Correct	8 (1.5%)	8 (1.5%)	
Incorrect	224 (43.1%)	280 (53.8%)	0.66
Knowledge about treatment			
Correct	97 (18.7%)	101 (19.4%)	
Incorrect	135 (26.0%)	187 (36.0%)	0.116
Awareness about precautions against COVID-19			
Correct	230 (44.2%)	287 (55.2%)	
Incorrect	2 (0.4%)	1 (0.2%)	0.41
Knowledge about spread of corona infection			
Correct	230 (44.20%)	279 (53.7%)	
Incorrect	2 (0.4%)	9 (1.7%)	0.075
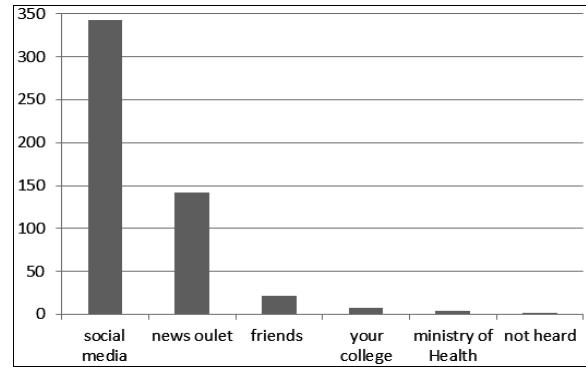
Figure-1: Sources of information about COVID-19

DISCUSSION

After its emergence, COVID-19 quickly ensnared the world, bringing everything to a standstill. As a result, healthcare and allied health professionals became frontline workers overnight, exposing themselves to infection to save others. This study was designed to compare and contrast the knowledge and attitudes of medical and allied students.

One of the most significant finding we discovered was that a good majority of students seem to have found out most of their information on social media (66%), demonstrating social media's importance in propagating information. In fact, most participants seem to have heard the word 'coronavirus' only after the outbreak taking place in China in December 2019, after which the number of posts, news articles and stories about the virus peaked. Another study carried out in India between medical and allied students also found that most students obtained their information from social media.[21] The fact that most participants had only heard about coronaviruses until then further exemplifies the significant role social media played. This indicates that healthcare workers and those qualified to give information about the virus should harness the power of the internet and social media when it comes to educating the public about important issues regarding health and keep them up to date with new research findings.

The correct answer rates of these questions ranged from 1.5–53.7%, lower than a study carried out by Zhong *et al*[22] where answer rates ranged from 70.2–98.6%. This was unexpected as we assessed medical and allied students and expected them to be more knowledgeable; however, this study was carried out when the pandemic was in its early stages so this may have affected how people answered. Another study done in Pakistan showed on the contrary that medical students fared outstandingly well. Sharing and acquiring knowledge for growth is frequently hampered by later unsatisfactory results. Mismanagement of knowledge, according to the literature, is a possible cause. If the barriers to knowledge sharing in acquired growth are not properly understood, such mismanagement can occur. In

general, it's rather surprising that non-medical students knew significantly more about the virus as compared to medical students in some areas, such as the route of transmission where more allied sciences students answered correctly compared to medical students, yet the number of correct answers was very low in both groups. This contrasts to similar finding from India where more than half (53.71%) of medical and allied health science students correctly identified COVID-19 transmission.[21]

About 44% medical and 52.1% allied students gave the correct response about the symptoms. However, this is lower than surveys where 98.63% Chinese and 90% Egyptian residents have accurately identified the symptoms of COVID-19.[22,23] This suggests that since most students use social media to get information, Pakistani media should find new ways of educating the public. Our study discovered that 46.9% allied and 41% medical students knew that there was no vaccine for COVID-19 available at the time of this study (before 2021). This finding is remarkably similar to a cross-sectional study carried out in Jordan, where 89% medical and non-medical university students knew about the then unavailability of the vaccine.[24]

About 48.5% allied and 41.9% medical students give the correct response of whether COVID-19 was contagious or not. Whereas our results were lower compared to 85.31% participants provided the accurate answer.[21] Barely 8.3% medical and 10.8% allied students had partial knowledge about the fatality rate of COVID-19. The result of this research is lower compared to 38.32% students in India choose the correct response.[21]

LIMITATIONS

The fact that we caused out the study by reaching out to participants via social media poses as a limitation to our study. This prevented us from approaching participants on the spot and may have produced the Hawthorne effect, giving participants time to lookup answers themselves before feeding answers in. This study was conducted in one university only, hence may not accurately represent knowledge levels of students in general. Due to restrictions and lockdowns the study took longer than expected to complete hence the opinions of these students might have changed.

CONCLUSION

The undergraduate allied medical sciences students showed a satisfactory level of awareness and with an obvious difference in awareness level between Medical and Allied Sciences. Better educational efforts with effective techniques are pointed to further increase the level of awareness and to suffice for the shortcomings. More efforts should be directed to all medical students specially, and allied health sciences generally.

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REVIEW ARTICLE
ROLE OF TRPM6 AND TRPM7 GENE POLYMORPHISMS IN RHEUMATOID ARTHRITIS: A SYSTEMATIC REVIEW

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Rheumatoid arthritis (RA) is a type of the autoimmune disease that adversely effects on the quality of life of the patient, primarily by affecting the joints. Different underlying immune system diseases through a common pathway may proceed to onset of rheumatoid arthritis. Magnesium (Mg) is one of the other essential minerals having significant role in the management of normal immune responses in inflammatory conditions. The exact role of magnesium deficiency (MgD) in pathophysiology of rheumatoid arthritis is still under debate, but several mechanisms are proposed through which, MgD leads to generation of inappropriate immune system functions. Most of the dietary Mg is absorbed in small intestine through paracellular passive mechanism while small amount is transported in blood through Transient Receptor Potential Channel Melastatin member 6 and 7 (TRPM6 and TRPM7). Genetic alterations in TRPM gene results in hypomagnesaemia, which may in turn, increase the risk of onset of pro-inflammatory mediators.
Keywords: Arthritis, magnesium, TRPM6, TRPM7, polymorphism

INTRODUCTION

Immune disorders are classified as a set of clinical abnormalities in host's defence system which are manifested by progressive production of pathological immune reactions against body's own antigens.[1] As per studies, the population-based data on determination of global burden of autoimmune diseases reveals that the incidence of these autoimmune diseases varies in different countries ranging from 5–10%, i.e., 9% in England, 6% in other European countries and approximately 7% in United States of America.[2] Among different autoimmune diseases, rheumatoid arthritis (RA) is one of the most common progressive autoimmune disease resulting in organ complications, failures, and disturbed life. The word rheumatoid arthritis came from a Greek word 'Rheumatos' which means 'flowing'. The suffix '-oid' means 'that mimics or resemble' while 'arth' means 'joint' and '-itis" means 'inflammation'.[3] This means that rheumatoid arthritis is a disease that resembles rheumatic fever disease which involves throat infections and further leads to joints inflammation and pain. The characteristics and clinical features of the disease are found in around 2,000–3,000 years old literatures. However, a clearer picture and description of the disease was first demonstrated by Sir Benjamin Collins Brodie, an English Physiologist, in 1819. In 1858, another English Physician Alfred Baring Garrod given the name rheumatoid arthritis to the disease.[4]

The physiological and pathological mechanism underlying disease onset is a complex process that is still being researched. However, several mechanisms and triggers are proposed, which ultimately lead to a common pathway in disease development. Anti-citrulline antibodies and other pro-inflammatory cytokines such as interleukin-1 and interleukin-6 are produced, resulting in chronic joint inflammation.[5] Furthermore, other extra-articular factors such as subcutaneous nodules, interstitial lung disease, pleuritis, endocarditis, and rheumatoid vasculitis may worsen the course of rheumatoid arthritis in the long run.[6] Inflammation of joints involves the activation of both natural and acquired immune systems. The chronic inflammation of joint synovium occurs due to interaction between synoviocytes and immunological cells such as macrophages, natural killer cells, T and B lymphocytes. Similarly, a complex interplay of complement and pro-inflammatory proteins also leads to progression of the disease, i.e., production of Anti-citrullinated peptide antibodies and TNF pathway.[7]

In our body, magnesium is the fourth most common mineral, involved in several biochemical and cellular processes.[8] Although the exact role of magnesium deficiency in inflammatory responses is still unknown, several mechanisms have been proposed. MgD increases thymic cellularity, which increases T-cell function and release of pro-inflammatory chemicals like IL-1, IL-6, TNF-alpha, and histamine, among others.[9,10] Similarly, MgD increases CTL cytotoxicity via an ATP-dependent mechanism.[11]

The recommended daily requirement of magnesium is 360–400 mg/day for an adult person which is completely acquired through exogenous sources, i.e., food.[12] Inside the body, the normal serum Mg levels is maintained through three different mechanisms, i.e., intestinal absorption, Mg storage in

bones and renal excretion. During Mg deficit conditions, Mg from bones is used for constant serum level.[13] About 80–90% of dietary Mg is absorbed in small intestine through paracellular passive mechanism while small amount is transported in blood through Transient Receptor Potential Channel Melastatin member 6 and 7 (TRPM6 and TRPM7).[14]

The main cause of hypomagnesemia related to gastrointestinal absorption is due to acute or chronic diarrhoea rather than vomiting since the concentration of magnesium in lower GI tract secretion is higher than secretion of upper GI tract secretions.[15] Some drugs may also reduce the absorptions of magnesium from GI tract such as proton pump inhibitors such as omeprazole for more than one year. The mechanism behind this malabsorption of magnesium from intestine is the inhibition of TRPM6 and TRPM7 magnesium channels.[16]

Besides these factors, genetic mutations in TRPM6 and TRPM7 genes, i.e., malfunctioning of TRPM6 channels causes hypomagnesemia with secondary hypocalcaemia (HSH) while downregulation of TRPM7 intestinal channel results in increased magnesium influx in colon cells.[17] Research studies have shown that genetic alterations in TRPM gene results in hypomagnesaemia and other related abnormalities which may in turn increases the risk of onset of pro-inflammatory mediators such as IL-1, IL-6, TNF-alpha and histamine.[18,19] The objective of current study was to review the association between TRPM6 and TRPM7 gene polymorphisms and hypomagnesemia leading to onset of rheumatoid arthritis.

METHODOLOGY

This review includes a comprehensive evaluation of the role of TRPM6 and TRPM7 Mg transport channels gene polymorphisms in the dysregulation of serum Mg levels. For achievement of this purpose, online computerized databases were searched manually such as PubMed (*http://www.ncbi.nlm.nih.gov/pubmed*), Cochrane (*https://www.cochranelibrary.com/*) and Amed (*https://www.ebsco.com/products/research-databases/allied-and-complementary-medicine-database-amed*) from January 1990 to December 2020. No language restrictions was imposed. The databases will be searched for terms such as *rheumatoid arthritis, pathogenesis, classification, nutritional elements, and rheumatoid arthritis, TRPM6* and *TRPM7 genetic alterations and serum Mg levels* etc.

The databases were searched for studies and articles in between January 1970 to December 2020. The studies before 1970 were excluded from our review because no study was performed on determination of role of TRPM gene polymorphisms in rheumatoid arthritis disease. Our review included case control, meta-analysis, and related studies while patients on anti-rheumatic treatment, suffering from other inflammatory diseases, case and medical reports were excluded from our review. The titles, abstracts and the content of articles will be selected based on rheumatoid arthritis introduction, name of the author, year of publication, sample size, epidemiology, causes, pathogenesis, factors involved in onset of the disease, immune response, immunomodulation, relationship between minerals and other food components with immune response, association between nutrients and rheumatoid arthritis, blood magnesium level regulation and odds ratio (OR) for risk of onset of rheumatoid arthritis. Data from these filtered studies on association between TRPM6 and TRPM7 gene polymorphisms and onset of rheumatoid arthritis was subjected to statistical analysis using computer-based software.

RESULTS

TRPM6 Gene Polymorphisms and Hypomagnesemia
Online databases as previously mentioned were searched for 'Role of TRPM6 gene polymorphisms and hypomagnesemia' including NCBI, Cochrane and Amed. As per our search, 10 studies were shown in the result in NCBI database, while in Cochrane library, 3 studies were found and in Amed library, 2 studies were found. Four studies were excluded due to article duplication. Out of remaining 11 studies, 5 studies were excluded because those studies were not on estimation of TRPM6 gene polymorphisms and hypomagnesemia. Only 6 studies which were considered for review were following the inclusion criteria as already mentioned, i.e., the studies showed significant results as per our inclusion criteria for studies. Among these studies, 4 articles were research articles while 2 articles were based on meta-analysis.

For 4 research articles[20–23], the calculated odds ratio for TRPM6 gene polymorphism and hypomagnesemia was found to be 1.32 (1.11–146). In these studies, a total number of 724 study samples were analysed in which both male and female subjects were equally distributed. For one meta-analysis of genome wide association study (GWAS) on TRPM6 gene polymorphism and its association with hypomagnesemia[4], the included study samples were more than 100,00 (29,880 RA cases and 73,758 controls) from both Asian and European population, the calculated risk factor (odds ratio) was 8.94 with 95% of confidence interval. According to this analysis, higher levels of serum magnesium level is associated with 8.84 fold increased risk of rheumatoid arthritis.

TRPM7 Gene Polymorphisms and Hypomagnesemia
Online databases as previously mentioned were searched for 'Role of TRPM7 gene polymorphisms and hypomagnesemia' including NCBI, Cochrane and Amed. As per our search, 68 studies were shown in

results in NCBI database, while in Cochrane library and Amed library, no such studies were found. Forty-eight studies were excluded because of the studies were not related to TRPM7 gene polymorphism and its association with rheumatoid arthritis. Twenty articles were screened and out of these, 16 articles were excluded from our review literature because those studies were not on estimation of TRPM7 gene polymorphisms and hypomagnesemia. Finally, only 4 studies which were considered for review were meeting the inclusion criteria, i.e., the studies showed significant results as per our inclusion criteria for studies. Among these studies, 3 were original research articles while one article was based on meta-analysis and was excluded from the study because the review was based upon TRPM7 gene polymorphism and its role in embryonic development. For three research articles[25–27], the calculated odds ratio for TRPM7 gene polymorphism and hypomagnesemia was 1.85 (95% CI: 1.09–3.14).

Association between Hypomagnesemia and Onset of Rheumatoid Arthritis
For meta-analysis of role of decrease serum level of magnesium in onset of rheumatoid arthritis in susceptible patients, we finally performed online databases search as previously mentioned for 'Association between hypomagnesemia and onset of rheumatoid arthritis' including NCBI, Cochrane and Amed. As per our search, 221 studies were shown in the result in NCBI database, while in Cochrane library and Amed library, no such studies were found. The articles were thoroughly screened, and 201 studies were excluded because those studies were not on determination of role of hypomagnesemia in onset of rheumatoid arthritis disease. Twenty articles were then included in our study. However, 11 articles were also excluded from the study because those studies were not related to determination of association between decrease magnesium intake in rheumatoid arthritis disease. Among the remaining 9 studies, 7 studies[28–33] were based on estimation of serum magnesium level in rheumatoid arthritis patients. The odds ratio for decrease magnesium level and onset of rheumatoid arthritis disease in these 7 studies was estimated as 2.8 (95% CI: 1.20–6.58, p=0.021). One study was based on estimation of serum magnesium level in patients suffering from rheumatoid arthritis conducted by Chavan *et al*[29], while the other study was on determination of effects of magnesium intake on radiographic osteoarthritis which was performed by Zeng *et al*[34]. In those two studies, 1,676 newly diagnosed patients were analysed from serum magnesium level. According to the study by Chavan *et al*[29], decrease magnesium level with dyslipidemia and hyperuricemia is a risk factor for rheumatoid arthritis

and cardiovascular diseases. In this study, other dietary factors such as calcium, potassium and phosphorus were also studied in patients suffering from rheumatoid arthritis. Along with this, serum bilirubin levels, i.e., both direct and indirect were also increased. The other study by Zeng *et al*[34], the study was not directly related to role of hypomagnesemia in rheumatoid arthritis, rather the case-control study was confined to the findings that increase intake of dietary magnesium is inversely associated with radiographic osteoarthritis and other knee joint problems.

DISCUSSION
Magnesium is one of the important elemental minerals which is required to the body for normal functions including maintenance of intact immune system responses. The exact mechanism involved in the role of magnesium in regulation of inflammatory responses is still unclear, but different indirect mechanisms are proposed through which magnesium plays its role in immunity. Magnesium deficiency is found to be involved in increase thymic cellularity that ultimately increases T-cells functions and release of pro-inflammatory chemicals such as IL-1, IL-6, TNF-α and histamine. Similarly, MgD also increases cytotoxic activity of T-lymphocytes (CTL) through ATP dependent mechanism.[35] According to Libako *et al*[36], due to hypomagnesaemia, there is an increase in intracellular calcium level which causes hyperactivation of phagocytes. Phagocytes are first line of host defence mechanisms against foreign pathogens. The function is achieved through production of cytokines, reactive oxygen species (ROS) and other cytokines which leads to intense inflammatory responses.[36] Similarly, Chavan *et al*[29] also found hypomagnesaemia in RA patients as compared to control subjects which shows that low blood magnesium level is significantly associated with development of RA disease (p≤0.01).[29] The genetic involvement in the regulation of serum magnesium level cannot be ruled-out.

Genetic influences can be understood by different mechanisms by which different genes can alter the normal physiological functions that regulate the normal body magnesium content. The most important mechanism is the regulation of dietary absorption pattern of magnesium. In intestines, there are two types of magnesium transport channels such as TRPM6 and TRPM7 transport channels that regulates the transfer of dietary magnesium from lumen to systemic circulation. These protein channels are transcripted from TRPM6 and TRPM7 genes. Any genetic alteration in these genes may alter the function of magnesium transport channels, which ultimately results into hypomagnesemia. According to Song *et al*, genetic mutations in TRPM6 gene may result in

diabetes mellitus in susceptible women whose magnesium content is deficient in the diet.[37] Coulter *et al* also found the novel mutations in TRPM6 gene results in decrease serum magnesium level which may be involved in other disorders in the body.[19] Malfunctioning of TRPM6 channels causes hypomagnesemia with secondary hypocalemia (HSH) while downregulation of TRPM7 intestinal channel results in increased magnesium influx in colon cells.[17]

In our study, 83 studies were included based on determination of association between TRPM6 and TRPM7 gene polymorphisms and hypomagnesemia, but only 9 studies fulfilled our inclusion criteria. As per our review, the odds ratio was found to be 1.32 and 1.85, respectively. This means that the genetic polymorphisms in TRPM6 and TRPM 7 gene are risk factor for development of rheumatoid arthritis in susceptible patients.

In the studies conducted on patients suffering from rheumatoid arthritis, along with magnesium level other parameters like uric acid, hyperlipidaemia, bilirubin level and other minerals were also measured. This reduces the quality of our review literature due to lack of evidence on possible association between hypomagnesemia and onset of rheumatoid arthritis. Similarly, we reviewed the role of decrease serum level of magnesium in onset of rheumatoid arthritis disease in susceptible patients, according to our systemic review, 9 case-control studies were found in which there was decrease serum concentration of magnesium in patients suffering from rheumatoid arthritis and one study was related to inverse association of magnesium intake with osteoarthritis. Both studies were case-control studies in which it was found that the patients suffering from rheumatoid arthritis and osteoarthritis have significant hypomagnesemia ($p<0.01$) and that the magnesium supplementation has a protective role in joint health. However, in these studies, other dietary components were also studied such as calcium, potassium, and phosphorus.

The increase serum level of direct and indirect bilirubin was also found to be elevated in study population. This means that the other dietary factors may act as confounding factor for onset of rheumatoid arthritis. Similarly, the hyperbilirubinemia may also be considered as a factor causing increase joint inflammation.

CONCLUSION

Any genetic alteration in TRPM genes regulating the functions of magnesium transport channels may lead to decreased absorption of dietary magnesium and cause hypomagnesemia which may be a factor for development of rheumatoid arthritis in susceptible patients.

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CORRIGENDUM

The name '**Naheed Akram**' may please be corrected, read and cited as '**Naheed Akhtar**' in the article:

ROLE OF PROPHYLACTIC ANTIBIOTICS IN CLEAN SURGERY
Ziyad Afzal Kayani, Sarmud Latif Awan, Muhammad Arshad Abbasi*,
Irum Gilani, *Naheed Akhtar*, Farzana Sabir**
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The correct citation of the article will be as follows:

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The authors' affiliations and other details remain the same. The Editorial Team regrets any inconvenience whatsoever caused by this unintentional mistake due to metadata upload on submission.

SHORT COMMUNICATION
PROBLEM-BASED LEARNING: AN OVERVIEW OF ITS PROCESS AND IMPACT ON LEARNING

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Problem-based learning (PBL) has a long history of advocating experience-based education. Psychological research and theory suggest that by having students learn through the experience of solving problems, they can learn both content and thinking strategies. Problem-based learning (PBL) is an instructional strategy that allows students to seek solutions to unstructured, real-world situations. PBL focuses student learning on a complicated topic with no one proper answer. Students collaborate in groups to determine what they need to know in order to solve an issue. Rather than imparting knowledge, the teacher's role is to aid the learning process. PBL aims to help students build flexible knowledge, effective problem-solving abilities, SDL skills, effective collaboration skills, and intrinsic motivation. The process of PBL includes identification of outcome, designing a scenario, introduction of PBL, research, performance and assessment. When compared to students in a lecture-based learning environment, studies on the effectiveness of PBL tend to be mixed, but they all suggest that students who have experienced PBL acquire similar or less learning gains when it comes to short-term information acquisition. However, in terms of long-term information retention, the results strongly favour PBL.
Keywords: Problem base learning, PBL, Effectiveness, Learning, Evaluation, Medical Education
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PBL OVERVIEW

Problem-Based Learning (PBL) is a teaching style in which students are taught concepts and principles through the use of challenging real-world issues rather than direct presentation of facts and concepts.[1] PBL can help students acquire critical thinking skills, problem-solving talents, and communication skills in addition to course content. It may also offer opportunities.[2]

Learning is usually triggered by an issue that needs to be solved in a PBL context. The origin of thinking, according to Dewey, is some 'perplexity, confusion, or doubt' triggered by 'something special that causes and provokes it'.[3,4] Many educators like PBL as a pedagogical technique because it provides an instructional framework that encourages active and group learning. It is based on the assumption that effective learning occurs when students construct and co-construct ideas through social interactions and self-directed learning.[4] A tutor, also known as a facilitator, serves as a guide to help students learn by scaffolding their learning, particularly in the PBL tutorial's issue analysis and reporting portions, and by facilitating students' inquiry paths as they make sense of their ideas through dialogue and sharing.[5]

PBL PROCESS

PBL is a student-centred, inquiry-based teaching method in which students work on a real-world, ill-structured topic that requires more research.[6] You can start designing, implementing, and assessing PBL in your own courses by breaking down the PBL cycle into 6 parts.

The first step is to identify the outcome. PBL is best for process-oriented course goals including teamwork, research, and problem solving. It can aid in the acquisition of content or conceptual information, as well as the development of disciplined habits such as writing and communication.

The scenario's second phase is to plan it out. After that, you design a PBL scenario with an incorporated challenge based on the brainstorming of students. Consider a real-life, complicated situation that relates to your course's subject.

The following stage is to introduce PBL. Start with a 'simple problem' such as a scenario with long lines in the dining hall, if students are unfamiliar with PBL. After grouping students and providing time for an abridged form of PBL, introduce the assignment expectations, rubrics, and timelines.

The next stage is to conduct research. PBL research begins with students describing the problem and determining what they already know about it (background knowledge), what they need to learn more about (research subjects), and where they can get data in small groups (databases, interviews, etc.).[7]

Fifth step is performance. Following their research, the students build products and presentations that combine their findings, solutions, and knowledge. You have complete control over format of the summative assessment. Students gather resources to gain background knowledge that will help them grasp the problem, and then present their findings to the class as research posters, along with suggested solutions.

The final step is assessment. Evaluate the products and performances of the groups during the PBL assessment process. Rubrics can be used to determine whether students clearly communicated the problem, background, research methodology, solutions (feasible

and research-based), and resources, as well as whether everyone in the group contributed meaningfully.[8,9]
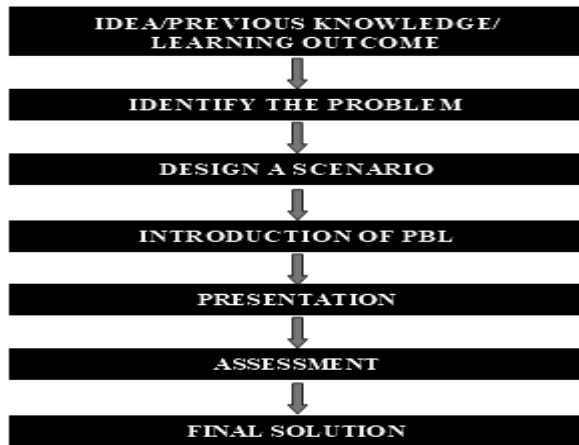
Flow chart showing the process of PBL

PBL AND ITS IMPACT ON LEARNING

The majority of the study on PBL's effectiveness has been done in the medical profession. The influence of PBL on student learning outcomes is increasingly being investigated in applied domains and professional education, providing new insights. Nursing education has devoted a large amount of study to investigating the efficacy of PBL in healthcare training in order to prepare nursing practitioners for an expanding spectrum of patient care services. According to a meta-analysis of studies on PBL's effectiveness in nursing education, it has good effects on student satisfaction with the course.[10]

Studies on the effectiveness of PBL appear to be variable when compared to students in a lecture-based learning environment, but they all show that students who have encountered PBL acquire similar or less learning gains when it comes to short-term information acquisition. However, the results favour PBL greatly in terms of long-term information retention. Several authors in the PBL literature have acknowledged that one of the PBL intended learning objectives is deep-content learning.[11] In fact, studies show that students who engaged in deep-content learning increased their overall learning results. It is well accepted that PBL components are linked to student performance and learning outcomes.

PBL was found to be more effective than traditional methods in developing students' knowledge of procedures or applications in several studies. Capon and Kuhn found that students in PBL had stronger procedural or application knowledge by demonstrating their ability to apply such information to a specific test situation. This was also demonstrated in a similar way.[12]

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UM: Introduction writing, Data collection
AS: Concept development and Data Analysis
BJ: Data collection, finalizing the draft, helped in getting approval from the review board

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