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EDITORIAL

Role of Pharmacists in Implementation of Pharmacovigilance in Clinical Settings of Pakistan

Rabia Bushra

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The disaster of cardiac drug Isosorbide mononitrate (batch number J093) led to the deaths of more than 200 heart patients in Punjab Institute of Cardiology, Lahore, Pakistan in 2012. In-patients were treated with a counterfeit drug that triggered severe adverse reaction due to the sudden drop in platelets count. After this tragic incident, Drug Regulatory Authority of Pakistan (DRAP) established an Act in 2012, to monitor the quality, administration and safety of medicines stringently all over the country[t].

Clinical trials are mainly conducted to determine the efficacy and potency of drugs usually on healthy subjects. But when the drug is marketed, it comes in contact with population including children, pregnant women, and elderly patients. The administration of trial/new drug in such population will provide the real scenario with respect to adverse drug effects and effectiveness both. Data dealing with drug safety is also one of the essential requirements for new drug application (NDA). Pharmacovigilance is especially significant during pre and post marketing stages due to limited volunteers' induction and conditions set during trials.

The Food and Drug administration (FDA) was constituted in 1906 to regulate the quality of manufactured drugs in all aspects. Pharmacopeias and codexes were also compiled to provide the methods, criteria and analysis for evaluation of active pharmaceutical ingredients as well as for pharmaceutical products[3].

According to the World Health Organization (WHO), "Pharmacovigilance is the cumulative activities of identification, evaluation, understanding and prevention of adverse drug reactions (ADRs) or any other drug related problem". WHO started the Program for International Drug Monitoring (PIDM) after the heartbreaking Thalidomide Tragedy (causing birth defects in children), emerged in early 20th century when patients were treated with the sulfanilamide elixirs prepared in a physiologically unacceptable diethylene glycol solvent[2].

WHO has always emphasized the development of national pharmacovigilance centers in countries all over the world. Internationally, Uppsala Monitoring Centre (UMC) in Sweden, was constituted to gather the information/data related to the drug associated adverse reaction to control future tragic incidences globally. Fortunately, Pakistan is currently a member of UMC after constituting national and regional pharmacovigilance centers in 2017-18[4].

It has been reported that the pharmacovigilance system is well established in clinical and hospital settings of developed countries while the developing countries are still in trial phases to construct as well as to implement the pharmacovigilance programmes practically. Healthcare professionals including physicians, pharmacists, and nurses have been involved in Adverse Drug Reactions (ADRs) documentation. ADRs of not only the new drugs but also the existing drugs are crucial to avoid risks and harmful effects of medications. These risks may also be consequent to increase the length of hospitalization, morbidity, and mortality[5]. Hence, it is highly recommended that each hospital whether small or large, must establish adverse drug reaction monitoring and reporting system.

Pakistan being a developing country also faces many challenges regarding healthcare facilities and units. Despite these hurdles, sustained efforts are being made for ADRs reporting and record keeping in clinical settings within Pakistan. The pharmacist, as a drug custodian, plays a vital role in preparation and administration of medicines with therapeutic monitoring in patients where necessary.

Traditionally, pharmacists were only confined to dispensing and filing of the prescriptions, but eventually ADRs, drug related problems and therapy failures endorsed the need of pharmacists in tertiary care settings. In developed countries, majority of pharmacists are also working on primary care with patient education.

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Currently, in large tertiary care settings of Pakistan, adverse drug reporting forms with electronic systems dealing with dose, frequency, adverse events, drug interactions and contraindications are operational. Extensive studies have been documented, highlighting the pharmacovigilance to be a core responsibility of physicians and pharmacists. The national and regional pharmacovigilance centers are taking the data of adverse and unwanted effects of drugs from the hospitals to avoid any future incidence.

Currently, many universities (Public and private sector) of Pakistan have been providing the concept, knowledge and practical exposure of 'Pharmacovigilance' to Pharm. D undergraduate students during their clinical rounds and visits. Nursing staff should also be educated and trained in respect of ADRs reporting and pharmacovigilance, as they are most likely available at the bed side of the patients. Because of this prior information, pharmacists can guide and train the nurses better.

There are many time constraints, logistical and financial issues in the development of infrastructure for the pharmacovigilance in hospitals. Teams of healthcare professionals (physicians, pharmacists, and nurses) need to put their efforts and assistance extensively to address harmful effects, hazards, drug safety, and effective treatment. This is extremely important when any new drug comes to the market. Data of adverse reactions of new marketed drugs should be collected by critically monitoring the patients. However; the workload of healthcare workers must also be balanced by hiring to facilitate adverse drug reporting and consequently to develop successful pharmacovigilance programmes.

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ORIGINAL ARTICLE

Association of Vitamin D and its Receptor (VDR) Gene Single Nucleotide Polymorphism (ApaI and TaqI) with Risk of Psoriasis

Muhammad Irfan Shereen[1], Mohsin Shah[1], Sami Siraj[2], Mohsin Ali[2], Muhammad Adnan Shereen[3] and Abeer Kazmi[*3,4]

ABSTRACT

Introduction: Psoriasis is one of the skin related inflammatory diseases that affects a low percentage of population around the globe. Vitamin D through Vitamin D Receptor (VDR) also regulates the function of white blood cells in psoriasis. Mutations in VDR gene have shown abnormalities in immune responses like psoriatic arthritis. To determine the possible association between Vitamin D Receptor (*ApaI and TaqI)* gene polymorphism and psoriasis**,** a case-control study was designed and conducted at the Institute of Basic Medical Sciences (IBMS), Khyber Medical University (KMU) Peshawar and health units of Peshawar.

Method: This multi-centre study included 220 samples (110 cases of psoriatic disease and 110 healthy controls). DNA was extracted using modified salting out protocol. VDR gene polymorphisms (*TaqI and ApaI*) were genotyped using amplification refractory mutation system using polymerase chain reaction (ARMS-PCR) method. Results were statistically analyzed.

Result: Our study showed significant association between VDR gene (*TaqI, ApaI*) polymorphisms and psoriasis with p-value of 0.009 (0.0019 and 0.0162) and odds ratios (95% confidence interval) for psoriasis of CC vs CT (TaqI) and AA vs AC (ApaI) were 2.963 (95% CI: 1.508-5.743) and 2.293 (95% CI:1.22-4.246) respectively.

Conclusion: Findings indicate that VDR gene polymorphisms (*TaqI, ApaI*) are significantly associated with onset and progression of psoriasis, and mutations in these loci are risk factors for development of psoriasis.

Key words: Psoriasis, VDR polymorphism, *TaqI, ApaI,* South Asia, Pakistan

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INTRODUCTION

Psoriasis is a type of chronic skin inflammatory disorder which affects 1-3 % of total world population and adversely affects quality of life. It is an auto-immune type of disorder. Symptoms include rapid growth of epidermal cells accompanied by inflammation of underlying dermal layer[1]. The word psoriasis was derived from Greek word "Psora" meaning "to be itchy"[2]. It is one of the two familiar immune-mediated skin inflammatory diseases. The other one is atopic dermatitis[3], characterized by skin inflammation and other symptoms including initial small and raised red patches covered by silver-coloured dead skin cells appearing as scales. About half of the patients experience the onset of symptoms below 25 years of age and mostly the females tend to be affected earlier than males[4]. Environmental factors such as infections, chemical agents, alcoholism, smoking, drugs as stress may provoke early events of the disease especially in those who are genetically vulnerable to psoriasis. Recent studies have shown that the early appearance of symptoms as compared to late onset involve genetic influence[5] which include mutations in different genes such as, TNFRSF9, IL-28RA, RUNX3, IL23R, LCE3B/LCE3C, REL, B3GNT2, IFIH1, ERAP1, TNIP1, IL12B, EXOC2, HLA-C, TRAF3IP2, TNFAIP3 and several others which are thought to be causing factors for initiation of early onset of disease[6]. Vitamin D is a steroidal hormone chemically known as 1, 25-dihydroxycholecalciferol[7]. The parent compound of vitamin D is cholecalciferol and found in vitamin supplements and dairy products. Inside our body, vitamin D exists in two forms i.e. 25 hydroxy VD (25-OH-VD) and 1, 25-dihydroxy VD (1, 25-[OH] 2 VD)[8].

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Both forms of VD are found in the blood and carrier proteins. About 10 to 15% are bound to albumin and 85 to 90% are bound to VD binding proteins (VDBP)[9,10]. Normal serum vitamin D level is around 20-50 ng/ml. Serum level less than 20 ng/ml is defined as vitamin D avitaminosis[11].

Vitamin D produces its action in body through its receptor (VDR). The receptor is present on long arm chromosome 12q13-12q14 near the centromere consisting of 11 exons that together with the associated introns makeup nearly 75kb of the DNA. The vitamin D receptor (VDR) mediates the effects of the physically active form of vitamin D 1,25- dihydroxy vitamin D3 (1,25(OH)2D3). Upon activation, VDR ligand/receptor complex regulates the transcription level of target genes which are involved in T helper (Th) cell improvement and Th cytokine profile change. The gene is involved in cellular growth, differentiation and activated inflammatory processes in keratinocytes. Vitamin D3 (calcitriol) through VDR gene regulates the functions of white blood cells like monocytes, T-cells, macrophages and dendritic cells. Based upon these mechanisms, the role of topical vitamin D formulations in curing psoriasis is still under investigation. These studies now give enough evidence for re-thinking vitamin D as possible candidate for the treatment of psoriasis[12]. The studies have shown that vitamin D (VD) and its active metabolite 1, 25-dihydroxy VD has a role in the pathogenesis of psoriasis including genetic mutations in VDR. Most common Polymorphisms in VDR gene are Cdx2, Fok I, TaqI situated in exon 1, 2 and 9 respectively while the BsmI and ApaI are in intron region between exon 7 and exon 8[13,14]. Therefore, in this study we analyzed and compared plasma levels of 25-(OH) D by ELISA in vitamin D deficient patients and normal healthy control samples along with VDR gene polymorphism (ApaI and TaqI) using allele specific polymerase chain reaction. This is the first report to show the role of VDR gene polymorphisms in the development of psoriasis in Pakistani population.

METHODOLOGY

The study was conducted on a total of 220 individuals. Out of these 220 individuals, 110 were the clinically diagnosed patients of psoriasis and 110 samples were taken from healthy control subjects. The cases included 48.18% females while in control group 49.10% were females. Similarly, the average ages of cases and controls were 57.36±6.25 years and 58.71±8.39 years, respectively.

Blood samples were taken from healthy individuals and psoriasis patients as per the inclusion criteria. Sampling was done at Khyber teaching Hospital (KTH) Peshawar, Lady Reading Hospital (LRH) Peshawar, Hayatabad Medical complex (HMC) Peshawar and experimental work was carried out in Institute of Basic Medical Sciences (IBMS), Khyber Medical University (KMU). Informed consents from the patients were taken. The study was approved from the Ethical Committee of the Khyber Medical University, Peshawar, Pakistan.

Clinically diagnosed psoriasis patients with complete medical history were included. There were no age and sex limitations. Patients with other skin diseases that mimic psoriasis were excluded from the study. Furthermore, patients not consenting and/or unwilling were also excluded from the study. Data of every sample was collected on a pre-designed pro forma. Apart from the demographic data, other information obtained from the patients included: name, age, gender, and ethnicity, duration of disease, family history of disease and history of other metabolic disorders.

Blood samples were collected in disposable syringes from every subject. A total of 3 ml of blood was withdrawn from the antecubital vein of every subject under sterile conditions. The blood was divided into two portions. Two ml of the blood was transferred to labeled plain vacuum tube and the remaining 1ml to labeled ethylene diamine tetra-acetic acid (EDTA) tube for separation of cell pellet. Within half an hour, the blood in plain tube was centrifuged at 3000 rpm for 10 minutes. A clear supernatant of serum was collected and transferred into a fresh Eppendorf tube and stored at -20°C for biochemical analysis. While the blood in EDTA tubes, was stored at 4°C for genotyping. ELISA Analysis: VD serum concentration was measured by an enzyme-linked immunosorbent assay (ELISA) kit (EQ 6411-9601 EUROIMMUN 25-OH VD ELISA kit manufactured by Medizinische labordiagnostika AG) Lot. No. E160916BH. The kit used solid phase competitive ELISA to assay the level of VD in human serum samples. The wells were pre-coated with monoclonal anti-25-OH VD antibodies. First the samples and calibrators/controls were diluted by adding 20 ul of each with 0.5 ml of working strength biotin. The solution was then incubated at room temperature for 10 minutes (+18^{O}C to +25^{O}C) and then pippeted into the microplate wells. 25-OH VD in the sample competed with biotin-labeled 25-OH VD enzyme conjugate for binding site. After washing completely, 3, 3', 5, 5'-tetramethylbenzidine (TMB) liquid substrate was added. The intensity of the colour produced was inversely proportional to the

concentration of 25-OH VD in the samples. The reaction was terminated by the addition of a stop solution (0.5 M Sulfuric acid) and the colour change was measured at a wavelength of 450 nm. All other steps of the assay were performed according to the manufacturer's instructions.

DNA Extraction: Before starting the DNA extraction procedure, all the plasticwares including tips, Eppendorf tubes, cell lysis buffer, nucleic lysis buffer and TE buffer were sterilized in auto clave. Chloroform and ethanol were pre-chilled at -20^{o}C. Total 0.6 ml of blood was dissolved in 1.2 ml of cell lysis buffer in 2 ml pre-sterilized Eppendorf tube. The solution was mixed thoroughly and centrifuged at 7000 rpm for five minutes. The supernatant was discarded carefully, and the pellet was washed 2-3 times with cell lysis buffer up to clarity. The set speed was 5000 rpm after first washing. The Eppendorf tube was kept for 2-3 minutes until the pellet was dried. To the pellet, 0.5 ml nucleus lysis buffer was added and after dissolving the pellet by pipetting, 0.15 ml of saturated NaCl and 0.7 ml of pre-chilled chloroform was added. The Eppendorf tube was centrifuged at 7000 xg for three minutes. The two visible layers were achieved after centrifugation. The supernatant (0.5 ml) was carefully? 12000 xg for 2 minutes to precipitate the DNA. The supernatant was carefully discarded, and DNA was washed with 70% ethanol and centrifuged at maximum speed (14000xg) for 10 minutes. The supernatant was removed, and the tubes kept for 10 minutes at room temperature. The DNA was re-suspended in 0.1 ml TE buffer and was kept at -20^{o}C.

PCR amplification: PCR assay was done for each DNA sample in a final reaction volume of 10 µl. Specific PCR primers were designed. The Control forward primers used for VDR-ApaI genotyping were 5'-CCA AAC ACT TCG AGC ACA AGG-3' and Control Reverse 5'-AGA GCA GAG TTC CAA GCAGAG G-3' with product size 592 bp. The allele specific reverse primer 5'- GGT GGG ATT GAG CAG TGA AGT-3' and reverse 5'-GGT GGG ATT GAG CAG TGA TGG-3' with product size 318 bp were respectively. Similarly, the control forward and reverse primers for VDR-TaqI genotyping were 5'-GCC AAA CAC TTC GGC AAG-3' and 5'-CGG TCC TGA ATG GCC ACA-3' respectively with product size of 592 bp. The allele specific forward and reverse primers were 5'-CAG AGC ATG GAC AGG GAG CAAG-3' and 5'-CAC ACTG CAG ACG TAC ATCC-3' respectively with PCR product size of 340 bp. The primers sequences are shown in Table 1 for allelic discrimination of both ApaI and TaqI polymorphisms in our study samples. The PCR product sizes are shown in Table 2 and the gel electrophoresis results are shown in Fig. 1 and Fig. 2. For each sample, four PCR tubes were labeled i.e. two tubes for VDR-TaqI and two tubes for VDR-ApaI PCR assays. The tubes were labelled as 'C' for C allele, 'T' for T allele for VDR-TaqI PCR assay and 'A' for A allele, 'C' for C allele for VDR-ApaI. Final reaction volume was prepared by taking 7 µl of deionized water, 0.8 µl of 10x PCR buffer, 0.3 µl of $MgCl_2$, 0.5 µl of 10mM dNTPs, 0.5 µl of template DNA, 0.3 µl of control primers, 0.3 µl of allele specific primers (C and T) in one PCR tube and 0.3 µl of allele specific primers (A and C) in the other PCR tube. The PCR tubes were vortexed for 5-10 seconds and then were run in a PCR thermal cycler (Multigene Optimax). The PCR conditions are mentioned in Table 2 and were as follows: denaturation at 94°C for 5 minutes, followed by 30 cycles of PCR: denaturation at 95°C for one minute, annealing at 64°C for 30 seconds, polymerization at 72°C for one minute and proof reading at 72°C for five minutes.

Stock Solutions Preparation: Total 46.1 gm of EDTA was dissolved in about 800 ml of distilled water and placed on magnetic stirrer. The pH was adjusted with sodium hydroxide (NaOH) to 7.5. After pH adjustment, sufficient quantity of distilled water was added to make up the volume 1000 ml; 121.14 gm of ultra-pure tris was dissolved in about 800 ml of distilled water and placed on magnetic stirrer. The pH was adjusted with hydrochloric acid (HCl) to 8.0. After pH adjustment, sufficient quantity of water was added to make up the volume 1000 ml. Then 12 mM Tris-HCl, 350 mM Sucrose, 6 mM $MgCl_2$ and 1% Triton X 100 were dissolved in 700 ml distilled water. After complete dissolution, sufficient distilled water was added to make up the volume 1000 ml.

Next, 12 mM Tris-HCL, 15 mM Ammonium Acetate 1.5 mM EDTA and 2% SDS solution were dissolved in 700 ml distilled water and after complete dissolution, enough water was added to make up the volume 1000 ml. NaCl 59 gm was dissolved in 150 ml of distilled water and after complete dissolution; sufficient water was added to make up the volume 200 ml; 5 ml 1M Tris-HCl solution and 2 ml 0.5M EDTA solutions were dissolved in 1000 ml of distilled water.

Statistical analysis: Statistical Package for the Social Sciences (SPSS) v23.00 software was used to determine the genetic association between psoriasis and VDR polymorphism. Chi-square test was performed to determine the association between alleles and genotypes while allele and genotype frequencies were assessed under Hardy-Weinberg Equilibrium (HWE). Odds Ratio (OR) was calculated with 95% Confidence Interval (CI).

RESULTS

The association study between VDR SNPs (apaI and TaqI) and psoriasis was conducted in 110 psoriasis cases and 110 healthy controls. The clinical demographic data of study samples is mentioned in Fig. 3. The average Body Mass Index (BMI) of both cases and controls was 25.79 ± 3.97 and 30.767 ± 6.59, respectively. Similarly, initial serum vitamin D levels were calculated in both cases and healthy control samples were 13.5±5.34 and 29.02±17.65, respectively. The results were statistically analyzed by using chi-square (x^2) test as shown in Table 3, which showed significant association between the VDR SNPs (ApaI and TaqI) and psoriasis (p = 0.0162 and 0.0019 respectively). To check either this significant association is a risk factor for the onset of disease, we calculated the odds ratio between the cases and controls. The results are mentioned in Table 4 which showed that although our study SNPs were significantly associated with psoriasis, but they are not a risk factor for onset of psoriasis. The odds ratios also showed that these polymorphisms are risk factors for development of psoriasis (Odds ratios of 2.29 and 2.96). Similarly, the serum levels of vitamin D in both cases and controls were statistically analyzed through chi-square test. According to our results, the low levels of serum vitamin D in cases (13.5±5.34 ng/ml) as compared to controls (29.02±17.65 ng/ml) with p-value less than 0.005 (<0.0001) shows a strong association with psoriatic plaques. The results are summarized in Table 3. Finally, the levels of vitamin D in both cases and controls were statistically compared with VDR SNPs i.e. ApaI and TaqI. The results showed that these VDR mutations are risk factors for early onset of psoriasis symptoms in local population of Peshawar, KPK, Pakistan and nearby areas (Table 5).

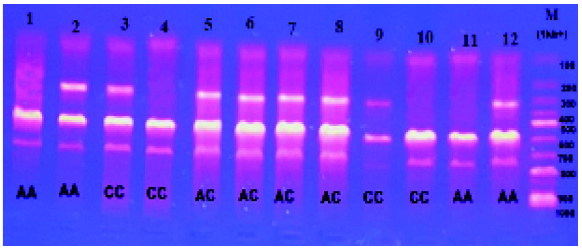

Fig. 1: PCR amplification ApaI

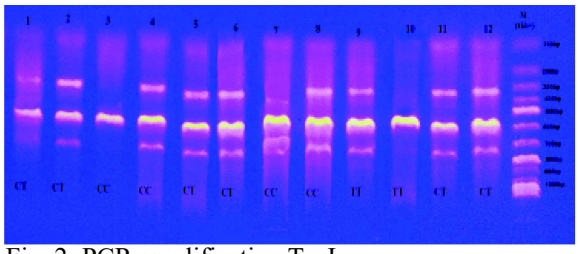
Fig. 2: PCR amplification TaqI

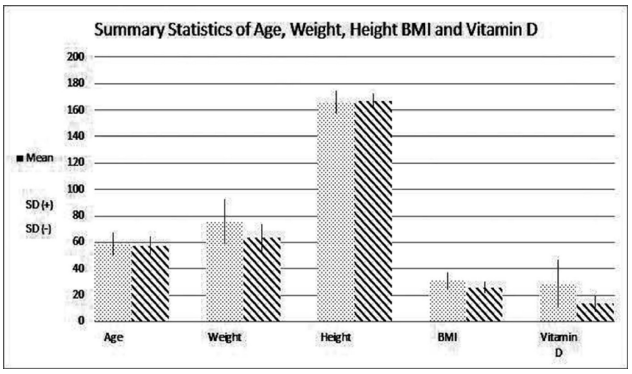

Fig. 3: Demographic Parameters of Controls and Cases

Table 1: ASPCR Primers with specific Sequences

Primers Type		Primers Sequence	PCR Product size
Apa I	Controls	Forward: 5° CCA AAC ACT TCG AGC ACA AGG 3° Reverse: 5° AGA GCA GAG TTC CAA GCAGAG G 3°	592 bp
	Allele Specific	Reverse: 5° GGT GGG ATT GAG CAG TGA AGT 3° Reverse: 5° GGT GGG ATT GAG CAG TGA TGG 3°	318 bp
Taq I	Controls	Forward: 5° GCC AAA CAC TTC GGC AAG 3° Reverse: 5° CGG TCC TGA ATG GCC ACA 3°	592 bp
	Allele Specific	Forward: 5° CAG AGC ATG GAC AGG GAG CAAG 3° Reverse: 5° CAC ACTG CAG ACG TAC ATCC 3°	340 bp

Table 2: PCR Amplification Profile

	Temperature (^{O}C)	Duration (minutes)	No of Cycles
Activation	95	5	1
Denaturation	95	1.0	30
Annealing	64	0.5	
Extension	72	1.0	
Final Extension	72	10.0	1
Soak	4	8	Hold

Table 3: Association of TaqI with

Psoriasis			
Genotypes	Controls (n)	Disease (n)	p-value
CC	48	18	0.0019
TT	17	42	
CT	45	50	
Apa-I with Psoriasis			
Genotypes	Controls (n)	Disease (n)	p-value
AA	42.72	21.82	0.0016
CC	19.10	34.55	
AC	38.12	43.64	
Vitamin D with Psoriasis			
Group	Number	Vitamin D ng/ml	p-value
Cases	110	13.5±5.34	<0.0001
Control	110	29.02±17.65	

Table 4: Odds ratio CC vs CT (TaqI) and AA vs AC (ApaI)

CC vs CT					
Genotype	Cases	Controls	CI 95%	Odds Ratio	p-value
CC	18	48	1.508 to 5.743	2.963	0.0019
CT	50	45			
AA vs AC					
Genotype	Cases	Controls	CI 95%	Odds Ratio	p-value
AA	24	47	1.22 to 4.246	2.293	0.0162
AC	48	41			

Table 5: Allele frequency of VDR Polymorphisms between cases and controls

VDR Loci	SNP	Genotype	Cases % (n = 110)	Controls% (n=110)	Vitamin D Level (ng/ml)		OR	p-value
					Cases	Controls		
Apa 1	A>C	AA	21.18	42.7	13.5	29.02	2.29	0.0162
		CC	34.5	19				
		AC	43.6	37.3				
Taq 1	C>T	CC	16.36	43.6			2.96	0.0019
		TT	38.18	15.45				
		CT	45.45	40.9				

DISCUSSION

Recent advancements in genetics have shown a relationship between psoriasis and gene mutations. Russel et al first time reported the association between HLA-B13 (Human Leukocyte Antigen serotype) and psoriasis[15]. Besides common type of psoriasis, genetic involvement in a rare type of psoriasis known as "Pustular psoriasis" has shown mutation in IL36RN[16]. This allele encodes IL36 receptor antagonist protein which reduces cytokines production. Mutation in IL36RN allele results in synthesis of abnormal protein and increase production interleukins (IL-8) from keratinocytes[17]. The type-2 psoriasis is a type whose genetic cause is not very much clear which shows the diversity of genetic variations associated with different types of psoriasis[6]. Previous study by Keren et al reported the role of vitamin D and its analogues in defensive mechanisms of skin and suggested the effective use of vitamin D in treatment of psoriasis[18]. Vitamin D influences the homeostasis of skin through its receptor i.e. vitamin D receptor.

The association between psoriasis and vitamin D polymorphisms and blood levels is not well understood as no such study had been performed previously. But vitamin D levels and VDR gene polymorphisms are associated with other autoimmune diseases like rheumatoid arthritis and multiple sclerosis. Hence, one can rationalize the possible association between psoriasis and vitamin D serum concentrations and VDR gene polymorphisms. To find possible association between psoriasis and VDR gene polymorphisms, we designed a case-control study for this purpose. The current study elucidates the role of VD and VDR gene polymorphism and showed significant difference between VD levels and genotypes of disease and control group. In our study, a total of 220 individuals of same age group were included. Out of 220, 110 were psoriatic patients and 110 were healthy controls. Level of vitamin D was analyzed for both case and control groups using commercially available kits. The levels of vitamin D were normal in control group (29.02 ± 17.65 ng/ml) while levels were below than normal in diseased patients (13.5 ± 5.34 ng/ml). The purpose of our study was to determine possible association between VDR gene polymorphism (TaqI and ApaI) and psoriasis. Genetic polymorphisms were checked by analyzing the restriction pattern of the polymerase chain reaction (PCR) products. The results were analyzed through chi-square analysis which showed a significant association between both TaqI and ApaI polymorphisms with psoriasis with a mean p-value of 0.009 (0.0019 and 0.0162). The odds ratios (95% confidence interval) for psoriasis of CC vs CT (TaqI) and AA vs AC (ApaI) were 2.963 (1.508-5.743) and 2.293 (1.22-4.246), respectively. The odds ratios show that VDR genes polymorphisms are risk factors for the onset of psoriasis. Similarly, vitamin D levels in psoriatic cases and healthy controls were analyzed through chi-square which also showed significant association between low vitamin D level and psoriasis disease (p-value < 0.005). Hence, through our study, we have shown that VDR gene polymorphisms (TaqI and ApaI) are significantly associated with the onset and progression of psoriasis. Furthermore, levels of vitamin D are very low in our case group that further complicates the psoriasis profile of cases and may be a risk factor for development of psoriasis.

CONCLUSION

Study concludes that VDR gene polymorphisms (TaqI and ApaI) were associated with the development of psoriasis. Vitamin D receptor SNP was associated with increased risk of psoriasis. Vitamin D deficiency may be a modifiable risk factor for developing psoriasis.

Authors' contribution: M.I.S, M.S, S.S, and M.A, conceptualized study design, collected, analyzed and interpreted data. M.A.S, and A.K interpreted data, prepared manuscript and did proof reading.

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ORIGINAL ARTICLE

Menstrual Hygiene Practices among Adolescent School Girls in Pakistan

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ABSTRACT

Objectives: To evaluate menstrual hygiene practices among teenage girls and to compare menstrual hygiene practices among secondary and higher secondary school girls in Pakistan

Methodology: A cross-sectional study of adolescent girls (grades 9 to 12) from different secondary and higher secondary schools across Pakistan was conducted between November 2017 and September 2018. A pre-tested and standardized questionnaire was administered using a non-probability sampling technique. The menstrual hygiene practices were evaluated and comparisons were made between secondary and higher secondary school girls about these practices. Data was analysed using SPSS version 22. P-value < 0.05 was set for being statistically significant.

Results: Out of a total of 2,000 adolescent girls, an equal number of adolescent girls (n=1,000) were chosen from secondary and higher secondary schools. The mean menarchal age was 12.5 ± 1.2 years. Majority of the participants (63.6%) belonged to public sector schools and (71.3%) responded that menstrual blood comes from the womb. One in five (19.3%) girls missed 2 days/month of school due to pain (54.5%). Two-thirds (68.6%) of the participants were using sanitary pads and one of three changed their pads three times/day, and forty-six percent of the girls were unable to carry out daily activities due to menstrual period. Statistically significant (P < 0.05) association was observed between knowledge of menstruation among secondary and higher secondary school girls.

Conclusion: The study showed that menstrual hygiene was understood well among young girls. However, a substantial association was noted between secondary and higher secondary school girls' hygiene practices.

Key words: Adolescent girls, Menstruation hygiene practices, Schools, Pakistan

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INTRODUCTION

Menstrual hygiene is one of the problems for young girls in middle-and low-income countries, particularly when in school[1]. Several factors including the female's knowledge influence menstruation[2]. Poor water sources, sanitation and hygiene facilities at schools, education in puberty and insufficient hygiene procedures make it humiliating and painful for adolescent girls to undergo menstruation[3].

Lack of knowledge about menstruation is associated with profound psychological and emotional problems[4]. Many schools do not help adolescent girls in maintaining menstrual hygiene that causes stress and humiliation dealing with blood-stained clothing[5]. Also it may lead to development of urinary and genital infections and even possibly infertility[6,7].

UNICEF reports that one girl out of ten does not go to school during her monthly cycle.

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Likewise, World Bank figures suggest that girls were absent from school every four days due to menstruation[8]. Statistics from India have shown that out of 113 million, 68 million adolescent girls go to schools, with inadequate menstrual hygiene habits and cultural taboos being considered the main barriers to school attendance[9,10]. Use of sanitary pads amongst Tanzanian women was found to be only 18 %, while remaining others were using toilet paper or cloth[11]. A study among Nigerian school girls found that 56% were utilizing pieces of cloth and toilet paper instead of menstrual pads[12]. In Ethiopia, it was found that most of the school girls knew about menstrual cycle before menarche, however only 37.6% used sanitary napkins, and a substantial proportion, 62.4 percent used rags and bits of cloth[13], and 11% changed their menstrual cloth once a day[14].

In Pakistan, many traditional and religious factors impose restrictions on social lives of women. Cultural taboos, misconceptions, isolation and restrictions regarding menstruating girls and menstrual hygiene prevent them from seeking help and restrain them from physical activities when menstruating[15].

Menstrual hygiene is a very significant factor in reproductive tract infections and a crucial element of teenage girls' health education. A study found that girls' anxiety and embarrassment from blood and body odor leakage is a major cause of school absenteeism and dropouts.[16] Most school girls do not have the resources for their own care and do not seek the help they need when facing problems, which not only hampers their ability to carry out daily activities but it can also lay the foundation for life long debilitation.

In Pakistan, studies regarding menstrual hygiene practices are scarce. This study aims to evaluate menstrual hygiene practices among teenage girls and to compare menstrual hygiene practices among secondary and higher secondary school girls in Pakistan.

METHODOLOGY

A cross-sectional analysis of adolescent school girls from different Pakistani provinces (Sindh, Punjab, Baluchistan, and Khyber Pakhtunkhwa) was done from November 2017 to September 2018. A total of 2000 girls (1000 from secondary and 1000 from higher secondary schools) who accepted and agreed to participate were interviewed. Using non-probability convenient sampling technique, post menarche girls from grades 9 to 12 were included. Girls who were not physically or mentally fit on the day of data collection were excluded. Hamdard College of Medicine & Dentistry Ethics Committee gave the approval to conduct the study.

The data was collected using structured, self-administered questionnaire written in English. To ensure its understanding it was translated in local language (Urdu) by a language professional. The questionnaire was validated and used in other studies previously to assess the menstrual hygiene practices among school girls[17]. Pilot study was carried out on 5% of the sample and relevant amendments were made.

After taking approval from the schools' administrators, girls are provided the questionnaire with their consent. Before the questionnaire was administered, the respondents were briefed about the intent and various aspects of the study. The responses from each class were taken separately in their dedicated sessions. Participants were asked to read each statement carefully and respond objectively. Girls also had the opportunity to ask questions. They were briefed that participants' identity would remain confidential, data cannot be traced back to participants, and it will only be used for research purpose.

The data was cleaned, coded and entered in SPSS version 22 after ensuring completeness of the filled forms. Descriptive statistics were done for all independent variables and Chi-square test measured inferential statistics to analyze the association of knowledge of menstrual hygiene activities between secondary and higher secondary school girls. Difference between types of absorbent material used during periods among secondary and high schools' girls was also determined. A p-value < 0.05 was considered as statistically significant. Binary logistic regression was applied to estimate the effect of different activities missed during menstruation. Multiple logistic regression analysis selected relevant variables to determine the adjusted odds ratio (OR).

RESULTS

Out of total 2000 adolescent girls, equal number of adolescent girls (n=1000) were selected from secondary and higher secondary schools. The mean age of the participants was 16.1 ± 1.8 years. More than half of the participants 1,272 (63.6%) belonged to public sector schools. The mean age at menarche was 12.5 ± 1.2 years. In terms of knowledge of menstruation, 1,793 (89.7%) participants replied that women stopped menstruation as they grew old, 1,502 (75%) thought that menstruation was a disease, 1,461 (73 %) said that pregnant women menstruate, 1,551 (77.5%) thought that menstrual blood comes from the gastrointestinal tract where food is processed, 1,425 (71.3%) replied that menstrual blood comes from the womb, 1,158 (58%) believed that menstrual blood contains dangerous

substances, 1,327 (66.4%) thought that pain during periods implies that somebody is not well and 1000 (50.0%) thought it was unsafe for a female's body if she runs during her menstruation cycle. A statistically significant ($p < 0.05$) relationship was found between secondary and higher secondary school girls' knowledge of menstruation. (Table 1)

with secondary and higher secondary school girls. The response that periods make you unable to walk far 1,130 (56.5%), was negatively associated with secondary and higher secondary school girls with OR=0.81, 95% CI = 0.68 - 0.98, $p < 0.05$.

Table 1: Difference of Knowledge Regarding Menstruation Between Secondary and Higher Secondary School Girls

Knowledge About Menstruation		Secondary School n (%)	Higher Secondary School n (%)	Total n (%)	p-value
Menstruation stops in very old women.	Yes	864 (86.4)	929 (92.9)	1793 (89.7)	
	No	136 (13.6)	71 (7.1)	207 (10.3)	<0.001*
Menstruation is a disease.	Yes	385 (38.5)	113 (11.3)	498 (24.9)	
	No#	615 (61.5)	887 (88.7)	1502 (75.1)	<0.001*
Pregnant women menstruate.	Yes	432 (43.2)	107 (10.7)	539 (27.0)	
	No#	568 (56.8)	893 (89.3)	1461 (73.1)	<0.001*
Source of menstrual blood is stomach where the food is processed.	Yes	340 (34.0)	109 (10.9)	449 (22.5)	
	No#	660 (66.0)	891 (89.1)	1551 (77.5)	<0.001*
Source of menstrual blood is womb.	Yes#	648 (64.8)	777 (77.7)	1425 (71.3)	
	No	352 (35.2)	223 (22.3)	575 (28.7)	<0.001*
Menstrual blood contains dangerous substances.	Yes	567 (56.7)	275 (27.5)	842 (42.1)	
	No#	433 (43.3)	725 (72.5)	1158 (57.9)	<0.001*
Menstruation pain means that someone is not well.	Yes	512 (51.2)	161 (16.1)	673 (33.6)	
	No#	588 (48.8)	839 (83.9)	1327 (66.4)	<0.001*
Running during menstrual cycle is harmful.	Yes	631 (63.1)	369 (36.9)	1000 (50.0)	
	No#	369 (36.9)	631 (63.1)	1000 (50.0)	<0.001*

*p-value < 0.05
#correct response

Most of the participants 385 (19.3%) missed two days of school in a month and the reasons of missing school by majority of them 1,089 (54.5%) were pain during menstruation and the fear of staining clothes 368 (18.4%). Absorbent material used by most of the participants 1,371 (68.6%) were sanitary pads and one-third of them 704 (35.2%) changed their pads thrice per day. Statistically significant ($p < 0.05$) relationship was observed between types of absorbent materials used during menstruation among public and private school girls. (Table 2)

Univariate analysis showed the difference of activities missed during menstruation among secondary and higher secondary school girls. Periods make you stay at home 1,089 (54.5%) with crude OR = 1.36, 95% confidence interval (CI) = 1.13–1.63, $P < 0.001$; periods make you unable to conduct daily activities 1,083 (54%) with OR = 1.60, 95% CI = 1.33 – 1.93, $P < 0.001$; adolescent girls who take regular baths during periods 1,102 (55%) with OR = 1.23, 95% CI = 1.02 – 1.48, $P < 0.05$; these variables were positively associated

Multivariable analysis reveals that the variables; periods make you stay at home, periods make you unable to conduct daily activities and periods make you unable to walk far; have been found to be significantly associated with secondary and higher secondary school students. After adjustment, the significance of these variables were adjusted as follows: OR = 1.24, 95% CI = 1.01 – 1.52, $p < 0.05$; OR = 1.58, 95% CI = 1.29 – 1.93, $P < 0.001$; and OR = 0.70, 95% CI = 0.58 – 0.85, $P < 0.001$. (Table 3)

DISCUSSION

In this study, the mean age at menarche was found to be 12.5 ± 1.2 years, which is consistent with other findings of Kumar NP study, which is 12.6 ± 1.08 years[16].

The study shows that the menstrual knowledge among young girls was adequate and most of the participants 89.7% knew that menstruation is a physiological process, and 71.3% knew that the blood comes from the womb, nevertheless 42% believed that menstrual

Table 2: Types of Absorbent Materials Used During Menstruation Among Public and Private School Girls

Absorbent Material Using During Periods								
n (%)	Cloths/Towels	Sanitary pads	Cotton	Toilet papers	Mattress (Foam)	Any other	Total	p-value
Public	345 (27.1)	830 (65.3)	46 (3.6)	20 (1.6)	26 (2.0)	5 (0.4)	1272 (100.0)	<0.001*
Private	107 (14.7)	541 (74.3)	38 (5.2)	12 (1.6)	23 (3.2)	7 (1.0)	728 (100.0)	
Total	452 (22.6)	1371 (68.6)	84 (4.2)	32 (1.6)	49 (2.5)	12 (0.6)	2000 (100.0)	

*p-value < 0.05

Table 3: Difference of Activities Missed During Menstruation Among Secondary and Higher Secondary School Girls

Activities During Monthly Cycle		Univariable Analysis		Multivariable Analysis	
		OR[a] (95% CI)	p-value	OR[b] (95% CI)	p-value
Housework missed	#	1			
	No	1.11 (0.92 – 1.34)	0.240		
Staying at home	#	1		1	
	No	1.36 (1.13 – 1.63)	<0.001*	1.24 (1.01 – 1.52)	0.034
Unable to walk far	#	1		1	
	No	0.81 (0.68 – 0.98)	0.034*	0.70 (0.58 – 0.85)	<0.001*
Unable to carry out daily activities	#	1		1	
	No	1.60 (1.33 – 1.93)	<0.001*	1.58 (1.29 – 1.93)	<0.001*
Regular bathing	#	1			
	No	1.23 (1.02 – 1.48)	0.026*		

= Reference category
*p-value < 0.05
OR[a] = Crude Odds Ratio
OR[b] = Adjusted Odds Ratio

blood contained dangerous substances. Knowledge about menstruation was also found to be high in another survey as most of the adolescent girls 88.9% replied that menstrual blood is not harmful[16]. A previous study done in Pakistan revealed that 50% of the girls were deficient in their understanding of the source of menstrual blood[15]. Significant association has been observed between knowledge about menstruation among secondary and higher secondary school girls. Similar findings have been observed from studies in Ethiopia[18] and India[19] showing that participants who were educated up to high school and above were more aware about menstruation.

Menstruation leads to absenteeism of school girls. In our study 17.5% participants missed one day per month and 19.3% missed two days per month from school during their periods and reasons were pain 54.5% and fear of staining clothes 18.4%. Indian study in 2018 reported that 41.6% girls missed their school because of menstruation and the reasons were; lack of proper place to change or dispose sanitary pads in school 56%, due to pain and discomfort 23.1% and due to fear of staining 18.1%[16]. However in other Indian studies, it was only 13.9%[20] and 12.6%[21] respectively.

It was found in this study that most of the girls 68.6% were using sanitary pads while 31.4% were using cloth/ towels or other absorbent material. Similar finding were recorded in an Indian study showing 94% of girls used sanitary napkins during periods[16]. However, several studies revealed lower usage of sanitary pads 20.8%[22], 34.7%[23] and 15.7%[20] and high usage of cloth 31.3%[22] and 44.1%[23]. In our study, significant association was observed between types of absorbent material used by public and private school girls. West Bengal study also found higher use of sanitary pads among girls in urban schools compared to rural ones[24]. This was also found in this study that 35.2% girls changed pads three times per day, 32.9% two pads/day, and 11% changed pads once a day.

Study from Lebanon reported that 40.4% of adolescent girls changed sanitary pads for every 3 to 4 hours each day[25]. In addition, study from Bengal showed that in 27% adolescent girls in urban and 30.4% in rural areas, the frequency of changing pads was only once per day[24]. Routine menstruation affects housework 55.3%, unable to walk far 56.5%, girls stay at home during cycles 54.5% and 46% are unable to perform daily activities such as cooking. Other studies also revealed

similar results 52%[16], 60%[26] and 54%[27]respectively. Study from Bengal showed that 78% girls were restricted from household work[24]. However, other studies indicated that most girls faced various barriers and restrictions[6,15].

Adolescent girls 44.9% bathed daily during periods. In contrast, a study conducted in Karachi showed that approximately 50% of the subjects were restricted from taking bath[15]. Another Indian study revealed that 84% adolescent girls bathed daily during their monthly menstruation cycle[6].

CONCLUSION

The study revealed sufficient knowledge of menstrual hygiene among adolescent girls, but significant association was observed between menstrual hygiene practices among secondary and higher secondary school girls. Education regarding menstrual hygiene practices should be conveyed by the mothers of adolescent girls and schools should also include health education programmes in their curricula regarding better management of menstrual hygiene.

Authors' contribution: NM designed the study, analysed statistically and edited the manuscript. HT contributed to study design and data collection. IA, A, and IN worked on data collection and manuscript writing. SMM analysed and interpreted data, edited and proofread the manuscript.

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ORIGINAL ARTICLE

Effectiveness of Educational Training related to Disaster Preparedness among Nursing Students

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ABSTRACT

Objective: To evaluate the effectiveness of educational training related to disaster preparedness among nursing students at Karachi

Methodology: This quasi-experimental study was performed in Indus College of Nursing and Midwifery in Karachi. The study was carried out from June to November 2019 over a period of six months. Total sample size was 40. Both male and female final year nursing students were included. The participants were approached by using non-probability purposive sampling method. Validated questionnaire was used to collect the data. Data was entered and analyzed using SPSS version 22.0.

Results: Out of total 40 subjects, 19 (47.5%) were males. A large number 23 (57.5%) of respondents' ages lied between 23 and 27 years. Nearly half 21 (52.5%) of the participants' educational qualifications were Matriculation. Majority 34 (85%) of participants did not have any formal education of disaster management. On the other hand, few 6 (15%) study participants had training of disaster management. Significance difference was found between pre and post knowledge score of disaster, burns, flood and earthquake practices among study participant and it is also statistically significant.

Conclusion: It is concluded that there is an effect of educational intervention on pre and post knowledge of disaster, burns, flood and earthquake practices among study participants.

Key words: Effectiveness, Educational Training, Disaster Preparedness, Nursing Students

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INTRODUCTION

Disaster is a situation which has severe consequences including disturbance of daily activities, destruction of infrastructure, electricity, roads and loss of lives[1-3]. There are two types of disasters, one is natural and the second is man-made disaster. Natural disasters include floods, droughts, volcanic eruptions, land sliding. While, man-made disasters include accidents, fires, poisoning, bomb blasts, and industrial accidents etc[4]. Time and place of disaster is unpredictable but the healthcare providers (HCPs) can reduce the number of casualties in a disaster by providing efficient care[5].

Globally, disaster rate is increasing which ultimately turns focus towards the preparedness of nurses to handle critical situation[6]. So, plans may be developed at nationwide, provincial and local levels for effective disaster response[7,8].

World Health Organization (WHO) reviewed the present situation and sought to improve nursing curriculum by adding management and prevention of disaster[9]. Hence, this education is given by several health agencies to increase competency in disaster relief[10]. Nursing students should be provided sound theoretical and practical knowledge for disaster readiness[11]. In the past, Florence Nightingale also identified this lacking and recommended for education in order to prevent complications[12]. Nurses must be able to observe nursing needs, develop plans, and collaboratively implement this plan within situational context[13]. The competencies of HCPs are measured on the basis of knowledge of skills, implementation of skills, learning attitude of

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individuals and success of institution, hence it explains the behavior of HCPs[14].

According to Global Climate Risk Index of Pakistan in 2014, it was reported that Pakistan is on number three across the globe, facing unwanted events of flood and earthquake[15], which caused destruction with massive casualties and HCPs struggled to provide relief to the people. As a result, lots of gaps were pointed out which need proper planning and preparedness[16]. These kinds of disasters can be handled with collaboration of different organization and illustrate the need of sound knowledge[17], to build self-confidence for sound decision making in emergency situations[18,19].

The Pakistan Nursing Council can play a vital role by adding disaster related topics in the curriculum and by encouraging research work at national disaster forum for its effective management[20]. Hence, this study was conducted to evaluate the effectiveness of educational training related to disaster preparedness among nursing students at Karachi.

METHODOLOGY

This Quasi-experimental study was performed in Indus College of Nursing and Midwifery in Karachi for a period of six months from June to November 2019. Subjects were approached by non-probability purposive sampling method.

The sample size was calculated on STATA 14 software with power 80%, significance level 5% of previous study pre and post mean and standard deviation (6.26±1.61 & 12.42±2.45) respectively, the required sample size was 4 but as the samples were easily accessible so total sample size was 40[3].

The willing students of final year nursing were included. Written informed consent was obtained from all the participants prior to induction in the study. Ethical approval was taken from Interactive Research & Development (IRD) with IRD_IRB_2019_07_003. Semi-structured questionnaire was used for data collection. Pilot study was conducted among 10% of sample size and changes were made as per findings. The questionnaire consisted of 39 questions that covered demographics, flood, earthquake, and burn practice.

A five-day intervention was provided. On the first day, introduction of research objectives, purpose was explained to all research participants, written consents were taken, and pre-test was obtained. On the second and third day of intervention, audio-visual aids were used in lecture for 60 minutes, in which knowledge and practice related to flood, earthquake, burns and first aid management was given and handouts were also distributed. On the fourth day, demonstration and hands-on-practice was performed. On the fifth day, post-test was taken. The questionnaire comprised 6 demographic questions, 8 disaster knowledge, 10 burns practices, 11 flood practices and 10 earthquake practice questions.

The data was entered and analyzed on SPSS version 22.0. Categorical data was managed by frequency and percentage. Whereas mean and standard deviation were computed for continuous data. Knowledge of disaster was assessed by independent t-test. While, the difference between pre- and post-knowledge was determined through paired t-test.

RESULTS

Table 1 exhibits the demographic characteristics of study participants. Out of total 40 subjects, 19 (47.50%) were males. Large number 23 (57.50%) of respondents' ages were between 23 and 27 years.

Nearly half 21 (52.50%) of the participants' qualifications were matriculation. Majority 34 (85%) of participants did not have any formal education of disaster management. On the other hand, a few 6 (15%) study participants had training of disaster management.

Table 1: Demographic Information of the Participants

Demographic Factors	N	%
Gender		
Male	19	47.50
Female	21	52.50
Age (in Years)		
18-22	15	37.50
23-27	23	57.50
Above	2	5.00
Qualification		
Matriculation	21	52.50
FSc	16	40.00
Others	3	7.50
Formal Education in Disaster Management		
Yes	6	15.00
No	34	85.00

Table 2 shows the pre and post-test mean knowledge of disaster, burns, floods and earthquake practices. The pre mean disaster knowledge score was 4.57±1.12 while after training post mean disaster knowledge score was 7.30±0.68 which is statistically significant (p-value<0.001). Pre-knowledge about burn practices score was 6.60±1.614 and after training post mean

knowledge of burn practice score was 8.70±0.88 which is also statistically significant (p-value<0.001). The flood related practices knowledge was assessed and its pre training score was 8.67±1.89 whereas post training mean score was 9.32±1.28. This variable was also found significant (p-value=0.021). The pre and post knowledge of earthquake practice was also measured, the pre training earthquake score was 5.85±1.35 and post training earthquake practice score was 9.20±0.93 which is also statistically significant (p-value<0.001).

Table 2: Difference in Pre and Post-Knowledge of Disaster, Burns, Floods and Earthquake Practices

Knowledge		Mean	t-test	p-value
Disaster	Pre test	4.5750		
	Post test	7.3000	-14.929	<0.001
Burn Practice	Pre test	6.6000		
	Post test	8.7000	-8.396	<0.001
Flood Practice	Pre test	8.6750		
	Post test	9.3250	-2.414	0.021
Earth Practice	Pre test	5.8500		
	Post test	9.2000	-12.124	<0.001

Table 3 showed gender-wise knowledge difference. It is highlighted that males had more pre-knowledge (mean knowledge 5.05±1.17) as compared to female participants (4.14±0.91) and it is also statistically significant (p-value=0.009). The post training knowledge in males and females is approximately equal, male participants (7.31±0.67) female participants (7.28±0.71) and also not statistically significant (p-value=0.892) which concludes that there is no mean difference of knowledge among the genders.

Table 3: Gender-Wise Knowledge Difference

Knowledge	Gender	Mean	SD	t-test	p-value
Pre-test	Male	5.052	1.177		
	Female	4.142	0.910	2.749	0.009
Post-test	Male	7.315	0.671		
	Female	7.285	0.717	0.137	0.892

DISCUSSION

In this study, 52.5% of the nursing students were females and maximum age was 23-27 years (57.5%). Similarity was observed with the study of Korean diploma nursing students in which (98.8%) were females with 21-25 years range of age[21]. Present study found 85% of participants had not attended any training previously. Likewise Egypt study also disclosed 88.8% not having received any formal training[22,23]. Before intervention, the male students' knowledge mean score 5.052±1.177 SD was slightly higher than female students 4.142±0.910 respectively with significant p-value 0.009, while female post-test knowledge mean score became approximately equal to male students (M) 7.315±0.671 & (F) 7.285±0.717 individually but statistically no significant p-value 0.892. On the other hand, a study identified females' pre-knowledge mean score (6.41±1.51) to be higher than male students (5.91±1.81) with significant p-value >0.05 and post score (12.34±1.53 & 12.61±1.20) but p-value >0.05 was significant in contrast with current study[24] hence it could be possible that male students have much exposure of dealing with emergencies. Another study reported noticeable difference in pre-test scores (13.83±2.26) and post-test knowledge scores (20.16±2.66)[25]. Furthermore, study conducted in Japan also revealed significant difference between pre and post-test score 10.38 & 14.68 respectively with 4.29 difference[26]. Additionally, another Korean study exposed similar results as mean score of pre and post-test 10.60±3.82 & 19.50±2.13 individually with significant p-value <0.001[27].

Research study presented obvious improvements in mean pre-evaluation and post-evaluation and difference provided in percentage of each item[28]. The present study found knowledge of nursing students regarding earthquake practice mean score as pre 5.850±1.350 SD and post 9.200±0.939 SD respectively. One study depicted comparable results mean 70.07±10.01 SD at moderate level[29]. Moreover, another study revealed much difference among pre and post means score 2.18±0.68 and 6.30±0.84 respectively with significant p-value 0.000, which is similar to present study[30].

RECOMMENDATIONS

1. Educational programmes add essential component for all undergraduates and graduates enhance further their understanding of disaster.

2. To manage the patients in hospital/community setting, the nursing curriculum should be revised and updated with disaster management.

3. More research work is needed to assess the effectiveness of teaching methodology among nurses and nursing students especially in disaster circumstances.

Limitations of the Study

The study was accomplished in single setting as well as in a private institution with small sample size, hence the findings of the study cannot be generalized.

CONCLUSION

Inadequate knowledge of nursing students on disaster prior to interventional session and improvement in knowledge and practice after educational activity reflects significant need for educational programmes to pay more attention to disaster related practices in curricula.

Authors' contribution: AG conceived the idea, B performed statistical analysis and proofreading, R searched the literature, SZA collected data and performed critical review. ARK collected data. AG, R, and ARK contributed to writing the manuscript.

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ORIGINAL ARTICLE

Knowledge, Attitude and Practices of Pharmaceutical Waste Disposal in Community Pharmacies of Karachi

Amna Khan[1], Nazish Jaffar*[1], Sadaf Razzak[1], Faiza Zeeshan[1], Asma Shabbir[1] and Syed Muhammad Ishaque[2]

ABSTRACT

Objectives: The aim of the study was to assess the knowledge of community pharmacies about recommended methods of drug disposal and hazardous effects of improper disposal of pharmaceutical waste and to determine the attitude as well as practice of community pharmacies about the proper disposal of pharmaceutical waste.
Methods: A cross-sectional study was performed with 139 community pharmacies in Karachi, Pakistan by using non-probability convenient sampling technique. Predesigned structured questionnaire was distributed to different community pharmacies in Karachi and filled questionnaires were collected after one day. Data was analyzed using SPSS 22.0
Results: About 65 (46.8%) of the participants reported to have a system for regularly removing the expired or unused drugs by returning them to contractors/distributors. More than half 86 (61.9%) of the participants disposed of solid, liquid, semi-solid, controlled, and p-listed drugs in the rubbish bin. Approximately half of the participants believed that standard method for disposal of solid, liquid, semi-solid, controlled, and p-listed drugs is by flushing them down the toilet/sink.
Conclusion: Our study showed that knowledge and practices of community pharmacies regarding disposal of unused and expired medicines were not satisfactory. However, most of the participants were well aware of the harmful effects caused by improper disposal of pharmaceutical waste on environment and showed positive attitude towards learning safe methods of management and disposal of unused and expired drugs.

Key words: Community pharmacy, pharmacists, disposal, environment, Karachi

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INTRODUCTION

Environmental pollution is one of the biggest threats which the world is facing today. One of the major contributing factors is improper disposal of unused and expired medications which is causing water as well as land pollution. It is adversely affecting the environment, health of human beings and animals[1,2]. Different types of drug preparations include solid (tablets), liquid (syrups) and semi-solid (ointments) formulations[3,4]. Drugs such as opioids, stimulants, hallucinogens, anabolic steroids capable of causing addiction and substance use disorder are classified as control drugs[5,6]. P-listed drugs such as warfarin, nicotine patches, and physostigmine are classified as acutely toxic drugs that can cause death and irreversible illness even at low doses[7].

In many countries, the recommended method to dispose of unused and expired medicines is to return them to pharmacies. However, it will be beneficial only if pharmacists also dispose them in a proper manner[8,9]. Improper disposal of medicines produces a lot of harmful effects in man and animals which may include accumulation of pharmaceutical residues in body after drinking contaminated water, poisoning in children and adults, antibiotic resistance, production of deadly mutant bacterial strains, substance use disorders, infertility in fishes, which will ultimately lead to disturbance in food chain etc[10,11].

The common methods of disposal of expired medicine are reported to be throwing them either into toilet, sink, or garbage[12,13]. Different studies reported well-established policies and programmes in developed countries for proper disposal and management of

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pharmaceutical waste, whereas, developing countries lack such programmes[8,9]. A study in Ethiopia reported flushing medicines down the toilet/sink as the common disposal practice[14].

According to a study in India, most of the leftover drugs belonging to different dosage forms were returned to the distributor, whereas one third of the pharmacists did not know the correct method to dispose of drugs[15]. Moreover, only a few studies in Pakistan and Afghanistan reported the practices of general public regarding pharmaceutical waste management[16,17].

According to the World Health Organization (WHO), a pharmacist is a caregiver, the decision maker, communicator, manager, lifelong learner, teacher, and leader[18]. To the best of our knowledge, no study has been conducted until now in Karachi considering the practices of community pharmacies regarding drug disposal. Therefore, the present study was aimed to assess the knowledge of community pharmacies about hazardous effects of improper disposal of pharmaceutical waste and to determine the attitude as well as practices of community pharmacies about the proper disposal of pharmaceutical waste.

METHODOLOGY

A descriptive cross-sectional study was conducted during June, 2020 with a total of 139 community pharmacies in Karachi by using non-probability convenient sampling technique. Ethical approval was obtained from Institutional Review Board of Jinnah Sindh Medical University (JSMU/IRB/2020/-301) and written informed consent was acquired before data collection. All consenting pharmacists/pharmacy technicians from Karachi practicing for the minimum duration of at least three years and aged more than 25 years were enrolled in our study whereas, all non-consenting pharmacists/pharmacy technicians and participants with ages less than 25 years were excluded. Data was collected by distributing a predesigned structured questionnaire to different community pharmacies in Karachi and filled questionnaires were collected after one day. The questionnaire was prepared after extensive literature review from Google Scholar and comprised four parts. The first part included socio-demographic data (age, gender, education, number of years for which he/she is practicing as pharmacist). Second part comprised their knowledge and attitude about the recommended methods of drug disposal. Third section inquired about hazards of improper methods of drug disposal. The fourth part included current practices of community pharmacies regarding disposal of unused and expired medication. Sample size was obtained using Open EPI software (www.openepi.com/Menu/OE_Menu.htm) Using confidence interval of 95% (z score=1.96) 90%[15] as prevalence from previous study and 5% allowable error of known prevalence, sample size obtained was n=139.

Data was analyzed using SPSS version 22.0. Descriptive statistics were used to determine mean and standard deviation for numerical variables. Categorical variables were expressed in frequency and percentages.

RESULTS

A total of 139 pharmacists/pharmacy technicians practicing in Karachi were included in our study. The majority 138 (99.3%) of the participants were males aged between 25-35 year. Mean age with standard deviation was 36.0±8.7. Most 93 (66.9%) of the respondents were practicing for less than 10 years. More than half 82 (58.9%) of the participants were neither graduates nor diploma holders. Less than half 68 (48.9%) of the participants were taught different disposal techniques during graduation/diploma whereas, approximately one-third of the participants felt the need to learn more about safe and environment friendly techniques for pharmaceutical waste disposal**.** (Table 1-2)

The frequency of enquiry from customers about advice on drug disposal was received in the frequency of never 50 (36.0%), sometimes 72 (51.8%), and always 17 (12.2%). (Table 1) More than half 98 (70.5%) of the participants took help of pharmaceutical sales representatives to get updated information for disposal of unused or expired medications. (Figure 1)

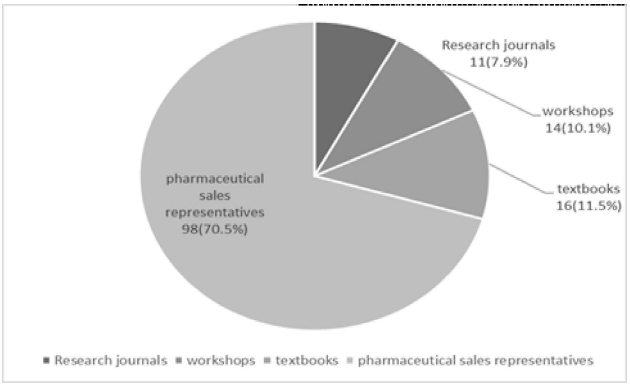

Figure 1: Different Sources of Updated Information for Participants Regarding Disposal of Pharmaceutical Waste N (%)

However, knowledge about recommended method of disposal of different dosage forms was inadequate as approximately half of the participants believed that standard method for disposal of solid, liquid, semi-solid, controlled and p-listed drugs is by flushing them down the toilet/sink. More than half of the participants

are aware of drug take back system whereas approximately one-third reported that they do not take back unused or expired medicines from the customers. (Table 1)

Knowledge of participants regarding environmental impact of improper pharmaceutical waste disposal was satisfactory. Many 115 (82.7%) participants admitted that improper drug disposal is linked to environmental pollution and contamination of drinking water. Furthermore, 101 (72.7%) reported that it also causes antibiotic resistance. Some of them 79 (56.8%) reported incineration as unfavorable method for pharmaceutical waste disposal. **(**Figure 2)

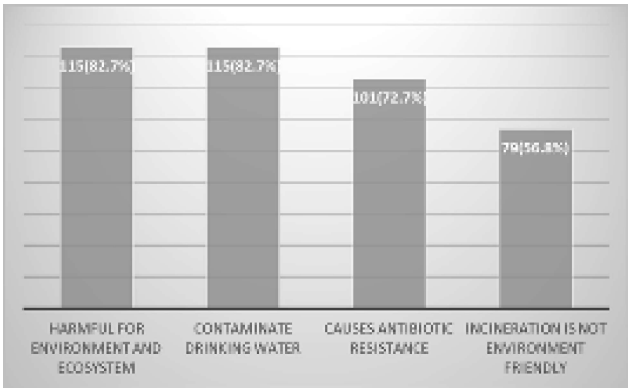

Figure 2: Knowledge of Participants Regarding Environmental Impact of Improper Pharmaceutical Waste Disposal N (%)

More than one-third of the participants asked for a proper system to regularly remove the expired or unused drugs by returning them to contractors/ distributors. However, approximately half of the participants practiced disposing of solid, liquid, semi-solid, controlled and p-listed drugs in the rubbish bin. (Table 2, Figure 3) More than half of the participants reported educating general public as the best way to minimize pharmaceutical waste. (Table 1)

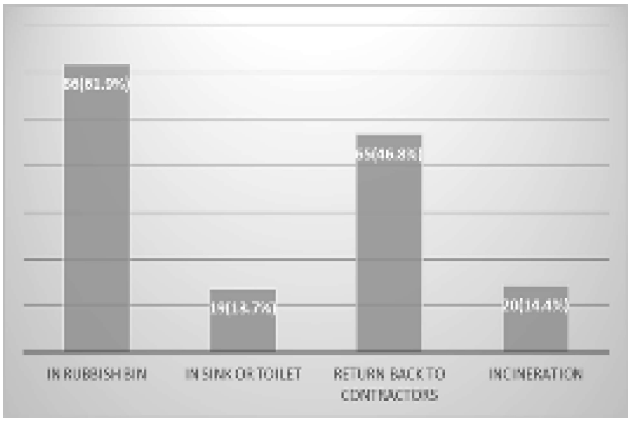

Figure 3: Different Methods of Disposal for Unused and Expired Medicines Practiced by Community Pharmacies N (%)

Table 1: Sociodemographic Data and Knowledge of Pharmacists/ Pharmacy Technicians About Recommended Methods of Drug Disposal:

Variables:	N(%)	Mean± SD
Age group:		
· 25-35	75(54.0)	(36.0±8.7)
· 36-45	44(31.7)	
· 46-55	17(12.2)	
· 56-65	3(2.2)	
Gender:		
· Male	138(99.3)	M: F ratio (138:1)
· Female	1(0.7)	
Number of practicing years:		
· Less than 10 years	93(66.9)	(10.4±7.3)
· 11-20 years	37(26.6)	
· 21-30 years	5(3.6)	
· 31-40 years	3(2.2)	
· Over 40 years	1(0.7)	
Qualification:		
· Graduation in pharmacy	20(14.4)	
· Diploma in pharmacy technician	37(26.6)	
· Others	82(58.9)	
Medicine disposal techniques were taught during graduation/diploma:	68(48.9)	
Did customer ever enquire about advice on drug disposal:		
· Never	50(36.0)	
· Sometimes	72(51.8)	
· Always	17(12.2)	
Customer returned unused or expired medication:	70 (50.4)	
We do not take back unused or expired medications:	69(49.6)	
I know about drug take back system:	87(62.6)	
Recommended method to dispose of solid dosage forms:		
· By disposal in landfills	32(23.0)	
· By incineration	12(8.6)	
· By flushing the medicine down the toilet/sink	70(50.4)	
· Take back system	1(0.7)	
· Don't know	24(17.3)	
Recommended method to dispose of liquid dosage forms:		
· By disposal in landfills	32(23.0)	
· By incineration	7(5.0)	
· By flushing the medicine down the toilet/sink	67(48.2)	
· Take back system	1(0.7)	
· Don't know	32(23.0)	
Recommended method to dispose of semi-solid preparations:		
· By disposal in landfills	29(20.9)	
· By incineration	9(6.5)	
· By flushing the medicine down the toilet/sink	63(45.3)	
· Take back system	1(0.7)	
· Don't know	37(26.6)	
Recommended method to dispose of controlled drug preparations:		
· By disposal in landfills	31(22.3)	
· By incineration	11(7.9)	
· By flushing the medicine down the toilet/sink	63(45.3)	
· Take back system	1(0.7)	
· Don't know	33(23.7)	
Recommended method to dispose of p-listed drugs:		
· By disposal in landfills	33(23.7)	
· By incineration	11(7.9)	
· By flushing the medicine down the toilet/sink	56(40.3)	
· Take back system	1(0.7)	
· Don't know	38(27.3)	
Best way to minimize pharmaceutical waste:		
· By drug take back system	17(12.2)	
· By donation	19(13.7)	
· By educating general public	74(53.2)	
· First sell drugs nearing expiry dates	29(20.9)	

Table 2: Practices and Attitude of Pharmacists/Pharmacy Technicians Regarding Pharmaceutical Waste Disposal:

Variables:	N(%)
1-How would you dispose of solid dosage forms?	
· In the rubbish bin	79(56.8)
· In the sink/toilet	18(12.9
· By incineration	12(8.6)
· Send back to pharmaceutical distributors or contractors	30(21.6)
2-How would you dispose of liquid dosage forms?	
· In the rubbish bin	65(46.8)
· In the sink/toilet	6(4.3)
· By incineration	13(9.4)
· Send back to pharmaceutical distributors or contractors	55(39.6)
3-How would you dispose of semi-solid preparations?	
· In the rubbish bin	62(44.6)
· In the sink/toilet	8(5.8)
· By incineration	8(5.8)
· Send back to pharmaceutical distributors or contractors	61(43.9)
4-How would you dispose of controlled drug preparations?	
· In the rubbish bin	61(43.9)
· In the sink/toilet	5(3.6)
· By incineration	10(7.2)
· Send back to pharmaceutical distributors or contractors	63(45.3)
5-How would you dispose of P-listed drugs?	
· In the rubbish bin	66(47.5)
· In the sink/toilet	4(2.9)
· By incineration	10(7.2)
· Send back to pharmaceutical distributors or contractors	59(42.4)
6-I would like to learn more about safe and environment friendly techniques for pharmaceutical waste disposal.	50(36.0)

DISCUSSION

Unused and expired medicines should be disposed of properly to avoid accumulation of toxic and harmful residues in the environment. As they are potentially harmful substances, improper disposal could cause toxicity in human beings and animals[19,20]. In Pakistan, there is limited literature on methods used by community pharmacies for management of pharmaceutical waste. The present study was designed to assess the knowledge of community pharmacies about hazardous effects of improper disposal of pharmaceutical waste and to determine the attitude as well as practice of community pharmacies about the proper disposal of pharmaceutical waste.

Results of our study showed that most of the community pharmacies do not have proper system for disposal of unused and expired medications and there is lack of knowledge about proper pharmaceutical waste disposal among pharmacists/pharmacy technicians. The best way to dispose of most types of old, unused, or expired medicines both prescribed and over the counter is to drop off the medicine at a drug take back site, location, or programme immediately. If it is on the FDA flush list, it should be immediately flushed. If it is not on flush list then it ought to be removed from original container and be mixed with any undesirable substance such as coffee grounds or kitty litter and placed in a sealable bag, empty can or other container to prevent any leaking of the drug. Controlled drugs such as opioids should be flushed to reduce unintentional or illegal use. Studies reported that environmental effects caused by flushing of FDA approved medicines are insignificant[21].

In the present study, majority of the pharmacists/ pharmacy technicians believed that flushing all medicines down the toilet/sink (whether solid, liquid, semi-solid or controlled dosage forms and p-listed drugs) is the recommended method of disposal. This finding confirmed an inadequate knowledge of participants regarding recommended methods of drug disposal. However, if drug take back programmes are not available, some medications listed by FDA can be disposed of in toilet/sink[22].

Most of the respondents were not taught about the recommended disposal techniques during graduation or diploma courses. Pharmaceutical sales representatives were considered as the guiding source for disposal of unused and expired medications followed by textbooks, workshops, and research journals. This is in contrast to a study conducted in India which showed workshops as the most common source of updated information regarding disposal of pharmaceutical waste[15].

Pharmaceutical residues enter the environment through primary route which is unintentional and unavoidable by means of excretion and bathing and secondary route i.e. disposal of pharmaceutical waste into trash and sewerage. Humans as well as aquatic animals are exposed to these trace residues as even modern water treatment plants do not remove them completely[8,14]. Non-steroidal anti-inflammatory drugs, hormones and antibiotics are the drugs that adversely affect the ecosystem[16]. Studies have reported that flushing of antibiotics down the toilet/sink causes mutations in different bacterial strains ultimately leading to antibiotic resistance. Improper disposal of oral contraceptive pills containing estrogen cause infertility in fish leading to disturbances in food chain. Controlled drugs such as opioids, stimulants, hallucinogens, anabolic steroids disposed of in rubbish bin may lead to substance use disorders[8,10,11,20]. Most of the participants in our study also agreed that improper pharmaceutical waste management is harmful for our environment and ecosystem and they would like to learn more about safe and environment friendly techniques for disposal of unwanted medicines. Only some of the participants

agreed that incineration is not an eco-friendly way to dispose of unused and expired medications. Studies showed that incineration is the most environmentally friendly method of pharmaceutical waste disposal however, it is prohibited in case of inhalers[14,21].

Very few pharmacists in our study expressed drug take back programme as the best way to minimize harmful effects of pharmaceutical waste on environment and ecosystem. However, majority of respondents were of the opinion that educating general public is the best possible solution to avoid pharmaceutical waste. Our findings are similar to study conducted in Ethiopia where only 1% of the respondents believed that returning unused and expired drugs to the pharmacies would be the best way to minimize pharmaceutical waste[8]. The purpose of drug take back programme is to help recipients in properly disposing of their prescribed and over the counter medications in order to avoid addition of harmful pharmaceutical residues in the environment. People should not buy medicines in excess and unused medications should be returned to pharmacies immediately or should be donated to hospital emergency departments.

Most of the pharmacists in our study reported disposal of different types of drugs in rubbish bin which is similar to practices of general public in Pakistan[16] and India[23,24] whereas, controlled drug preparations were sent back to pharmaceutical distributors or contractors. It might be because only some of the respondents had ever received proper training on pharmaceutical waste management. In Nepal, most of the unused and expired medicines were disposed of via municipal garbage truck whereas, some of the participants also discarded them into garbage dumps and informal waste collectors[25].

Our study also observed that most of the community pharmacies did not take back unused and expired drugs from the customers probably because they were unaware about drug take back system. However, community pharmacies following drug take back system reported that they return unused or expired medications to the distributors or contractors six months before expiry therefore, medicines brought by customers with expiry date of less than six months were not accepted.

Our study findings emphasized the need for development of programmes and policies to ensure collection of unused and expired medicines from general public and community pharmacies and then subsequently disposing them in an environmentally friendly manner.

LIMITATIONS:

Some of the participants might not have reported actual practices due to the fear of being exposed for unprofessional conduct. Findings of our research cannot be generalized as it is a single center study.

CONCLUSION

We conclude that knowledge and practices of community pharmacies regarding disposal of unused and expired medicines is not satisfactory. However, most of the participants are well aware of the harmful effects caused by improper disposal of pharmaceutical waste on environment and showed positive attitude towards learning safe methods in order to properly dispose of unused and expired medications. Proper system is required for collection of old, unused, expired medicines with subsequent disposal according to guidelines.

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ORIGINAL ARTICLE

Knowledge, Attitude and Practices of Undergraduate Medical and Nursing Students Regarding Basic Life Support Training

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ABSTRACT

Objective: To assess and compare the Knowledge, Attitude, and Practices of Medical and Nursing Students regarding Basic Life Support Training

Methodology: This cross-sectional study was done at the Shalamar Institute of Health Sciences, Lahore in a period of three months. Students from all years of MBBS and BS Nursing were taken as subjects. Participants satisfying inclusion criteria were requested to fill out a self-structured pre-tested questionnaire after informed consent. Data was analyzed using SPSS v.21.

Results: There were 330 participants from MBBS and BS Nursing (50 from each class of MBBS and 20 from each class of BS Nursing). The age of participants ranged from 18 to 30 (Figure 1). Only 250 subjects had heard about BLS. Out of 330 participants, only 204 had good knowledge about BLS (Table 2) and 126 had poor knowledge of BLS where score of higher than 3 out of 7 was considered as good. Knowledge of Medical and Nursing students was compared and the p-value came out to be 0.088, which was considered insignificant. Only 34.5% of the participants had learned BLS by training and only 13.3% of the participants (Table 3) had practically done BLS. Most of the people gave no reason for not attending a training session for BLS.

Conclusion: It was concluded that medical and nursing students had satisfactory knowledge of BLS and more attention was needed towards this important life-saving skill.

Key words: Cardiopulmonary Resuscitation, Medical, Undergraduate, Knowledge, Attitude, Practices, Nursing

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INTRODUCTION

Basic Life Support is a set of life-saving techniques like cardiopulmonary resuscitation (CPR) and basic airway management[1]. The matter at hand is the Knowledge, Attitude and Practices of medical and nursing students related to this crucial aspect of the field that is Basic Life Support (BLS). Cardiac Emergencies are one of the most common emergencies seen in the ERs of any hospital. BLS can increase the chances of survival of patients in such emergencies[2].

Medical students in Pakistan lack cognitive awareness though they consider it to be an important part of curriculum[3]. As medical students and nurses lack knowledge of BLS, attention needs to be paid to teaching it and providing opportunities to students for hands-on practice of BLS where possible[4].

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BLS has not yet played a significant role in the care of a critical patient as it should have mainly because of a lack of resources and awareness among students. Other than that, those students who have been trained, do not adequately exercise their skills during emergencies and teach their expertise to the younger generation of doctors and nurses[5]. The discussion about high-risk patients and the benefit they can get through proper Basic Life Support measures and training is an eye-opener and also gives the idea about how it is essential for non-risk patients as well. A study conducted on the eligible patients showed the BLS resulted in a 27% decrease in cardiac arrests, a 33% decrease in deaths due to cardiac arrests, and a 17% decrease in overall in-hospital deaths[6]. One can only imagine how these percentages can be increased by implementing the knowledge on Basic Life Support and conducting continuous feedback to make it better at every step. Proper administration of first aid and resuscitation is essential following extreme conditions of shock and cardiac arrest. But unfortunately, our medical and nursing students are not familiar enough with the

training of Basic Life Support despite the fact that they are studying to become medical professionals. The focus needs to be on the skills and techniques which make BLS effective. There needs to be a balance between confidence and competence of the health workers in order to address this inadequacy[7].

The objective of this study was to comprehend and evaluate the attitude of doctors and nurses towards Basic Life Support. Only that way an ideal and improved plan can be designed and initiated. The setbacks to accurate CPR administration and emergency ABC protocols must be tackled after the current situation and educational status about BLS should be recognized. Even though, the topic is less common to be discussed but as far as its importance is concerned, it should be given more attention. In addition to the medical personnel, the friends, and family members of high-risk patients should be trained too. BLS training must be given to every person medical or non-medical provided they are willing and competent[8].

METHODOLOGY

It was a cross-sectional study conducted in Shalamar Institute of Health Sciences, Lahore. MBBS and BS Nursing students were the target population. The duration of the study was three months. Sociodemographic variables and Knowledge, Attitude and Practices of BLS were the variables. Students of MBBS Year 1-5 and BS Nursing Year 1-4 willing to be a participant of this study were included. Students who were repeating the session were excluded. Students from courses other than MBBS and BS Nursing were excluded. Students not consenting to the study were excluded.

Our target population was MBBS and BS Nursing students of the undergraduate level of Shalamar Institute of Health Sciences. The minimum calculated sample size using Open Epi Version 3 was 274 with Confidence Level of 95% and Margin of Error 5%. We took 330 students, out of whom 250 students were from MBBS (50 each from 1st, 2nd, 3rd, 4th and final year) and 80 were from BS Nursing (20 from each year) with a non-response margin of 17%.

The data was collected by circulating self-structured pre-tested printed questionnaire (Annex-1) developed from American Heart Association Basic Life Support Guidelines 2016, among the students in person and also through Google Forms.

Participants were approached and after taking informed consent, they were given the questionnaire(Annex-1). Participants then returned the questionnaire to the researchers. The data was also collected online through Google Forms. Before filling an online form, informed consent from the participants was taken. Statistical analysis was performed using SPSS version 21. The knowledge, attitude and practices of participants were assessed through a 14-item questionnaire. Every correct answer was awarded 1 point making a maximum score 7 (for knowledge related questions). Chi-square and Fisher's Exact Test was applied in which a p-value of less than 0.05 is considered significant.

Ethical approval was obtained from the college IRB committee (letter reference number SMDC/IRB/08-07/181).

RESULTS

The data was collected from 330 participants including students from both MBBS and BS Nursing. The age range of participants was from 18 years to 30 years (Figure I). The participants were from 1st Year of MBBS till the Final Year of MBBS and from 1st Year till 4th Year of BS Nursing. Females enrolled were 53.03%. p value of less than 0.05 was considered significant.

The Knowledge was tested using seven questions and the frequencies and percentages of each option are mentioned with the p value for each question in Table 1. The Knowledge score was calculated for each of the participants out of seven and a score of lesser than or equal to three was considered as having poor knowledge while a score of greater than three was considered as good knowledge. The results are cross-tabulated in Table-2. Chi-Square and Fisher's Exact Test was applied to find out the association of MBBS and BS Nursing Class to Knowledge about BLS and to compare the knowledge and p-value were calculated which came out to be 0.088 which was considered as statistically insignificant.

Furthermore, the attitude and practices of participants was also tested using various questions and the frequency of each answer is given in Table 3. Out of the 330 students, 250 had heard of BLS, while 80 had never heard of BLS. More MBBS students had heard about BLS than BS Nursing (Table 3). As many as 114 (34.5%) participants had learned about BLS by attending a training session while 216 (65.5%) had not attended a training session.

No particular reason was given by 100 (30.3%) participants for not attending a training session while others stated lack of opportunities, laziness and being busy with their studies as their reasons (Table 4).

Table 1: Answers of Questions about Knowledge of BLS and the Frequencies and Percentages of Each Option

Question	MBBS (N=250)		BS Nursing (N=80)		P value
Who should be trained in BLS?	**Frequency**	**Percentage**	**Frequency**	**Percentage**	
Citizens	12	4.8	3	3.8	
Doctors	12	4.8	4	5.0	
Hospital Staff	4	1.6	4	5.0	0.349
Nurses	1	0.4	1	1.3	
All of the above	221	88.4	68	85.0	
Where BLS measures can be performed?					
ICU only	4	1.6	1	1.3	
Hospital only	23	9.2	10	12.5	
Anywhere	216	86.4	67	83.8	0.792
Cardiac Ward Only	4	1.6	2	2.5	
Others	3	1.2	0	0	
Where hands of BLS performer are to be placed for chest compression in adults?					
Below Clavicle	22	8.8	12	15	
Upper Half of Sternum	90	36.0	25	31.3	
Lower Half of Sternum	90	36.0	21	26.3	0.972
Xiphisternum	39	15.6	20	25	
Others	9	3.6	2	2.5	
What is the number of chest compressions in adults?					
12	27	10.8	2	2.5	
20	66	26.4	18	22.5	0.003
25	67	26.8	17	21.3	
100-120	90	36.0	43	53.8	
How deep chest compressions should be in adults?					
2 inches	77	30.8	39	48.8	
1.5 inches	97	38.8	17	21.3	0.263
5 inches	29	11.6	7	8.8	
2.5 inches	47	18.8	17	21.3	
What is chest compression to breath ratio in adults?					
30:2	120	48	50	62.5	
30:4	52	20.8	4	5.0	0.871
15:3	65	26.0	13	16.3	
5:2	13	5.2	13	16.3	
Are emergency medicines required for BLS?					
Yes	106	42.4	33	41.3	0.857
No	144	57.6	47	58.8	

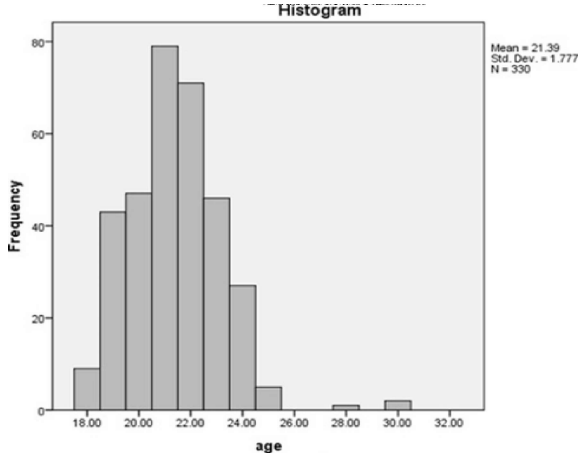

Figure 1: Age of the Participants

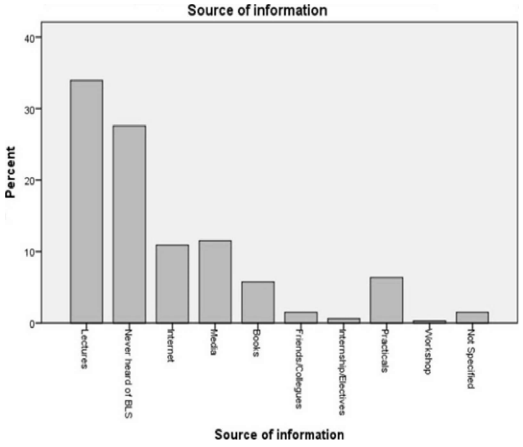

Figure 2 : Source of Information about BLS

Table 2: Cross Tabulation for MBBS and BS Nursing Students Divided as Poor Knowledge and Good Knowledge

	Poor Knowledge (Lesser than or Equal to 3)	Good Knowledge (Greater than 3)
MBBS	102	148
BS Nursing	24	56
Count	126	204

Table 4: Reasons for No Training of BLS

	Frequency	Percentage
Learned BLS in training session	126	38.2
No reason is given	100	30.3
No opportunity was given	95	28.8
Laziness	1	0.3
Busy	1	0.3
No knowledge about BLS	7	2.1
Total	330	100.0

Table 3: Questions Regarding Attitudes and Practices of BLS and Frequencies and Percentages of Each Answer

Question	MBBS (N=250)		BS Nursing (N=80)		P value
Have you ever heard about Basic life support (BLS)?	Frequency	Percentage	Frequency	Percentage	
Yes	179	71.6	71	88.8	
No	71	28.4	9	11.3	0.002
Have you learned BLS in training session?					
Yes	67	26.8	47	58.8	
No	183	73.2	33	41.3	<0.001
Have you ever practically ever done BLS in adults?					
Yes	31	12.4	13	16.3	
No	219	87.6	67	83.8	0.379
Will you perform mouth to mouth breathing to an unknown person?					
Yes	152	60.8	33	41.3	
No	98	39.2	42	52.5	0.036
In your opinion, should BLS be included early in syllabi of MBBS and BS Nursing along with periodic assessments?					
Yes	222	88.4	75	93.8	
No	28	11.2	5	6.3	0.200

DISCUSSION

Our institute Shalamar Medical and Dental College, is admitting 150 medical and 50 nursing students annually. Our college has progressively been taking steps to produce good doctors through exercises like problem-based learning, encouraging the culture of research in early years and holding trainings like BLS which are essential to produce good doctors. Our study was done among undergraduate Medical and Nursing students to assess and compare their Knowledge, Attitude and Practices about BLS. This is a unique study that compared the Knowledge, Attitude and Practices of Medical and Nursing Students which has been done previously in very few studies and emphasizes about importance of BLS training in Undergraduate Medical and Nursing curriculum. We found out that BLS was not being given its due importance. It was alarming to see that medical and nursing students who would become healthcare professionals coming directly in contact with patients, had the least knowledge and only 34.4% of participants had attended a training session for such an important skill. This indicated the need for making this training compulsory for every student studying to become a healthcare professional.

Our study showed that 81% of participants had heard about BLS while a study done in Saudi Arabia showed 93.8% of the participants had heard about BLS[9]. The reason for this can be that the study was done on healthcare interns and we did our study on undergraduate students and there is a gap of knowledge among pre-clinical and clinical students due to different class levels. It can also be due to the fact that BLS training is not a mandatory skill to be learnt by the undergraduates or interns in Pakistan.

A study done in Karachi showed 90.9% of the people knew that BLS can be performed anywhere while our study showed 85.8% of the people knew this[10]. This gap was perhaps due to a lack of training sessions and workshops and neglecting BLS in our institute and because this study was done on House Officers, Medical Officers and other senior faculty.

Our study revealed that 34.5% of the people had attended workshops while a study done in Allama Iqbal Medical College; Lahore showed only 24.3% had attended a workshop[11]. This might be due to practical classes for BLS held in our institute and due to a workshop given which was attended by only a few of the participants. Our study also showed that 38.18% of the people had poor knowledge about BLS while another study done in Karachi showed 22.8% which might be due to the same reason as cited above[9].

Our study showed 90% of the participants thought that BLS training should be included as a compulsory part of the curriculum while another study done in India showed that 98% of the participants thought that BLS should be incorporated as an integral part of the curriculum[12]. This is probably due to the reason that there is increased awareness about importance of BLS in the early years in Indian Medical and Nursing Institutes.

CONCLUSION

It was concluded that medical and nursing students had less than satisfactory knowledge of BLS and more attention was needed towards this important life-saving skill. Moreover, it was felt that training sessions should be arranged more often to teach BLS which should be made compulsory to attend for all medical and nursing students so that more students can benefit from it. This can save countless lives.

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Authors' contribution: ON conceptualized the study, developed questionnaire, worked on data entry and interpretation, wrote results and discussion. SA participated in data collection and entry, wrote introduction and reviewed the manuscript. RT collected and entered data, wrote the discussion. MA helped in literature search, data entry and revision. SN worked on data collection, analysis, revision and referencing.

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REVIEW ARTICLE

Arsenic: A Cause of Cardiometabolic Syndromes

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ABSTRACT

Objective: The objective of the study is to identify major pathological biomarkers and their possible mechanisms in arsenic-induced cardiometabolic syndrome.
Methodology: The present review summarizes the data and literature taken from previously done studies from Research Gate, Science Direct, PubMed, PubMed Central, Medline, and some other scientific databases emphasizing the role of arsenic in cardiometabolic syndrome. The results obtained through this database were assembled, composed, critically elucidated and presented in explanatory and tabular form.
Results: The major pathological target of arsenic is antioxidant defense system and increase in Reactive Oxygen Species reduces the Nitric oxide production, reduction in vascular permeability, increases adhesion and production of inflammatory mediators (IL-6, TNF-á,), TCs, TGs, LDL-C, lipid peroxidation, â-cells dysfunction. The persistent accumulation and generation of these pathological biomarkers in blood vessels can induce hyperlipidemias, dyslipidemias, atherosclerosis, hypertension and diabetes.
Conclusion: There is a need to propose natural antioxidant with minimum side effects in the treatment of CMTs.

Key words: Arsenic, endothelial dysfunction, NO production**,** hypertension, atherosclerosis, hyperlipidemia, dyslipidemia

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INTRODUCTION

Arsenic is the most abundant toxic metal on earth and is considered as a major health concern for 200 million people worldwide and major source of this contamination is through drinking water. The aim of present review is to figure out the various pathological and possible mechanisms in arsenic-mediated cardiometabolic syndromes. For this purpose, previously published 45 articles from Science of the Total Environment, African Journal of Microbiology Research, Environmental Science and Technology, International Journal of Environmental Research and Public Health, Current Environmental Health Reports, and American Journal of Epidemiology were assembled, composed, critically elucidated and presented in explanatory form. The data expedites the previously done pre-clinical, clinical, and in vitro studies mainly describing the pathological biomarkers and possible mechanisms involved in the development of arsenic-mediated cardiometabolic syndrome. Natural substances can be introduced to combat the pathological biomarkers involved in the development of CMTs.

Arsenic is considered as a major health concern for 200 million people worldwide[1].The International Agency for Research on Cancer ranked arsenic as Group 1 carcinogenic agent. Arsenic is also on top of the list of toxic substances according to The Agency for Toxic Substances and Disease Registry. The World Health Organization (WHO) described arsenic as the largest poisoning agent in human history. The exposure of arsenic to human population occurs through various ways e.g., industrial wastes, contaminated food, and, air[2]. However, the major source of toxicity is drinking water.

Arsenic exists in two forms (trivalent and pentavalent). The pentavalent form of arsenic in the body is converted to trivalent form via methylation in the liver in the presence of glutathione (GHs) and S-adenosylmethionine produces metabolites (monomethylarsonous (MMA) and Dimethylarsinous (DMA)[3]. This methylated trivalent form of arsenic is known to be responsible for toxicity. Reported 0.01 mg/L of arsenic is consumable range for humans[4]. Countries that are exposed to arsenic-intoxicated drinking water (above 0.01 mg/L) include Bangladesh[5], India[6], Pakistan[7],

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China[8], Taiwan[9], Mongolia, Mexico, Argentina, Chile[10], and some areas of the United States[11,12]. These countries are suffering from serious health related issues due to arsenic toxicity. Recently, arsenic concentration in ground water of various regions of Pakistan was found to be more abundant than expected[13,14]. Another report stated that, more than 50 million people in Pakistan are at risk of arsenic contaminated water[15]. The 95% of population of Pakistan is dependent on the ground water, which most probably exceeds the stated WHO safe limit of arsenic in drinking water[16].

In view of above introduction, there is a need to summarize the literature on this subject for further research and understanding. Therefore, the present review is organized to identify major pathological biomarkers and their possible mechanisms in arsenic-induced cardiometabolic syndrome.

METHODOLOGY

The present review summarizes the data and literature taken from previously done studies from Research Gate, Science Direct, PubMed, PubMed Central, Medline, and some other scientific databases emphasizing the role of arsenic in cardiometabolic syndrome. The results obtained through this database were assembled, composed, critically elucidated and presented in explanatory and tabular form.

The systematic survey of ground water in a study revealed that arsenic-contaminated water and drinking water resources in 2000, 2001, and 2003. Studies in the provinces of Punjab and Sindh revealed that 3% of community was exposed to arsenic above 50 ug/L in drinking water, while 20% of the community was exposed to arsenic above 10 ug/L[15]. The data of the province of Sindh showed even worse statistics of arsenic-exposed water. The 16% of community was exposed to above 50 ug/L and 36% of the community was exposed to 10 ug/L of Arsenic contaminated water[7].

Multiple epidemiological studies have disclosed the chronic arsenic exposure has been subjected to numerous cardiometabolic syndromes (CMTs) including, atherosclerosis[17,18], hyperlipidemias, dyslipidemia[19], endothelial dysfunctions[20], coronary artery disease (CAD), stroke, and Diabetes Mellitus (DM). Neurodegenerative disorders (NDDs)[21], dermatological disorders (DTD), and cancer (mainly of liver, kidney, lungs, and bladder)[22] have also been reported. Chronic arsenic consumption is associated with serious cardiometabolic syndromes[23,24]. The present review expedites the possible mechanism and pathophysiological biomarkers in the development of CVDs.

The entire human vascular system is covered with the endothelium. Endothelial cell structure integrity is an important factor in maintaining the circulatory functions. It exerts autocrine, paracrine and endocrine actions[25]. Endothelium is responsible for maintaining balance between the vasodilatory and contracting factors to regulate the vascular homeostasis[26]. Release of various mediators are regulated by endothelium. The most important and major vasodilatory substance is Nitric Oxide (NO). NO is generated in endothelium during the conversion of L-arginine to L- citrulline in the presence of eNOS (nitric oxide synthetase). eNOS is activated in the presence of ca^{2+}-calmodulin, flavin adenine nucleotide (FAD), flavin mononucleotide (FMN), tetrahydrobiopterin (BH4)[27].

Endothelium dysfunction can happen when an imbalance occurs in the vasoconstriction and vasodilation, which can further reduce NO production, eNOS expression, antioxidant status and increase oxidative stress, deposition of Low-Density Lipoprotein, very Low-Density Lipoprotein, Total Cholesterol, Triglycerides (LDL-c, LDL-c, TC, TGs). It further propagates the release of inflammatory mediators, cell adhesion molecules, and acute phase serum protein. VED is involve in pathogenesis atherosclerosis[28], heart failure[29], diabetic neuropathy[30], and hypertension[31].

Arsenic-Mediated Vascular Deterioration

In a preclinical study, Arsenic (1.5mg/kg, i.p, for a period of two weeks) was found to be associated with significant increase in the aortic superoxide anion generation and in the serum level of thiobarbituric acid reactive substance (TBARS: a biomarker of lipid peroxidation). The nitrile production and superoxide dismutase (SOD) was found to be reduced significantly. Tumour necrotic factor-á (TNF-á: biomarker of inflammation) was also found elevated. Histopathological studies also revealed loss of integrity of vascular endothelium cells.

Vascular reactivity study revealed the arsenic significantly reduced Ach-mediated endothelium dependent relaxation, but sodium nitroprusside-induced (SNP) endothelium independent relaxation was not affected by arsenic. The possible mechanism of arsenic-induced endothelial dysfunction can be concluded as increase in ROS in aorta by activation of NADPH oxidases and produces oxidative stress. The excessive ROS can further react with NO to reduce its production and upregulate the inflammatory mediators in the aorta, one of them is as mention above TNF-á. The damaging effects were visible in the aorta of rat by histopathological lesions[32].

In another study in which arsenic (2, 10, 20, and 50 mg /L) was given to rats for sub-chronic duration of three months, the level of arsenic in urine and water was found to be elevated. Dose-dependent damage was seen. The body weight of Arsenic treated group showed no significant difference compared to control group. The histopathological study of aortic arch showed structural variations of endothelial nuclei in arsenic-exposed (50mg/l) rat group, cytoplasm was loose, and swelling was very evident, erythrocytes were agglutinated in this area. vWF was elevated in all groups exposed to arsenic but, dose-dependent reduction was seen in NO production. The TNOS activity was also lower in same trends of NO production. The concentration of apoptotic cells was also found to be elevated in aortic arch. The rate of apoptosis was more in high exposure 50 mg/L group compared to 5, 10 mg. The serum level of pigment epithelial-derived growth factor was found to be significantly decreased in arsenic group compared to control.

The immunohistochemical analysis done on aortic Fas, FasL, P-53, P-p38 revealed higher level in high dose arsenic group (10 and 50 mg) compared to control group but not significantly higher in the low dose group (5 mg). The MDA level was much elevated and SOD level was lower in all arsenic exposed group. The possible mechanism of arsenic-induced endothelial dysfunction revealed major role of PEDF in arsenic-exposed endothelial dysfunction, optimal level of PEDF may act as antioxidant in VED. Lower level of it may cause higher ROS, that can initiate oxidative stress and lipid peroxidation as evident from the higher level of MDA and lower level of SOD.

Other possible mechanism could be the apoptosis of aortic arch by P-p38. PEDF protect P-53, P-p38 induced apoptosis. So, lower level of PEDF is involve in the apoptosis of aortic arch[33]. Arsenic was given to rats (25, 50, and 100 ppm in drinking water) for sub-chronic duration of 90 days. A dose-dependent damage was seen. The food intake, body weight, and water consumption were found to be reduced. The physical appearance was not changed significantly except the rough coating of hair of rat (exposed to 100 ppm). The relative and absolute weight of kidney and liver were not changed in (25, 50 ppm) compared to (100 ppm) group where weight of kidney and liver was found to be elevated.

Other vital organs (brain, heart, lungs, spleen, testis) weights were not significantly different as compared to control group. The biomarker of lipoprotein oxidation MDA and ROS was significantly higher in dose dependent manner compared to control. Antioxidant enzymes SOD and catalase activity was significantly lower in arsenic exposed rat compared to control. The dose-dependent reduction was also seen in Glutathione Reductase (GR), Glutathione Peroxidase (GPx), and GHS compared to control. The possible mechanism attributes to arsenic- induced deterioration of vascular redox homeostasis and physical health was due to oxidative stress (excessive ROS generation) and decrease in antioxidant enzymes (GSH, GPx, GR) which leads to oxidative damage to DNA, proteins, lipids. The cascade of lipoprotein oxidation begins here[34].

Sub-chronic study on arsenic (100 ppm in drinking water) was done and different parameters were evaluated. Vascular reactivity experiment revealed Ach-induced endothelial-dependent relaxation was found to be reduced but, the SNP-induced relaxation and Phenylephrine- induced contraction was not affected by arsenic exposure. Furthermore, the pro-inflammatory cytokines (IL-1â and IL-16), chemokines (MCP-1), Cell adhesion molecule (sICAMP-1, VCAMP-1), acute phase protein (CRP) were also found to be elevated by arsenic exposure. Arsenic-exposure reduced the concentration of eNOS protein, Phosphorylated eNOS concentration and, down-regulate the eNOS gene expression which in turn reduced the nitrite production in the tissues.

On the other hand, the iNOS mRNA expression and iNOS-mediated Nitrile production was found to be enhanced. The possible mechanism of arsenic-induced endothelial dysfunction is majorly associated with inhibiting NO production and suppressing eNOS, up-regulating the iNOS expression, as a result the vascular homeostasis is lost. The lining of endothelium becomes adhesive for the circulatory mediators of inflammation cytokines, cell adhesion molecules, acute phase serum protein[35].

Arsenic-Mediated Hypertension

Chronic arsenic consumption is a threat to the cardiovascular system and has strong association in the development of hypertension. Oxidative stress is the major factor in development of arsenic-mediated hypertension. A pre-clinical study assessed the arsenic (100 ppm via drinking water for 90 days). Weekly blood pressure was determined. The systolic, diastolic and mean arterial blood pressure was significantly elevated in rats on week 6 and 7 respectively. The aortic antioxidants enzymes superoxide dismutase, catalase, reduced glutathione, glutathione peroxidase was found to be significantly elevated on 91st day. The serum LDL-C, total cholesterol and triglycerides were found to be significantly elevated. The iNOS

expression was up-regulated and down-regulated the eNOS expression. Reactive oxygen species and lipid peroxidation was found to be significantly elevated. HDL-C was reduced. Several gene expressions aortic Nox-4 and p22Phox mRNA was prominently elevated. The possible mechanism of elevated blood pressure could be an increase in the reactive oxygen species and shutting down the antioxidant defense system. These ROS react, target the endothelium, and reduce the production of Nitric Oxide.

The possible mechanism of arsenic-mediated hypertension could be through alterations in the redox signaling and over production of ROS and this further propagates the accumulation of total cholesterol, triglycerides and low-density lipoprotein cholesterol and reduction in the high-density lipoprotein cholesterol and thus enhance the blood pressure[36]. In another study, the relationship between the arsenic mediated hypertension and role of antioxidant defense and CYP system was evaluated. Arsenic salt (arsenate and arsenite 50 ppm for 200 days) was administered in rats through drinking water. The systolic blood pressure was evaluated each 20th day at the same time. The blood sample sand tissues were also collected for the determination of antioxidant status, CYP4 and angiotensin converting enzyme activity. The weight gain and organ weights were also determined and compared with rats. The systolic blood pressure was significantly raised, antioxidant defense system showed time-dependent variations. The systolic blood pressure remained high until 200th day. The arsenic-exposed rats showed slight variations in body weight gain but, these were significant until day 200. The most common biomarker ACE was not affected significantly by arsenite. However, the CYP4A was expressed in higher concentration in arsenic-administered group which might have been more crucial in the development of hypertension.

The possible mechanism of arsenic-induced hypertension could be the overexpression of CYP4A and antioxidant system shut down thus elevate the systolic blood pressure[37]. Previously done studies have reported that arsenic via drinking water is associated with hypertension. Several mechanisms of arsenic-induced hypertension have been reported. It is suggested that arsenic may induce hypertension by enhancement of calcium sensitization in the blood vessels, myosin light chain phosphorylation-mediated vasoconstriction, disruption of antioxidating defense mechanism, increased â-receptor adrenoreceptor stimulation, potentiate peripheral vascular resistance, vasoactive agent stimulation, enhanced pressor response to preganglionic stimulation and increased expression of ET-1(endothelin-1)[38].

Arsenic-Mediated Diabetes

Previously done studies show that prevalence of type-2 diabetes mellitus is linked with high level of inorganic arsenic in drinking water. The pathophysiology of arsenic induced diabetes is similar to type 2 diabetes. Preclinical studies report that 50 ppm of arsenic intake through drinking water for eight weeks produce glucose intolerance. Studies have shown that rats and mice are less susceptible than humans to the diabetes induced by chronic arsenic intake. The reason may be the effective clearance of arsenic and its metabolites from tissues[39]. Several mechanisms of arsenic-mediated diabetes have been purposed previously[42].

Some of those mechanistic studies are purposed here for the understanding of pathological biomarkers and mechanism involved in the pathology of arsenic-induced diabetes. The pathophysiology of arsenic induced diabetes is similar to type 2 diabetes. A study disclosed the mechanism of arsenic-mediated diabetes. According to this study, the possible mechanism by which arsenic induces diabetes is through improper metabolism which in turn causes oxidative stress and affects the signal transduction pathway, impairs the metabolism of glucose, the transport of glucose is inhibited and gene expression is modified or altered[40]. Another study purposed the mechanism and the role of intermates in the pathogenesis of diabetes. Study claims that arsenic generates multiple intermediates which have potential to induce toxicity. Arsenic inhibits glucose metabolism, the secretion of insulin from â- cells is impaired. On the other hand, in signal transduction, the cells which were exposed to arsenic cause the inhibition activation of PKB/AKT. It is a necessary component of insulin induced signal transduction pathway. Thus, insulin-dependent signal transduction at the PKB/AKT level is inhibited and leads towards hyperglycemia. Furthermore, the uptake of glucose in skeletal muscles and adipocytes are inhibited. The chronic exposure of arsenic causes the upregulation of two cytokines, oxidative stress is increased and the expression of tumor necrosis factor alpha (TNF alpha) and interleukin-6 (IL-6) is upregulated. Both have their well-studied role in insulin resistance[44].

Another study reported the molecular mechanism of arsenic-mediated diabetes. It states that arsenic forms covenant in disulfide bridges present in the molecule of insulin and enzyme like pyruvate dehydrogenase and alpha-ketoglutarate dehydrogenase which are involved in glucose metabolism thus normal function of these is interrupted[43]. One more mechanistic study has reported that relates the pancreatic â cells dysfunction in the pathology of diabetes. Arsenic

mediates the pancreatic â cells damage by increased gluconeogenesis oxidative damage in liver; this oxidative stress can cause amyloid formation in the pancreas which may destroy insulin stimulating â cells. The impairment to beta cell function and resistance towards insulin by activating the nuclear factor-kappaB (NF-kappaB) has been reported to be linked with oxidative stress)[45].

CONCLUSION

The major pathological target of Arsenic is antioxidant defense system, and increase in Reactive Oxygen Species, reduce the Nitric oxide production, reduction in vascular permeability, increase in adhesion and production of inflammatory mediators (IL-6, TNF-á,), TCs, TGs, LDL-C, lipid peroxidation, â-cells dysfunction. The persistent accumulation and generation of these pathological biomarkers in the blood vessels can induce hyperlipidemias, dyslipidemias, atherosclerosis, hypertension and diabetes. There is a need to propose natural antioxidant with minimum side effects in the treatment of Cardio Metabolic Syndromes (CMTs).

Authors' contribution: AU conceived, searched for literature and wrote the manuscript. KZ reviewed the methodology, data and manuscript.

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SHORT COMMUNICATION

Frequency of ABO Blood Groups and RhD Factor in Donors of District Nowshera

Hamzullah Khan

ABSTRACT

To screen the donors for the frequency of different blood groups, reason for deferral and comparative analysis of the donation in two consecutive years in the blood bank of Qazi Hussian Ahmed Medical Complex Nowshera, a cross sectional study was carried out from April 25, 2017 to May 5, 2019. A total of 3,429 donors were included in the study. The mean age of donors with standard deviation was 35± 3.24 years. Three thousand eighteen (88%) of the donors were males. The distribution of RhD+ and RhD- blood groups was 89.90% and 10.09% respectively. The frequency of 'RhD+ blood groups in target population' was B: 1226 (35.75%), O: 807 (23.53%), A: 754 (22%) and AB: 296 (8.63%). The frequency of 'Rh Negative blood groups' was: B: 139 (4.05%), O: 95 (2.77%), A:84 (2.45%) and AB: 28 (0.82%). The packed cell wastage rate in our blood bank was 185 (5.4%). The donor deferral rate was 0.7%. Hepatitis B Virus was the major cause of deferral that was reported in 14 cases followed by HCV Virus in 13 cases. It was concluded that the frequency of 'Rh-positive blood group' was B,O,A and AB respectively. Blood Group B was noted in 35% of the donors that counted to be the major prevalent Rh positive blood group in our population. Regarding the Rh Negative blood group, again the frequency was B,O,A and AB. Blood group B- was prevalent as a major negative blood group in our population that was recorded in 4.05% cases. The seroprevalence of hepatitis in the donors was 0.7%.

Key words: Transfusion medicine, blood grouping, blood borne infections

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INTRODUCTION

Blood Banks evolved to save lives in various emergencies. The term Blood Bank refers to the process of collecting the blood, separating the blood contents for different clinical usage, and storing of blood. Healthy and safe blood transfusion is vital, as according to American Red Cross statistics, each year about 5 million people are transfused in the United States only. So far about 400 red cells antigen have been identified in literature. The inheritance of these blood group antigens is by Mendelian Fashion. In literature, the ABO blood group system was first reported followed by the Rh blood group system. These both systems are vitally important for the purposes of blood transfusion.[1]

A study on the prevalence of ABO and Rh Group was conducted in tertiary care hospitals of Peshawar and reported that the Rh Positive blood group distribution in their population was BAO, AB (31.2%), (10.1%), (27%), (31.7%) respectively.[2] Another study from Islamabad reported that overall distribution of different blood groups in the target population of 1,521 donors was 35.50%, 28.27%, 26.89% and 9.34% for blood groups O, B, A and AB respectively[2].

The prevalence of Hepatitis B and C viral infections in our country cannot be ignored. Transfusion of infected blood is one of the important causes of the spread of hepatitis. A study from Rawalpindi reported the seroprevalence of Hep B and C in blood donors was 2.52%[3].

Present study was conducted to screen the donors for the frequency of different blood groups, and the reasons for deferral in the blood bank of a tertiary care hospital of Nowshera.

METHODOLOGY

A total of 3,429 donors were studied in the blood bank of the Qazi Hussain Ahmed Medical Complex, Nowshera. Duration of study was from April 25, 2017 to May 5, 2019. Out of the total donors, 3,018 (88%) were males.

Selection criteria followed in our blood bank was: age between 18 to 60 years; weight more than 50kg; and haemoglobin of >11g/dl. Exclusion criteria was any

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pervious history of viral disease like Hep B and C and HIV, drug abuse, body tattooing/needling/piercing, previous transfusion of whole blood or blood component in the past 6 months and or any renal, cardiac, pulmonary or hepatic diseases.

All the donors were screened for HBsAg, Anti HCV antibodies and HIV antibodies was done through ELISA using COBAS 311 (ROCHE) version.

Data was entered in SPSS version 25. Numerical variables like age of patients were presented with mean and standard deviation while the categorical variables like type of blood group and infection was presented with percentages.

RESULTS

A total of 3,429 donors were received in the blood bank. The age range of the donors was from 18 years to 52 years with mean age of 35 (±3.24) years. Three thousand and eighteen (88%) donors were males and 411(12%) were females. The frequency of RhD+ and RhD- groups among the donors was 89.9% and 10.1% respectively. The frequency of 'Rh Positive blood groups' was B: 1226 (35.75%), O: 807 (23.53%), A: 754 (22%) and AB:296 (8.63%). The frequency of 'Rh Negative blood groups' was: B: 139 (4.05%), O: 95 (2.77%), A:84 (2.45%) and AB: 28 (0.82%) (Table 1).

Table 1.Frequency of ABO and Rh D Blood Groups of Donors Presenting to the Blood Bank of Qazi Hussain Ahmed Medical Complex, Nowshera

Blood Group	No. of Donors	Percentage
B+	1226	35.75
O+	807	23.53
A+	754	21.99
AB+	296	8.63
B-	139	4.05
O-	95	2.77
A-	84	2.45
AB-	28	0.82
Grand Total	3,429	

Table 2. Cause of Deferral of Donors

Reason for Deferral	No. of cases	Percentage
Hbs Ag positive	14	50
HCV Ab Positive	13	46.43
MP Positive	1	3.57
Grand Total	28	

Out of total 3,429 donations, 185 (5.4%) bags expired in the last two years, 24 (0.7%) was donor deferral rate due to positive virology. A total of 3,034 (88.48%) bags were issued for transfusion after verification/ screening and crossmatch. Hepatitis B Virus was the main cause of deferral that was reported in 14 cases followed by HCV Virus in 13 cases. In one case, malarial parasite was reported on smear and was deferred to be transfused (Table 2).

DISCUSSION

Blood transfusions have been used in medical practice since 1930 for various clinical indications. Soon after the introduction of blood banks for better storage and safe transfusion, the use of blood became more common in clinical set ups. In Pakistan, more than 1.5 million bags of blood are donated each year. Among these donors, 65% are the relatives of the patient, that is, replacement donor, while 25% are volunteer donors and about 10% are professional blood donors.[4-6]

We observed the mean age of 35 years with male predominance 3,018 (88%). In present study, the frequency of 'Rh Positive blood groups' was B: 1,226 (35.75%), O: 807 (23.53%), A: 754 (22%) and AB:296 (8.63%) while the frequency of 'Rh Negative blood groups' was: B: 139 (4.05%), O: 95 (2.77%), A:84 (2.45%) and AB: 28 (0.82%).

Another study from Rawalpindi-Islamabad coincides in its findings with ours. It reports that among volunteer blood donors, 3,519 (79.5%) were males. B+ve blood group was the most common (31.2%). Frequency of ABO groups was A+ve, AB+ve, O+ve, A-ve, B-ve, and O-ve was 21.5%, 9.8%, 29.7%, 1.8%, 2.9%, and 2.5% respectively. The distribution of Rh+ and Rh- blood groups was 92.2% and 7.8% respectively in their population[7].

Going through the international literature, as study from Tanzania reported that the most common blood group in their population was O (52.3%) and the rarest was AB (3.18%). A total of 97.7% of the donors in their set up were Rh positive and 2.3% were Rh negative. Most donors were in the age range of 19-29 years. The male to female ratio among the donors was (88.1%):11.90[8] that strongly coincides with our findings.

Another study from Islamabad agreed with our findings. They showed that among ABO blood groups, the most prevalent was B (33.5%), followed by O (31.3%), A (22.5%) and AB (12.4%)[9].

The sero-prevalance of hepatitis in our study was 0.7%. Hepatitis B Virus was the main cause of deferral that was reported in 14 cases followed by HCV Virus in

13 cases. A study from Rawalpindi and Islamabad reported that the prevalence of Hepatitis B in their donors was 2.45% with a male gender predominance while that of Hepatitis C was 2.52%[4]. A retrospective study from Iraq reported that out of the total sampling of 495,648 blood donors, only 3258 (0.6%) were positive for hepatitis B and 933 (0.3%) were positive for hepatitis C,[10] that coincides with our findings.

In our study, the packed cell wastage rate was 5.4%. The average expiry rate in blood bank is reported for packed cell wastage in hospitals ranging from 1.93% to 30.7%[11], which coincides with our findings.

CONCLUSION

The descending order of the frequency of "Rh-positive blood group" in the target population was B, O, A and AB. Regarding the Rh Negative blood group, again the descending distribution of blood group was B, O, A and AB. To control the spread of viruses in blood transfusions, there is a need for public awareness through advocacy, communication and social mobilization and health education activities. Selection of healthy and young blood donors should be encouraged.

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CASE REPORT

Do Family Wishes Trump Verbal Advance Directive? An Ethics Case Report Amidst COVID-19

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INTRODUCTION

Covid 19 pandemic brought many challenges for public and healthcare workers. Healthcare workers all around the world are at the heart of this dreadful situation. While providing critical care to Covid 19 patients, they have faced many clinical as well as ethical dilemmas. The novelty and profound uncertainty associated with the clinical course of the disease introduced a dimension to the context of ethical dilemmas faced in clinical practice. The patients and families were being bombarded by information/misinformation from various forms of media. This, with the clinical uncertainty associated with the course of illness, led to widespread confusion and conflicts. We are reporting a case, managed by a team of experienced clinicians at a local tertiary care hospital, in which the patient's wishes conflicted with those of the family. The case was brought to the clinical ethics committee and Karachi Bioethics Group for discussion.

The Case:

A 54 years old paediatrician was admitted to a tertiary care hospital with severe Covid 19 in April 2020. More than ten days of intensive treatment failed to improve his oxygenation. High flow Oxygen and non-invasive ventilation were offered. He remained fully oriented during these ten days. Primary team consultants, along with anaesthetists, sought his permission for intubation and mechanical ventilation. In all his senses, his confident reply was: "Doctor, please don't intubate me! I will get better in the next two days." He was urged to sign the Do Not Intubate (DNI) form, as per his wishes, which he did not. His wife remained neutral throughout. Many of his colleagues called to keep a check on his well being. Two of his close friends, also physicians, earnestly advised him to opt for assisted ventilation.

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On the morning of the 11th day of admission, the patient was confused, fatigued, and was progressively developing respiratory failure. His wife promptly met him and was distraught. She was counseled again, informed about the adverse outcome and the decision made by the patient about DNI. She insisted on providing assisted ventilation for him. After much ado, the clinical team started assisted ventilation. The patient suffered a fatal cardiac arrest after 16 hours.

The case prompts many questions about respecting the patient's wishes and the family's wishes. Physicians' prerogative and legal value of advanced directives represent matters of concern. The situation, nevertheless, created a degree of moral distress among healthcare providers. The key question is, whether it is ethically permissible to disregard patient's wishes and follow the wishes of the family? The purpose of this case report is to discuss all these aspects in light of principles of bioethics, socio-cultural and religious values, and legal rights.

DISCUSSION

Patient's Autonomy:

The values and social context of the patient and family are extremely important to be considered while executing decisions for critically ill patients. The patient, a paediatrician, had sound medical knowledge and we can reasonably assume that he was involved in discussions with his clinical team. He also understood the clinical significance of 'ventilation' or Assisted Life Support (ALS). With all his clinical acumen, in full consciousness, he asked his clinical team to 'not intubate' him. It was an autonomous decision. This required the clinical team to respect the moral right of the patient to make his informed decision. Autonomy, a principle of Bioethics, has been described by Beauchamp and Childress as the right of individuals to express their values and choices about the medical care they receive. According to a Bioethicist, Mary B. Mahowald, the respect for the autonomy of a patient even outclasses the principles of beneficence and non-maleficence, described by Beauchamp and Childress.

In Pakistan, however, patients are generally not completely aware of their rights, and the consent is often not a truly 'informed' one. Although in this situation, the patient himself was a physician, he did not comply with a written advanced directive of Do Not Resuscitate (DNR) or Do Not Ventilate (DNV).

Family's Wishes

A family typically has a socially and culturally significant role in decision-making in Pakistan. The expressed wishes of family members, particularly, in situations where patients are incompetent to make decisions for themselves, are valued by clinical teams while making tough decisions. In this case, the patient's wife was communicating with the clinical team. As the patient's clinical condition deteriorated, she steadfastly insisted to 'do everything possible', including ventilation or ALS. The clinical team shared the wishes of the patient and discussed details of an anticipated dismal prognosis with her. Her stance remained steady. An empathetic response to her profound grief would need time and that has been one of the scarcities clinicians have been facing during this pandemic. An anxious spouse, startled by a sudden decline in her husband's condition and afraid of losing him to this scary illness, reacted in the most imaginable way. Covid 19 pandemic and the loss associated with it, has profoundly affected humans incredibly. She was certainly not prepared to let him go, as any family member would in such an unpredictable situation. The clinical team respected her wish.

Clinicians' Dilemma

The clinical team was caught in between the patient's directives, the family's wishes, and legal challenges. The clinical team reasonably believed that the patient would most likely not benefit from ALS. They also wanted to respect the choices determined by the patient himself, but lack of written documentation posed a risk of litigation. This is not an uncommon scenario in Pakistan, where legal documentation of advanced directives is not practiced. The uncertainty associated with the illness itself was enough to cast a shadow of doubt on the clinical judgments. In the earlier part of the pandemic, the clinical information and guidelines of Covid 19 infection were constantly being updated. The demise of the patient, just 16 hours after receiving ALS, supported the clinical judgment. It was not arbitrary to assume that the previously healthy patient, even though a physician himself, did not anticipate his illness to get worse so briskly. The clinical team, however, suffered from moral distress due to this situation. The fact that they had provided ALS 'against' the wishes of the patient consumed them considerably. Some of the team members believed they caused more harm to the patient than benefit by providing ALS. They presented this case for discussion to the Clinical Ethics Committee.

Role of the Clinical Ethics Committee

The case not only highlights many ethical issues faced by clinicians but also underscores the key role of Clinical or Hospital Ethics committees. Clinical settings in Pakistan provide deficient support for such committees. There is a dearth of Clinical Ethics consultation. Inconsistent ethics education for clinicians frequently leads to moral distress. The prominent role of such committees is consultation and mediation in cases of conflict, such as the one discussed. Ethics consultants perform the role of mediators when stakes are high. Approaching such committees or consultants, if available, earlier in such situations may help mitigate some of the formidable challenges faced by clinicians. The committee reviews institutional as well as national policies, if any. These facilitate in bridging communication gaps inadvertently developed in such intensely emotional and critical situations with need to identify ethical dilemma and devise early decision-making strategies. The families equally benefit from such consultations. They frequently get appropriate answers and closure which helps them through their process of grief. In this case, the ethics committee, with the help of in-house team of psychologists, offered grief counseling to the family. The clinical team members also received support to mitigate moral distress.

Recommendations

Covid 19 has brought about a complex array of clinical as well as ethical challenges for the healthcare community around the world. The case under discussion may be approached from legal, social and, clinical perspectives. However, the lessons learned range from an individual to the policy level. Effective communication and adequate documentation of every discussion with the patients or families are crucial. Early decision-making should be tacitly encouraged among patients and their families. All autonomous decisions of critically ill patients should be shared with their immediate families and legally documented. The involvement of ethics experts or committees should be considered as early as possible. The institutions are responsible for developing and encouraging clinical ethics consultations. Reasonable policies should be implemented and disseminated by healthcare organizations. National resources should be adequately developed by promptly introducing formal clinical ethics education for all clinicians.

CONCLUSION

The issue of the validity of a verbal advanced directive by the patient was called to attention when the patient lost the capacity and the wishes of his family conflicted. The clinical team was placed in a tight spot because they sincerely wanted to respect the wishes of the patient but were unable to do so. Against all odds, the clinicians provided the most competent care possible to the patient and respected family's wishes. In the end, the family lost a loved one, and the clinicians developed moral distress. Legal documentation and early recognition of an ethical dilemma are imperative for empathetic clinical care in particularly grueling times such as the Covid 19 pandemic. Development of clinical ethics consultation and national policies is obligatory for healthcare organizations and the state to provide quality clinical care.

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Authors' contribution: Dr. Sarosh Saleem Concive the idea, design, drafting, composition and review of the article. Dr. Nuzhat Irfan Malik Concept, design and critical review of the final version. Dr. Nida Ilyas Shamsi Concept, design and critical review of the final version

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LETTER TO EDITOR

Importance of Face Masks during COVID-19

Umaima Khan[1], Ahsan Inayat[2] and Marrium Hassan[3]

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Corona virus disease also known as COVID-19 is a respiratory infection that is caused by the virus SARS-CoV-2, that spreads among the people by respiratory droplets usually produced by sneeze and cough, and by touching an object or surface used by an infected person. As COVID-19 pandemic is increasing, the best way to limit the outbreak is the use of face masks[1,2] which helps in preventing transmission of the virus from a person to another by creating a barrier between the oral cavity and the nose of the wearer with the potential contaminants in the surroundings.[3]

WHO has also provided comprehensive guidelines on using facemasks at home, public places and clinical setups.[4] Hence, several countries including Pakistan have already included face masks in their COVID-19 control plans. As a result of the unexpectedly high demand for face masks, it has been very difficult for low-income countries to provide N95 and surgical masks. Therefore, people have started using cloth masks, while surgical masks are being used by healthcare workers. The re-use of surgical masks is being seen frequently in Pakistan.

According to a previous study, N95 and Surgical Masks, both were successful in the prevention of the spread of influenza. Surgical masks are basically used in protecting an individual from contagious droplets in clinical setups. Although, it was not found to be useful in stopping the spread of other such diseases, for example MERS/SARS.[5] These surgical masks do not fit well on the face around the edges, therefore small air-borne microbes and its particles can escape through these edges. N95 masks are more effective than the surgical mask because these fit tightly on the face and can filter out both small and large particles, therefore, prevent the individual from inhaling smaller, airborne infectious particles.[6] There is insufficient data to prove that using a surgical mask prevents everyone from COVID-19. Some studies also report that both cloth and surgical masks were unsuccessful in the prevention of the spread of SARS-CoV-2 with the respiratory droplets of COVID-19 patients to the surroundings and the external surface of the mask.

Moreover, the appropriate use of these masks is important as inappropriate use may eventually raise the transmission rate of this virus. Evidence shows that even the health care workers do not have enough awareness and their practices of wearing masks are inappropriate.[7] Some studies suggest that cloth masks may help in the reduction of transmission of larger respiratory droplets. Their effectiveness may depend on the fabric used as well as how well they fit on the face of the wearer, but some studies have already reported about the effectiveness of cloth masks for SARS CoV-2.[8,9]

Public awareness is important regarding the appropriate use of face masks and future studies are also needed for investigating the effectiveness of face masks in filtering respiratory viruses and preventing viral release from an infected person.

Authors' contributions: Dr. Umaima Khan drafted the manuscript and did final review. Dr. Ahsan Inayat reviewed and did the corrections in the manuscript. Dr. Marrium Hassan drafted the manuscript and searched for literature.

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