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Use of Bacteriocin Producing *Lactobacilli* Strains from Fruits and Vegetables in Food Preservation

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ABSTRACT: *Bacteria, that generates bacteriocin plays an important role in the preservation of food. Lactobacilli strains that produced bacteriocin were acquired from various vegetables and fruits from the markets of Multan, Pakistan. Bacterial growth was observed on MRS broth as well as agar, which is a Lactobacilli-specific media, and their metabolic activity as well as antibacterial activity against diverse pathogens were investigated. The endeavor of bacteriocin at different parameters i.e., pH, temperature, and proteolytic enzymes were explored for further validation of bacteriocin generating Lactobacilli. These bacteria's antibiotic activity against ampicillin, ciprofloxacin, streptomycin and tetracycline, was also tested. Ten Lactobacilli strains were identified based on their morphology as well as biochemical characterization and six were further selected to perorm study. Among them, 80% strains exhibited inhibition zone and the significant zones were illustrated by Ct_2 isolate (14 mm) embracing E.coli (9473) and A_3 isolate(14 mm) against Enterobacteria spp. Bacteriocin ability of these designated strains was maximum at 7 pH and 35℃. T_1 strain was sensitive to ciprofloxacin, ampicillin as well as streptomycin although T_2 strain was resistant to streptomycin, ciprofloxacin as well as tetracycline. The most notable strains were identified for the preservation of fruit juice, where Lactobacilli that produce bacteriocin inhibit the growth of other bacteria that may spoil food.*

Keyword: *LAB, MRS media, Lactobacilli, Antibacterial activity, Bacteriocin*

INTRODUCTION

A large number of bacteria that produced lactic acid are present in nature and their industrial uses are also recognized (Dhamale et al., 2015). Lactic Acid Bacteria (LAB) belongs to "Gram-positive" group and have clusters of physiological, morphological and metabolic categorization (Mohankumar

and Murugalatha, 2011). They also showed positive results for the Indole test, Methyl-red and nitrate reduction test while, gives a negative result for Oxidase production test, Catalase test and Voges-prosker test (Dhamale et al., 2015). For the fermentation of feed and food, lactic acid bacteria assumes a critical duty and generally recognized as safe microbe (GRAS) microbe, hence under controlled conditions its use as a starter culture is also well known (Yang et al., 2012). Lactic acid is produced through either homo-fermentation or hetero-fermentation mechanisms. Nature has generously disseminated this bacteria all around and even found in human digestive track (Mohankumar and Murugalatha, 2011).

Lactobacillus and *Leuconostoc* are the members of LAB and they hold industrial importance and utilized extensively in the food industry. *Aerococcus* and *Vagococcus,* are also used in the biopharmaceutical industry. *Carnobacterium* and *Pedicoccus* are used in the production of bacteriocin (Huys et al., 2012). Use of *Tetragenococcus* as a probiotic is also well known (Rodpai et al., 2021). Other LAB bacteria like *Streptococcus* and *Lactococcus* are also frequently used in the dairy industry (Holzapfel et al., 2006). These are the bacteria that are known for their contributions in various industries and productions like, color, taste, texture and smell of several food items after its fermentation. Another bacteria i.e. *Lactobacillus acidophilus* is a part of the natural flora of the intestine. It is actually a non-pathogenic bacterium which is known for its use in medicine, as well as industrial fields. It has been reported that the production of acid causes reduction of yeast and bacteriain the intestine. It also aids in digestion of milk (Ahmed et al., 2010). Bio-preservation bacteria contains metabolites or even the living cells of microbe in humans and animals. They have a queer stimulatory effect on the digestive and immune systems of their hosts (Wang et al., 2010). Through the preservation of food the shelf life of food products is increased and ultimately there is an increase the supply. The food products that hold a lesser shelf life and are at a risk of perishing can be preserved for several days and sometimes upto weeks. Throughout the year, seasonal foods become accessible and results in the increase in variety of food (Zacharof and Lovitt, 2012).

Another use of LAB bacteria is that it is a safer microbe that prevents the food item from developing fungal or even bacterial pathogens. Food-borne

diseases, such as bacterial and fungal infections, infect about 30% of the population. In the year 2000, over ten million individuals died worldwide as a result of a diarrheal sickness (Black et al., 2003)

To restrict or limit the growth of disease-causing food borne pathogens, safe preservation items such as essential oils, surfactants, and bacteriocin derived primarily from lactic acid bacteria are used (Ahmad et al., 2014).

Food preservation is an important requirement of life. To increase the shelf life of the product has now become a necessity. The most crucial compound that is used for preservation of food is bacteriocin. It is an ancient technique to preserve vegetables using the method of lactic acid fermentation. It involves lactic acid bacteria that mainly produce lactic acid and other components like bacteriocin. Bacteriocin has antibacterial activity against a variety of different microorganisms.

LAB is renowned for their ability to commonly create bacteriocin. However, LAB strains and their produced bacteriocin need tostudy in detail to see effective activity against the microbes. Strangely it showed a lesser activity in comparison to the antibiotics. Nowadays much use of antibiotics results in resistant strains against the antibiotics. The present study was designed to isolate bacteriocin from collected samples of fruits and vegetables. The strains were tested at variable temperature, pH proteolytic activity and also used against different pathogens.

MATERIALS AND METHODS

Collection of Samples

Samples of fruits and vegetables were collected from divergent locations throughout Multan, Pakistan, and transported to the department of Microbiology and Molecular Genetics' research facility/laboratory. The samples were kept at 4°C until further processing.

Isolation and characterization of Lactobacilli

The fruits were washed in autoclaved sublime water and grinded with an aseptic mortar and pestle. *Lactobacilli* strains were obtained using MRS broth as well as agar. For the goal of pre-enrichment of *Lactobacillus* spp., squeezed fruits were enumerated in MRS broth. For each sample, inoculum from the broth was spread on MRS agar plates individually. Streaking was performed to isolate a purified colony of *Lactobacilli* strains (Ravi and Subramanyam, 2011). Gram staining and biochemical tests were implemented

as stated by Bergey's Manual of Bacteriology (Holt, 1994).

Pathogens used for antibacterial activity

Pathogenic species i.e. *E.coli* (9473), *Salmonella spp.*, *Klebsiella*, *Enterobacteria spp.*, and *MRSA*, were collected from The Women University Multan, Pakistan.

Bacteriocin production

Bacteriocin producing isolates were incubated at 37ºC for 48 hours in MRS broth and centrifugation was done for 10 minutes at 5000 × g to acquire separation of cells from by-products (Kumar and Kumar, 2015).

Antibacterial activity test

Antibacterial activity was scrutinized by well diffusion method aerobically. 500 µL of indicator microorganisms were inoculated in agar plates after incubate them in a nutrient broth and diluting them adequately. Muller-Hinton (MH) agar was used for this purpose in which 6 mm wells were compelled in plates of MH agar and 150 µL of crude bacteriocin (supernatant) was poured into separate well and the plates were incubated at 37ºCfor 24 hours. Antibacterial activity was deliberated by assessing the inhibition zone diameter (Kumar and Kumar, 2015).

Purifying bacteriocin

Divergent quantity of ammonium sulphate were added in crude bacteriocin and retained by stirring and was preserved overnight at the temperature of 4ºC bereft of disturbing. Then centrifugation was done for 10 minutes at 10000 × g, further precipitates were gathered subsequently and diffused in 20 mM potassium phosphate (KH_2PO_4) buffer (pH 7) (Udhayashree et al., 2012).

Bacteriocin Characterization

a) Heatlabile

5 ml bacteriocin was baited in distinct test tubes and labelled accordingly. Test tubes were subsequently heated at divergent temperatures i.e. 35ºC, 50ºC, 70ºC for 15 minutes and was further checked against pathogen (Kumar and Kumar, 2015).

b) Effect of pH

5ml partially purified bacteriocin was baited in divergent test tubes and their pH was regulated to 2, 7 and 9 separately making use of NaOH or HCl and were placed at room temperature. Their antibacterial activity was scrutinized against divergent pathogenic species (Kumar and Kumar, 2015).

c) Effect of proteolyticenzyme

5 ml partially purified bacteriocin was baited and treated with enzyme papain at 7 pH. Test tubes with and without enzyme (control) were incubated at 37ºC for 2 hours and were further heated for 3 minutes at 100ºC with the aim of denaturing enzyme and were checked for antibacterial activity against variant pathogens (Kumar and Kumar, 2015).

d) Effect of antibiotics

Lactobacilli that was grown overnight, was swabbed on soft agar plates of MRS media and discs of antibiotics were allocated on the surface media. Discs that were used for this purpose includes Ampicillin, streptomycin, tetracycline and ciprofloxacin (Kumar and Kumar, 2015).

e) Efficacy of bacteriocin as bio preservative

The bio-preservation efficacy of the strains was scrutinized by adding 5% bacteriocin in apple juice and was refrigerated. Serial dilution of 10^6 was prepared with the sample and incubated for 3 days at 37°C. The colony count (CFU) was noted and compared with and without bacteriocin control (Udhayashree et al., 2012).

RESULTS

Isolation and characterization of Lactobacilli isolates

Isolated strains were conceded as *Lactobacilli* after biochemical characterization that was carried out in accordance to Bergey's manual. The isolated strains were Gram positive, non-motile and non-spore formers as shown table1.

Antibacterial activity

Screening of *Lactobacilli* isolates was done and results were noted. T_1 and T_3 exhibited significant results abutting every pathogen included in the present study i.e. *E.coli* (9473), *Methicillin-resistant Staphylococcus aureus* (*MRSA*), *Salmonella spp*., *Enterobacteria,* and *Klebsiella.* Nevertheless strain A_2 and A_3 exhibited zone of inhibition abutting all pathogens excluding *MRSA 6* strain. O_2 and Ct_2 manifested the inhibition zone abutting all the pathogens besides *Klebsiella* as shown in table 2. Six strains that showed best results were selected for further test of bacteriocin.

Table 1: Isolated *Lactobacilli* strains and their characterization

Sample	Locality	Origin	No. of purified colonies	Selected strains	Gram reaction
01	Fruit shop Sabzazar Multan	Apple	2	A_1	+ve
02	Fruit shop near Multan Cantonment	Olives	1	O_1	+ve
03	Fruit shop in Muzaffargarh	Olives	1	O_2	+ve
04	Fruit shop near Khanewal Road Multan	Tomatoes	2	T_1	+ve
05	Fruit shop on BZU Road Multan	Apple	2	A_2	+ve
06	Fruit shop near Chowk Shah Abbas	Apple	1	A_3	+ve
07	Vegetable shop near Gulshan market Multan	Carrots	1	Ct_1	+ve
08	Fruit shop near children's Hospital Multan	Tomatoes	1	T_3	+ve
09	Vegetable shop near Chungi No. 06 Multan	Carrots	1	Ct_2	+ve
10	Fruit shop near Kalma Chowk Multan	Olives	1	O_2	+ve

Table 2: Screening of *Lactobacilli* strains against various pathogens.

Strains	**Zone of inhibition**				
	***E.coli* (9473) (mm)**	***MRSA* (mm)**	***Klebsiella* (mm)**	***Salmonella* (mm)**	***Entero bacteria spp* (mm)**
A_1	12	-	-	10	11
A_2	13	-	11	11	12
A_3	13	-	10	11	14
Ct_1	11	-	-	11	11
Ct_2	10	13	-	12	13
O_1	11	-	-	11	11
O_2	11	12	-	13	11
T_1	12	12	12	11	12
T_2	12	12	12	12	11
T_3	11	11	10	10	12

Characterizing bacteriocin

Following parameters were scrutinized for the characterization of bacteriocin:

a. Distinct pH
b. Distinct Temperatures
c. Proteolytic activity

Strains that showed activity at all temperatures, pH levels, and enzyme activity were thought to have the best bacteriocin activity.

Characterizing bacteriocin at distinct temperatures

Bacteriocin were examined at distinct temperatures including 35ºC, 50ºC and 70ºC to inspect that at which temperature bacteriocin exhibit best performance. At 35°C, strains A_1, Ct_1, O_1 and O_2 did not show any anti-*Klebsiella* activity and A_1, Ct_1, O_1 did not show any activity abutting *MRSA*. Apart from all

pathogens chosen for this study, strains T_1 and T_2 demonstrated bacteriocin activity at 50°C, and each strain showed activity abutting E.coli (9473). Apart from pathogen, most strains were ineffective at 70°C and showed no zone of inhibition. Conversely, T_2 strain was efficacious against all the pathogens selected for this study and the table 3 summarized the findings.

Characterizing bacteriocin at distinct pH

Strains were scrutinized against pathogens at distinct levels of pH including 2, 7 and 9. Apart from the pathogens (in this study), strains Ct_1, O_1, and T_2 showed activity under acidic condition i.e. pH 2. At pH 7, T_1 and T_2 strains exhibited bacteriocin activity against all pathogens under study however, strain T_1 and T_2 exhibited bacteriocin activity at pH 9. Results were recorded in table 4.

Table 3: *Lactobacilli* strains' bacteriocin activity at diverse temperatures

Test Strains	Zone of inhibition				
	***E.coli* (9473) (mm)**	***Salmonella*spp. (mm)**	***Klebsiella* (mm)**	***MRSA* (mm)**	***Enterobacteria* (mm)**
			At 35°C		
A_1	12	10	-	-	11
Ct_1	11	11	-	-	11
O_1	11	11	-	-	11
O_2	12	13	-	12	11
T_1	12	11	12	12	12
T_2	12	12	12	12	11
			At 50°C		
A_1	11	-	-	-	10
Ct_1	10	-	-	-	9
O_1	12	10	-	-	11
O_2	11	-	-	11	-
T_1	10	10	10	10	10
T_2	11	11	11	11	12
			At 70°C		
A_1	9	-	-	-	11
Ct_1	-	-	-	-	-
O_1	-	-	-	-	-
O_2	10	-	-	-	-
T_1	-	-	10	9	-
T_2	10	9	7	7	10

Table 4: Lactobacilli strains' bacteriocin activity at diversepH

Test Strains	Zone of inhibition				
	***E.coli* (9473) (mm)**	***Salmonella spp.* (mm)**	***Klebsiella*spp. (mm)**	***MRSA* (mm)**	***Enterobacteria* (mm)**
			pH2		
A_1	-	-	-	-	-
Ct_1	10	-	12	11	10
O_1	11	11	10	12	-
O_2	12	9	-	-	9
T_1	-	-	-	-	10
T_2	11	10	9	10	-
			pH 7		
A_1	12	10	-	-	11
Ct_1	11	11	-	-	11
O_1	11	11	-	-	11
O_2	11	13	-	12	11
T_1	12	11	12	12	12
T_2	12	12	12	12	11
			pH 9		
A_1	9	10	9	11	-
Ct_1	10	9	-	-	10
O_1	9	-	-	-	-
O_2	11		11	-	-
T_1	8	11	11	10	12
T_2	9	10	9	11	10

Characterizing bacteriocin by proteolysis

O_1 and O_2 strains showed bacteriocin activity in the presence of papain enzyme abutting every pathogen included in this study. Results were recorded in table 5.

Antibiotic resistance test

Resistance of strains was scrutinized besides various antibiotics

such as ampicillin, ciprofloxacin, streptomycin and tetracycline. Effects of antibiotics were noticed after 24 hours of incubation. Strain A_1 was found to be resistant to ampicillin and streptomycin; Ct_1 was resistant streptomycin; O_1 was resistant to ciprofloxacin and all the findings were summarized in Table 6.

Efficacy of bacteriocin in preservation of food

Each strain exhibited bacteriocin was compared with the control plate after incubation and was noticed that A_1 showed better prevention from spoilage as compared to Ct_1, T_1, T_2 and O_1 whereas O_2 showed the best protection against food rotting (Fig. 1).

Table 5: Proteolytic activity of Lactobacilli strains' bacteriocin

Test Strains	Zone of inhibition			
	***E.coli* (9473) (mm)**	***Salmonellaspp* (mm)**	***Klebsiella spp* (mm)**	***MRSA* (mm)**
A1	-	-	9	-
Ct1	11	13	12	-
O1	10	12	9	10
O2	12	11	9	9
T1	-	-	9	11
T2	-	10	10	-

Table 6: *Lactobacilli* strains with antibiotic resistance

Strains	Ampicillin	Ciprofloxacin	Streptomycin	Tetracycline
A_1	R	S	R	S
Ct_1	S	S	R	I
O_1	S	R	S	R
O_2	R	I	S	S
T_1	S	S	S	R
T_2	S	R	R	R

S= sensitive, R= resistant, I= intermediate

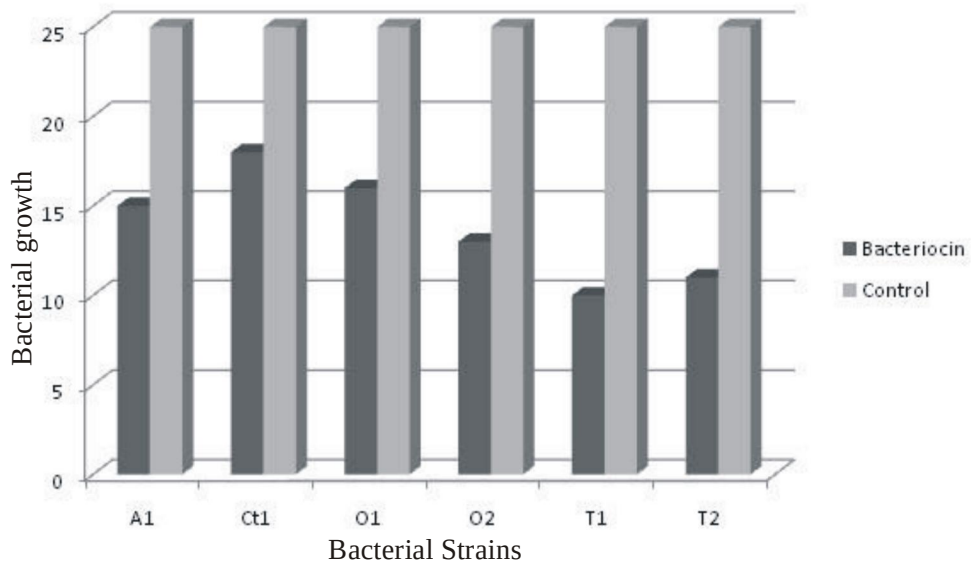

Fig. 1. Variation in bacterial growth amid with and without bacteriocin

DISCUSSION

Fruits and vegetables were selected because a large number of people in Multan love to take fruits and vegetables in their diet. Scrutinizing the probable activities of fruits and vegetables is the second purpose of the study. MRS media is differential medium for *Lactobacilli* growth used in this study. Strains that were isolated previously were normal flora of chicken and intestine and were *Bacillus* in shape and some were cocci nevertheless, *Pedicococcus, Tetragenococcus halophilus* (Udhayashree et al., 2012). In our study isolated strains were *Lactococcus* and some were *Lactobacillus* which was further tested by biochemical tests. The isolated strains were designated as *Lactobacillus* based on various morphological, biochemical and physiological attributes. In accordance with the results of our study, strains were gram positive rods. The chief motive for selection of fruits mainly apples is that it contains ample quantity of lactic acid bacteria which are beneficial in delivering immunity and probiotic potential to the host's body. Fruits and vegetables containing LAB are wide ranging but in our study carrot, grapes, tomatoes, olives, apple were used to check the presence of lactic acid bacteria and its utilization in preservation of food.

There is a wide range of fruits with varying degrees of microbial loads. Overall, concealed by agar well dispersal of two pathogenic organisms, *Klebsiella* and *MRSA* (*Methicillin-*

resistant Staphylococcus aureus) were immune to 50% of the selected isolates since *MRSA* is immune to many other drugs, but in this study, T_1, T_2, T_3,Ct_2, and O_2 were effective against *MRSA*, but *Klebsiella* was sensitive to T_2, T_3, A_2, A_3. In summary, our broad screening indicates that *Lactobacillus* species can produce anti-microbial compounds such as bacteriocin. Following a general screening, the six best strains for bacteriocin representation were chosen. Bacteriocin was characterized by testing it at various temperatures, pH levels, and with a protein degrading enzyme. Bacteriocin organization was nearly identical at 50°C to that of bacteriocin activity at 35°C, however bacteriocin was unproductive at 70°C because it is a protein, which degrades at very high temperatures. After the formation of bacteriocin was disrupted, its efficacy was also changed at the same level at pH 2 (acidic), and its effectiveness was the same with little changes of unevenness, whereas at pH 7, it has its normal effectiveness, and at pH 9, its activity against lab pathogens (*Klebsiella*, *Salmonella*, *MRSA*, *P. aeruginosa*, *E.coli*, and others) was influenced due to disordering of peptide bonds. The findings revealed that isolated bacteriocin was a proteinaceous substance (Udhayashree et al., 2012).

For food preservation, the best-characterized bacteriocin-producing strains were chosen.

CONCLUSION

The study's major goal was to explore the antibacterial activity of *Lactobacillus* spp. in order to inhibit the growth of bacteria (*E.coli*, *Klebsiella*, *Salmonella*, *P. aeruginosa*, *MRSA*). It was concluded that extracted chemical has antibacterial activity against the bacteria that have been infected. The current study also focused at the crushing of spoilage bacteria by counting CFU (Colony forming unit) prior to and following adding bacteriocin as a preventive agent and compared it to power (without bacteriocin). The activity of the extracted compound was further observed in the presence of various anatomical and enzymatic variables. Augmented or lowered that condition may lead to decreased activity. Low temperature and around the pH reveals its activity, decreased or increased that condition may guide to reduced activity. Finally, due to the proteinaceous nature of antimicrobial compounds, proteolytic enzymes such as amylase, lipase, chymotrypsin, and trypsin were not produced. It's probable that the lactic

acid bacteria growth during fortification chocked the growth of other damaged microorganisms, however needs to be investigated further. This method of preservation was one of the most natural process to keep food away from spoiling.

CONFLICT OF INTEREST

There is no potential for a conflict of interest.

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Determination of Dietary Knowledge among Patients Suffering from Chronic Renal Failure

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ABSTRACT: *Chronic renal diseases are usually mentioned as decreased functioning of kidneys. The main symptoms includes in this disease are kidney damage, greater loss of albumin in urine and a reduced glomerular filtration rate (GFR). The present study was aimed to determine the dietary knowledge about the patients that suffered in chronic renal failure along with various other impediments. A transverse study was performed for 4 months in Sir Ganga Ram Hospital and data of one hundred patients with chronic kidney failure and impediments were selected. A non-probability sampling method was used to conduct this study. Out of the total 100 patients, 36% of patients knew the renal diet. While, only 28% of patients knew about potassium, 16% about phosphorous and 20% about salt restrictions. Similarly, 57% knew about red meat and 56% knew about pulses restriction. The symptoms of studied disease was more prevalent in males as compared to females which showed gender as major factor for the renal failure. The majority of sufferers were unaware of the renal diet and electrolyte restriction in diet. Moreover, patients were also using excessive fluid against the advice of doctors. It was concluded there is need to conduct such studies to aware the necessity of appropriate dietary in control of chronic renal failure.*

Keywords: *Chronic, kidney, failure, symptoms, electrolyte, restrictions*

INTRODUCTION

Chronic kidney diseases (CKD) are prevailing worldwide as a major health issue. Ten to fourteen percent of the world's adult populace is put up with this disease (White et al., 2008; Saher et al., 2020). As per WHO's Global Burden of renal disease study (2015), 1.2 million people lost their lives because of renal failure worldwide. While, an average of 5-10 million people lost their life yearly due to this disease. In various

countries such as America (16.8%), Norway (10.2%), Taiwan (7%), and Iceland (5%) the occurrence is found high (Eriksen and Ingebretsen, 2006; Hsu et al., 2006). Only in Pakistan, 21 million people are hurting in third and fourth stage CKF (Seher et al., 2020). The danger to suffer in CKF enhanced with certain other diseases such as diabetes mellitus, hypertension, obesity, and cardiovascular diseases. A number of other factors like environmental pollution, excessive use of pesticides, painkillers, and use of unregulated food additives etc. (Ayodele and Alebiosu, 2010; Agyei-Mensah and Aikins, 2010; Engelgau et al., 2011). Regular screening of blood and urine can help in the prevention of these kidney diseases (Jha et al., 2013). Hypertension and diabetes mellitus are the major risk factors of CRF. About 75% of people with CRF also have hypertension. Moreover, lifestyle contributes a lot to the development of CKF, hypertension and diabetes mellitus. Smoking and alcohol should be prohibited in renal patients to avoid renal failure (Unger and Scherer, 2010). A moderate exercise of 30-60 minutes can help lower blood pressure and blood sugars level. Exercise can help maintain blood glucose levels (Vaziri and Norris, 2011). Victims of CKF can be suggested with a low Potassium (K) diet because as glomerular filtration rate decreased the levels of potassium because kidneys are the site for the filtration and secretion of potassium (Noori et al., 2010). Phosphorous from animal sources and food additives have high availability, whereas plant source phosphorous has a low availability. Therefore, CKF patients should be given a mixed animal and plant sources diet that is rich in phytic acid. Phosphorous from additives should be strictly restricted in CKF patients (Wolf, 2009). Victims of CKF faced various nutritional shortages as on hemodialysis they mostly suffer in Vitamins ($B_{6,}$ and B_{12}), folate and iron. The presence of these nutrients becomes very necessary to compensate the deficiencies (Kalantar-Zadeh et al., 2010). The main causes that lead to severe anemia are deficiency of hormone erythropoietin produced by kidneys that has a key role in the production of red blood cells, malnutrition, inflammation, iron deficiency and according to some recent studies Vitamin D deficiency (Moll and Davis, 2017). Intravenous supplements of iron can improve iron levels in hemodialytic patients. Similarly, the administration of oral supplements of Vit B_6 leads to an improvement in its levels (Kalantar-Zadeh et al., 2009).

The present study was carried out to find out the dietary knowledge of patients suffering from chronic renal failure who also have diabetes mellitus and hypertension. After the determination of the dietary knowledge of patients, awareness through health education could be given to patients about healthy food choices. So that the burden of renal disease could be reduced and patients may have a better quality of life.

METHODLOGY

Ethical statement

For the present study ethical approval was taken from the Institutional Review Board (IRB) of the University of Lahore (UOL).

Study design

A cross-sectional study was conducted in Sir Ganga Ram Hospital, Lahore, for the 4 months. 100 adult patients of both genders of chronic renal failure with diabetes mellitus and hypertension were selected by non-probability convenient sampling. Data were collected from a pre-tested questionnaire. Prior to study a written informed consent was taken from all the participants.

Statistical Analysis

Data were tabulated and analyzed with the help of SPSS version 23.0. The quantitative variables like age, income, etc. was assessed by using mean, standard deviation and standard errors. The qualitative variables were reported using percentages and frequencies.

RESULTS

In the present study it was noticed that majority of patients were unaware of the diet that should be given to patients with chronic renal failure. 36% of patients knew the type of diet for renal patients, 47% knew about dairy restriction, 57% knew about red meat restriction, 48% knew about foods restricted in the renal diet and 56% knew about pulses restriction. Analysis of results of the conducted study showed that 28% of patients knew about potassium restriction, 24% knew about food sources of potassium, 12% about food sources of phosphorous, 16% about phosphorous restriction, 20% about salt restriction, 10% about hidden sources of salt, 24% soaked vegetables before cooking, 96% knew that soaking vegetables before cooking leach extra potassium from vegetables, 12% knew about the high amount of salt in preserved foods and 7% patients knew about the high amount of phosphorous additives in soft drinks, as shown in Fig. 1.

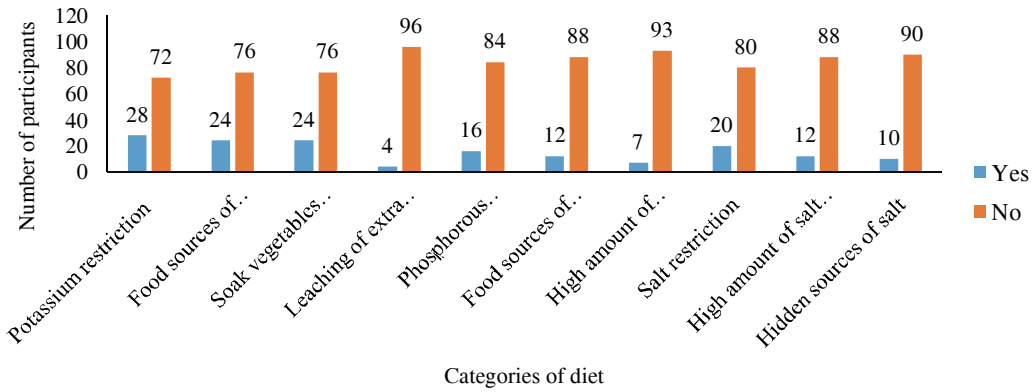

Fig. 1. Knowledge of Patients regarding Dietary and Electrolyte Restriction

Analysis showed a significant association of patients' knowledge about renal diet with socioeconomic status, p = 0.018. Whereas, there was an insignificant association of patient's knowledge about renal diet with gender (p=0.146) and body mass index (BMI) (p =0.255), as shown in Table 1.

Table 1: Association between Patient's Knowledge about Renal Diet and socio-demographic variables

Sr. No	Socio-demographic variables		Knowledge of Patients about Renal Diet		p-Value
			Yes	No	
1.	Socioeconomic Status	Low	8	29	0.018
		Middle	26	35	
		High	2	0	
2.	Gender	Male	22	48	0.146
		Female	14	16	
3.	Body Mass Index (BMI)	Underweight	13	35	0.255
		Healthy	9	15	
		Overweight	12	12	
		Obese	2	2	

DISCUSSION

The lack of knowledge about dietary restrictions is usual among CKD patients. Knowledge about renal diet and dietary restrictions is necessary for renal patients to improve their quality of life. Decreased urine output is a major indicator of a disturbed renal system. Analysis of the current study revealed that only 36% of patients knew renal

diet. A study was conducted by Karavetian et al. (2015) showed that 15%-40% of patients knew renal diet. However, that knowledge increased up to 90% after providing education by a dietitian. This study also showed that the serum potassium levels of patients dropped significantly after providing them knowledge about restrictions in renal diet (Karavetian et al., 2015). A study conducted by Betz et al. (2021) showed that the knowledge of patients regarding electrolyte restrictions was low and has no significant association with their intake of restricted items. The patients who knew and those who did not knew about restrictions were equally consuming a high amount of restricted items. The intake of phosphorous was 59-70% high and that of sodium was 67-91% high (Betz et al., 2021). Another study also reported similar to our findings that there is little knowledge in the renal patients were found regarding diet (Srinivassan, 2014). The current study also analyzed that the patients were having very little knowledge about electrolyte restriction and were consuming a high electrolyte diet on daily basis. The study showed that only 12% of patients knew about salt restriction and 16% about phosphorous restriction. Hoever, Vulpio et al. (2021) reported that advising in nutrition has ability to improve with renal failure on maintenance hemodialysis (MHD). So, MHD patients' knowledge about nutrients and subsequently dietcomposition and phosphate intakewas needed to improve.

CONCLUSION

The study concluded that chronic renal failure was more prevalent in males as compared to females and genetic factor was a common risk factor of renal failure. The majority of patients were unaware of the renal diet and electrolyte restriction in diet. Most of the patients were consuming a low caloric diet due to lack of appetite and were underweight. So, there is need to aware patients regarding improvement in diseases while using appropriate diet is very important.

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Effectiveness of Face Masks and Respiratory Aid Devices for Prophylaxis against COVID-19

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ABSTRACT: *The COVID-19 epidemic was spread rapidly around the world in 2019, and has greatly affected people's general interaction, their work habits, daily lives and businesses. A personnel protection against the spread of COVID-19 is not only beneficial but is critically important especially for those working in medical fields. Current studies were performed to review the use and benefits of face mask and respiratory aid devices in order to control the spread of COVID-19. The respiratory aids devices (RADs) may be recommended as anti-COVID-19 masks rather than surgical masks. However, RADs having P and R series filters do not prevent the infiltration of coronavirus due to its smaller size as compared to the filtration capacity of filters. The N99 and N100 filters cause a difficulty in breathing; it is difficult to wear such sort of respirators for a longer time. N95 respirators are recommended by medical professionals as they provide lesser (almost half) respiratory resistance. However, wearing of face masks is also involved some risks and side effects which may include physiological and disturbing effects, difficulty in breathing, affecting the volume and quality of sound, decrease of innate immunity, cardiopulmonary overload and increase in contamination chances due to development of humid habitat inside the mask.*

Keyword: *COVID-19, Epidemic, Virus, Respiratory aid devices, Face Masks*

INTRODUCTION

Currently, a lot of challenges are faced to the environment for which various suitable treatment technologies are being adopted (Ambreen et al., 2019; Rehman et al., 2019; Hussain et al., 2021) and widely investigated (Chaudhari et al., 2019; Iqbal et al., 2019; Ullah et al., 2019). The global outbreak of coronavirus disease 2019 (COVID-19) has affected every part of human lives and has shown numerous adverse effects on the climate and environment. The measures taken to control the spread of the virus and the slowdown of economic activities have significant effects on the environment. Other negative consequences of COVID-19 also include the disposal of gloves, masks and disinfectants and burden of untreated wastes continuously endangering the environment (Rume and Islam, 2020). The COVID-19 infections, caused by anovel coronavirus, were first time accounted for in December 2019 in the city of Wuhan, China (Hou et al., 2020). The World Health Organization (WHO) has described this disease as "serious intense respiratory plot COVID 2" (SARS-CoV-2) (Organization, 2020). WHO has announced the SARS-CoV-2 as a pandemic illness which is adversely affecting masses around the globe (Castagnoli et al., 2020). Particularly, old-aged people with affected immune systems are more prone to the disease caused by the coronavirus (Corse et al., 2020; Sinclair and Abdelhafiz 2020; Stankovska et al., 2020). According to the worldometer figures, the COVID-19 has reached to almost 223 countries of the world with 229,835,231 confirmed cases of infections and 4,713,636 reported deaths till September 21, 2021 (Annaka, 2021). People are using face masks and some other respiratory aid devices as a shield to keep themselves protected against the COVID-19. Mostly, face masks are fabricated from cotton and nonwoven fabric. The diameter of fibrous layer of the face masks is in the microns range (Li and Gong, 2015). Activated carbon is also being employed in different respiratory aid devices for the said purpose (Gupta et al., 2020; Rasheed et al., 2020).

Current studies were performed to overview the effectiveness of face masks and different respiratory aid devices to prevent the spread of COVID-19.

RESULTS AND DISCUSSION

Masks and respirators as protective tools against COVID-19

Due to their different resistance to the fluids, surgical face masks are

categorized into 80, 120 and 160 mm Hg (Park, 2020). Surgical face mask is a loose fitting and a disposable device which provides a barrier and separation from the nose to mouth of a user and thus provides protection against the coronavirus (O'Dowd et al., 2020). Respirator is another medical device and is different from the mask. The respirators are fabricated from a non-woven fabric that is comparatively thick in size (Li and Gong, 2015). Respirators are utilized to sift out airborne particles such as microorganisms and thus infections by producing a seal around the nose and mouth. They are employed when viral and dangerous particles or fumes are present in the environment. The US National Institute for Occupational Safety and Health (NIOSH) has classified Sifting face-piece respirators (SFRs) into nine different categories(as shown in Table 1) (Dorman, 2014). Fig. 1 demonstrates the importance of using face masks to minimize the exposure to infectious aerosol particles (Chatterjee et al., 2020).

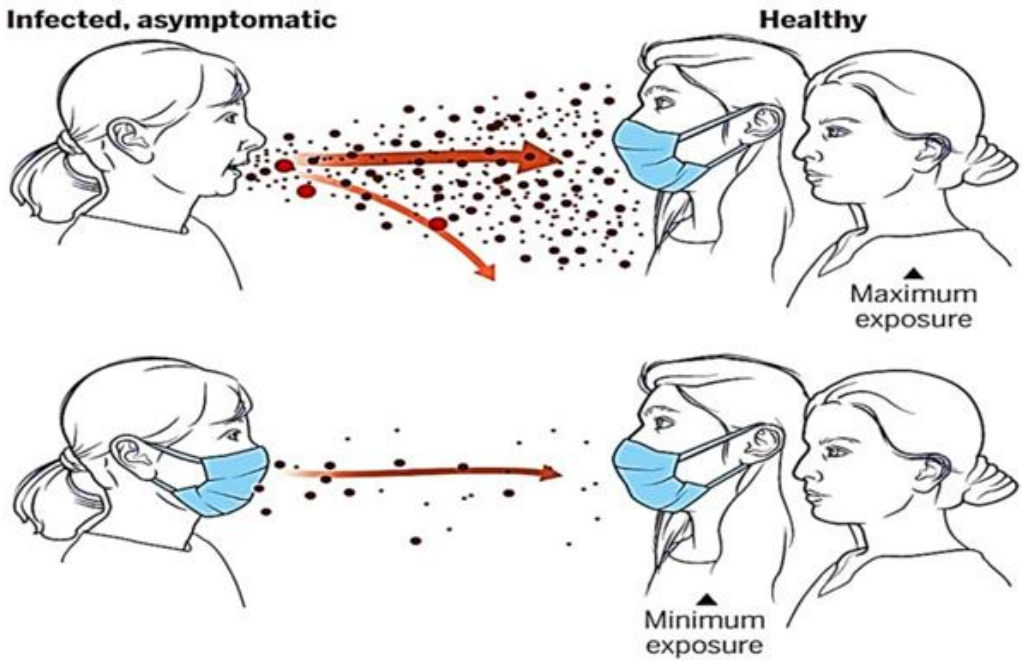

Fig. 1. Use of face mask helps preventing the spread of airbornetransmission of viruses (Chatterjee et al., 2020).

Depending on the aerosol type utilized in testing, filter series is divided into "N", "R", or "P" categories. A "95", "99", and "100" class efficiency is labeled against the 95%, 99% and 100% removal of aerosol particles. N-type

filters are tested against a mildly degrading NaCl aerosol to ensure the protection of workers from solid particles. R-type filters are tested against a highly degrading dioctylphthalate (DOP) oil aerosol to check their resistance against liquid particulates. P-type filters demonstrate high resistance to degradation and are therefore examined against DOP until their performance (in terms of filter efficiency) reaches at its lowest (Safety and Health, 1995). The respirators present in N-series can filter up to 0.079 ± 0.020 μm particle size and the P-series and R-series respirators block the particles having size up to 0.180 ± 0.020 μm (Dorman, 2014).

Table 1: NIOSH's Certified Respirators (Dorman, 2014)

Respirator	Filtered particles of Aerosol	Contradict to Oily substances	Minimal Particle (μm)	Service Life (hour)
P95	93%	Strongly	0.180 ± 0.025	40
R95	93%	Weakly	0.180 ± 0.025	8
N95	93%	Do not	0.079 ± 0.025	8
P99	97%	Strongly	0.180 ± 0.025	40
R99	97%	Weakly	0.180 ± 0.025	8
N99	97%	Do not	0.079 ± 0.025	8
P100	100%	Strongly	0.180 ± 0.025	40
R100	100%	Weakly	0.180 ± 0.025	8
N100	100%	Do not	0.079 ± 0.025	8

Spreading phase of coronavirus

The COVID-19 is mostly transmitted through little droplets or aerosol, but in many cases the virus is transmitted by a direct interaction with contaminated surfaces or infected bodies (Feng et al., 2020; Zuo et al., 2020; Azimi et al., 2021; Ram et al., 2021). Both ways of transmission of COVID-19 are considered when personal protections are taken care of (Tang et al., 2006). WHO reported that sneezing of a patient yields up to 45,000 droplets nuclei having a diameter range of 0.4-13 μm size (Borak, 2020). Such a tiny droplet can be transmitted with the speed of 100m/s (Simonds, 2020). These tiny droplets come from a patient's mouth or nose by coughing or sneezing and transmit directly to

ahealthy recipient (Sheriff, 2021).The transmission of droplets depends on their sizes and the distance between the recipient and the source. To minimizethe spread and transmission of coronavirus, a minimum of six feet distance is suggested between the source and the recipient (Tang et al., 2006; Chartier and Pessoa-Silva, 2009; Chigurupati et al., 2020).

A study by the National Institutes of Health (NIH) concluded that the COVID-19 viruses remain stable for a long time on the surface of aerosols present in the environment (Fears et al., 2020; Ren et al., 2020; Yang et al., 2020). The COVID-19 can also be transmittedthrough the eyes of an infected person (Chu et al., 2020; Manigandan et al., 2020; Sadhu et al., 2020). A dire need was felt for the detailed studies on the mode ofactual infection caused by the COVID-19 disease (Han and Yang, 2020).

Indications for the surgical face masks

Considering the situation of COVID-19 pandemic, a surgical face mask wearing must provide protection tothe followings (Worby and Chang, 2020):

- Persons having indications of respiratory infections and (or) confirmed COVID-19.
- Health care workers (HCWs), medical transport individuals and first-aid persons who somehow came into contact with any of the individuals mentioned above.
- HCWs having exposures to those with indications of respiratory infections.

In healthcare settings, the non-ill fraction of the population is not supposed to wear surgical masks to avoid from the above infections (Buckrell et al., 2020). Considering the spread of coronavirus and staying of population in isolation, the face mask wearing could provide an additional measure other than physical distance, hand hygiene and barrier measure (Bekele and Yitayih, 2020; Howard et al., 2021; Liao et al., 2021). In such situations, a standard cloth made mask is preferably used. A proper use of a mask by an asymptomatic person may reduce greatly the probability of virus transmission as a mask protects the wearer's surroundings. However, the chances of infections are still there if a healthy individual comes into a close contact with a person having respiratory signs (Howard et al., 2020). Table 2 summarized the situations under which different surgical face masks are recommended by health professionals (Lepelletier et al., 2020).

Table 2: Surgical facemask suggested by medical professionals (Lepelletier et al., 2020)

Professionals	Care situation	Indicating for wearing mask	Types of Masks		
		Patient Infection Status	Patients Surgical	Professionals Surgical	FFP2
COVID-19[I]	Aerosol Generating Procedures (AGPs) and invasive care[II]	Symptomatic patients[IV] having symptoms	✓	✓	
Pharmacists		Patients having a risk of severe type of virus[III]	✓	✓	
Pharmacy dispensers		All symptomatic patients[IV] regardless of infectious status	✓	✓	
HCWs [I]	Simple procedures including	An infected patient having arisk of severe infection of COVID-19[III]	✓	✓	
	Consultations but excluding	Infected patients[IV] regardless of infectious COVID-19[III]	✓	✓	
	AGP sand invasive care[I]	COVID-19 patients			✓
	AGP sand invasive cares[II]	All patients having infection of virus	✓	✓	

[I]Doctors, dentists, nurses, midwives, masseur-physiotherapists, ambulatory medicine and medico-social establishments and in healthcare settings

[II]Non-invasive ventilation, bronchofiberscopy, tracheal intubation, endotracheal suction, invasive open expiratory ventilation, nasopharyngeal sampling, autopsy, aerosol therapy, aerosol-generating chest physiotherapy, causing sputum, etc.

[III]hypertensive, diabetic.

[IV]Respiratory infection symptoms: cough, sneeze, fever etc.

Effectiveness of surgical face mask against COVID-19

The face masks fabricated from non-woven material haveonly 33.1% filtration capacity against the particles ofsize 2.5 μm ($PM_{2.5}$). The size of coronavirus is almost 0.1 μm (Das et al., 2020; Wu et al., 2020; Klumpp and Poudel, 2021) which is approximately less than the size of $PM_{2.5}$. In addition, the Centers for Disease Control and Prevention (CDC) indicated that since these materials are not effective against SARS-CoV-2, therefore even a careful coveringcannot effectively prevent the virus from escaping (Wuthisuthimethawee and Khorram-Manesh, 2021).

Effectiveness of respiratory aid devices forCOVID-19

The respiratory devices having Pand R series filters do not preventthe infiltration of coronavirusdue to its smaller size as compared to the filtration capacity of filters. The N99 and N100 filters cause adifficulty in breathing; it is difficult to wear such sort of respirators for a longer time. N95 respirators are recommended by medical professionals as they providelesser (almost half) respiratory resistance (Paxton et al., 2020). Compared to ~ 36% of surgical masks, the filtration capacity of N95 is approximately 95% (Roberge, 2008). Another study found that the maximum inhalation rate through N95 can produce less than 90% of the filtration capacity of a 0.25 micron virus aerosols (Tcharkhtchi et al., 2021). In another study, it has been shown that the N95 masks are highly effective against *E. coli* T4 virus, bacteriophage MS2 and *Bacillus subtilis*. The size of MS2 ranges from 0.03 to 0.08 μm, where 0.1 μm represents the flow size of *E. coli* T4 and *Bacillus subtilis* viruses. The sizes of T4 and *Bacillus subtilis* are ideal for COVID-19 case studies. For a normal inhalation flow rate, Q (30 liters / min), the percentage penetration (PP) values for T4 and *Bacillus subtilis* are 0.23 and 0.58, respectively (Fig. 2 and Fig. 3). For heavy duty, the suction flow (85 L/min) of T4 and the PP value of *Bacillus subtilis* are 0.95 and 1.9, respectively (Mahdavi, 2013).

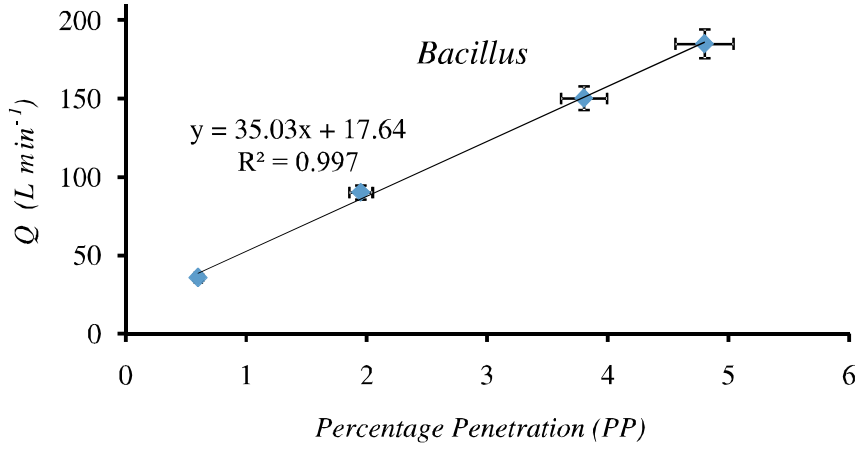

Fig. 2. A graph between the percentage penetration, PP and the inhalation flow rate, Q for *Bacillus subtilis* virus (Mahdavi, 2013)

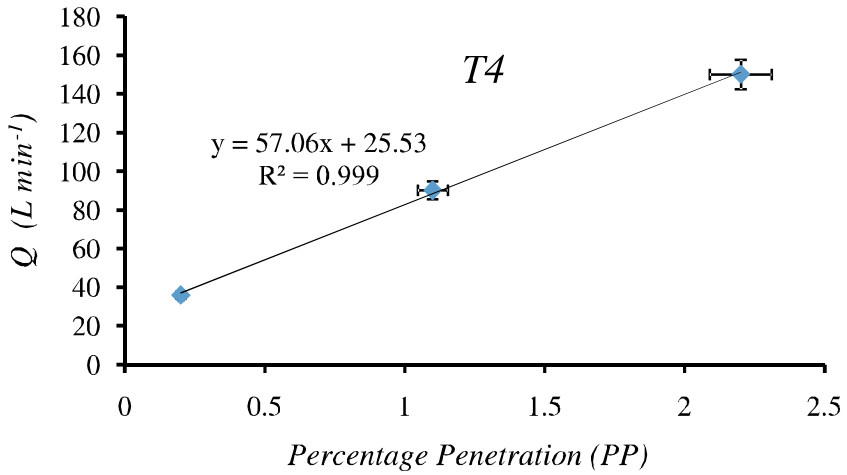

Fig. 3. A graph between the percentage penetration, PP and the inhalation flow rate, Q for the T4 virus (Mahdavi, 2013)

The average human respiratory rate is 60.9 g/min or 12 kg/day (Lepelletier et al., 2020). Statistical analysis is important when calculating PP based on average human respiratory rate. The regression line presented (Fig. 2 and 3) allows us to conclude that under normal rates of human respiration, penetration of *Bacillus subtilis* and T4 aerosols is not observed. However, there is no adequate clinical study on the safety of surgical and anti-COVID-19 masks, but another study has been concluded that such type of surgical masks was tested against influenza virus (which is similar to COVID-19). The department of Medical Research of Imperial University suggested that the use of

such respirators or respiratory aids can reduce the possibility of COVID-19 disease (Ortiz-Prado et al., 2020). When a patient sneezes, 0.5 μm nuclear droplets are formed which, because their size is greater than the filtering capacity of the surgical mask, can penetrate the outer surface of the surgical mask due to their low filtering capacity (the filtering capacity of surgical masks is only 36%). On the other hand, respiratory aids have an ability to block virus aerosol particles, thereforethese respiratory devices may be recommended as anti-COVID-19 masks rather than surgical masks.

Sideeffects of wearing the face masks

With the important role of face masks in prevention of COVID-19, its use (wearing) is also associated with some health risks. The volume and quality of sounds between people wearing masks is affected and people may come unconsciously closer to each other. Moreover, the exhaled air may go into eyes due to face mask so an impulse is created to touch the eyes with hands. Consequently, if contaminated hands are touched with eyes, then infection chances are more increased. The face masks may also make breathing process difficult (Kyung et al., 2020). In addition to this, some exhaled carbon dioxide may also go back along with inhaled air into the lungs in each successive respiratory cycle. It may enhance the breathing frequency and as a result, the infected people wearing masks may spread more contaminated air; the enhanced breathing may also push the viral load deep into the lungs (Chen et al., 2018). When a person weaning the face mask continuously breaths then water vapors provided continuously by breathing may develop a humid habitat inside the mask, which may further increase the viral load and other infections as well as decrease of innate immunity (Potts et al., 2006). The use of face masks has also been reported to be associated with some physiological and disturbing effects. Studies were conductedon 100 healthy volunteers in a tertiary hospitalon September 2020 and January 2021. The individuals having smoking habit, cardiopulmonary disease and impaired walking, were not considered for this study. To evaluate the mask uncomfortableness, the persons with and without surgical masks were given a six-minute walking test (6MWT). It was demonstrated in persons wearing surgical masks during 6MWT that end-tidal carbon dioxide (EtCO2), heart rate (HR) and respiratory rate (RR) were significantly increased ($p<0.001$) whereas SpO2 level ($p=0.002$) and

walking distance ($p<0.001$) were decreased. In Mask-Discomfort Questionnaire, itching ($p=0.001$), fatigue ($p<0.001$), odour, salinity, resistance, temperature, and humidity scores were enhanced after 6MWT with mask. It was concluded that cardiopulmonary overload may be caused by masks (Dirol et al., 2021).

CONCLUSION

From the above discussion, it can be concluded that the surgical face masks are not very effectiveto stop the spread of viral coronavirus. They can only be helpfulagainst bacterial infections and surgical wounds. The respiratory aid devices (including N95) offer low inhalation rate and therefore can provide a better shield against the spread of viral coronavirus. A use of full-face respiratory aid device is highly recommended in areas where the chances of spread of coronavirus are high (such as in hospitals and in other medical fields). Eye protection is also extremely important for the health field workers to avoid direct interaction with the infected bodies while dealing with such type of patients. The RADs having P and R series filters do not prevent the infiltration of coronavirus due to its smaller size as compared to the filtration capacity of filters. The N99 and N100 filters cause a difficulty in breathing; it is difficult to wear such sort of respirators for a longer time. N95 respirators are recommended by medical professionals as they provide lesser (almost half) respiratory resistance. However, wearing of face masks is also involved some risks and side effects which may include physiological and disturbing effects, difficulty in breathing, affecting the volume and quality of sound, decrease of innate immunity, cardiopulmonary overload and increase in contamination chances due to development of humid habitat inside the mask.

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DPPH Assay and Reducing Power Activity of Water Extract of (*Mentha longifolia*) Mint

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ABSTRACT: *Antioxidative properties of plants may be associated with oxidative stress defence in different human diseases. Oxidative stress and free radicals can be neutralized by antioxidants which are of great significance in preventing the expansion of these diseases. Many studies have investigated the toxic effect of synthetic antioxidants, thus to avoid these toxic effects new antioxidants of natural origin have been deliberated in recent years. Mint (Mentha longifolia) is extensively used as medicine, spice, food and flavouring. In the current work, an aqueous extract of mint leaves was determined using the DPPH assay and reducing power activity. Strapping and sound antioxidant effects were observed in 2,2-diphenyl-1-picrylhydrazyl radicals at concentrations of 0.1-0.5 mg/ml, ranging from 20.32 ± 0.35 % to 65.75 ± 1.5 %, while standard antioxidant BHT possess % Inhibition (DPPH) 30.41 ± 0.65 % to 83.50 ± 2.3 % at same concentration. A similar effect was found in the reducing power assay, which exhibited absorbance of M. longifolia water extract ranging from 0.1501 ± 0.010 to 0.5845 ± 0.042 and BHT exhibited 0.3221 ± 0.026 to 0.8197 ± 0.124 at 700 nm. The conclusion recommends that M. longifolia has confirmed vital benefits due to high concentration of antioxidants and has vast impending for claim in the preparation of useful food entities.*

Keyword: *M. longifolia, water extract, antioxidant activity, DPPH assay, RPA assay*

INTRODUCTION

More recently, dietary supplements made from various fruits, vegetables and herbs have emerged as a natural therapeutic and protective strategy to prevent disease and poor health. In developing countries, there is insufficient information on the nutritional value of several native plants, which may be beneficial for different

purposes. Herbal plants and their natural extracts can offer promising treatments for different diseases (Karimian et al., 2013; Kozłowska et al., 2021). Mint (*Mentha longifolia*) belongs to the family *Lamiaceae* and in temperate regions of the world widely grown (Fig. 1) (Hussain et al., 2010). Mints have traditionally been used for centuries as flavorings, condiments, herbal teas, fresh vegetables, infusions, decoctions and distillates (Dzamic et al., 2010; Krzyzanowska et al., 2011). They are also used as carminative, breath fresheners, anti-infective, choleretic, anti-inflammatory, antispasmodic, anticatarrhal, antiallergic laxative, diuretics, gastric tonics and antinociceptive (Zeinali et al., 2005; Naghibi et al., 2010). Therefore dried and fresh mints are used widely used in herbal medicine to treat a variety of health problems and discomforts (Younis et al., 2004; Sharopov et al., 2012).

Fig. 1. *M. longifolia* leaves and powder

(https://www.indiamart.com/proddetail/mint-leaves-powder-21944690991.html)

It is thought that free radicals trigger oxidation, which contributes to number of diseases in human being (Ścibisz et al., 2021). Antioxidants are a group of compounds that protect cells from free radicals and can help reduce different diseases such as aging and cancer (Spréa et al., 2020). Plant antioxidants are important because their presence in the human diet can help the body neutralize free radicals and reduce oxidative stress damage. In contrast, synthetic antioxidants may have carcinogenic effects (Suhaj, 2006; Tajner-Czopek et al., 2020). Various studies depicted that mint extracts have potent antioxidant properties (Gulluce et al., 2007; Mkaddem et al., 2009; Kadhim et al., 2020).

Therefore, this study was conducted to appraise the antioxidant potential of aqueous extract of *Mentha*

longifolia (Mint) leaves. DPPH assay and reducing power of activity were performed to check the antioxidants potential in water extract Mint leaves powder.

MATERIALS AND METHODS

Plant Material and Pretreatment

The leaves of *M. longifolia* had been collected from the local market. In order to remove all traces of dust and insects, the leaves were rinsed under tap water and cleaned. Afterword; the plants Leaves were squeezed, dehydrated at 50-60°C in dehydrator for one day. Then it was ground in grinder mill to convert it in to a powder form with a size of 80 meshes. The dried *Mentha longifolia* leaves were kept in airtight bottles to be used for extraction (Mohammed et al., 2020).

Chemicals and Reagents

2,2′-diphenyl-1-picrylhydrazyl, $NaHPO_4$, NaH_2PO_4, $FeCl_3$, $K_3Fe(CN)_6$ and TCA were purchased from Sigma Chemical Co. In this study all other chemicals were analytical grade.

Preparation of extracts

The ethanol extract of the aerial parts of was obtained using maceration method. An amount of 20 g *M. longifolia* powder was extracted using 200 mL of water. The extraction was performed by shaking at room temperature during 12 h. Finally, the extract was passed through a paper filter and the filtrated solution was concentrated by a rotary evaporator at 40°C.

DPPH Assay

The water extract of *M. longifolia* was subjected for dogged of antioxidant activity based on free radical scavenging activity using DPPH assay as described by Brand-Williams 1995 with some modifications (Saeed et al., 2021). Mix 0.1 mL aliquots of extraction solution (1-5 mg/ml) with 3.0 ml DPPH (0.004% in methanol). The mixture was shaked vigorously and incubated for 1/2 hour at 25°C and water used as a control. By using a spectrophotometer UV-Vis (1700, Shimazdu, Japan) the absorbance at 517 nm of water extract of *M. longifolia* was deliberated. Results were expressed as % inhibition and calculated by following formula.

% Inhibition = $A_{control}–A_{sample}/A_{control} \times 100$

Reducing power activity

Oyaizu (1986) method was used for the determination of the reducing power activity of water extract of *M. longifolia* with minor modifications (Liu et al., 2012). 1 ml of water extract of *M. longifolia* at 1, 2, 3 ,4 and 5 mg/ml

concentrations was mix with phosphate buffer (pH 6.6) of 2.5 ml (0.2 mol/l) and 2.5 ml of 1% $K_3Fe(CN)_6$. Incubated it for 20 min at 50 °C and stopped the reaction by addition of 10% tri-chloro-acetic acid (2.5 ml) and for 10 min the mixture was centrifuged at 6000 g. Finally mix the upper layer 2.5 ml with 2.5 ml H_2O and 0.5 ml (0.1% $FeCl_3$) and absorbance was measured at 700 nm by using spectrophotometer (UV-Vis: 1700, Shimazdu, Japan).

Statistical analysis

Data were statistically analyzed using SPSS v.16.0 and is presented as mean ± standard deviation (SD). All experiments were performed in triplicate.

RESULTS AND DISCUSSION

Plant extracts, which contain different classes of compounds such as polyphenol, flavonoids, anthocyanins, are very important source of antioxidants for the food industry. Therefore, in this study we investigated antioxidant properties of *M. longifolia* water extracts as a source of natural antioxidants. Lamiaceae family plants are rich in antioxidant compounds and a large number of which, like sage, oregano, thyme, basil and mint exhibit strong antioxidant activity (Ozgen et al., 2006; Zeljkovic et al., 2021).

Antioxidant activity by DPPH assay

Diphynyl-picryl-hydrazine is a stable free radical and has been extensively acknowledged as a tool to appraise the antioxidant activity of extracts. It is a simple, rapid, reliable and a widely used assay which is sensitive to some Lewis bases and requires organic solvents and non-physiological radicals (Sirivibulkovit et al., 2018). The ability of *M. longifolia* water extract to donate proton to DPPH free radical and change its color from violet to yellow is accessed in this assay (Benkhaira et al., 2022). In Fig. 2, mint water extract of 0.1-0.5 mg/ml were prepared to evaluate the antioxidant activity. The method relies on the reduction of violet to yellow diphenyl-picryl-hydrazine and the scavenging DPPH (Katalinic et al., 2006; Ghafoor et al., 2010). The respective scavenging capacities were ranged from 20.32± 0.35% to 65.75 ± 1.5 %. A dose-dependent approach was pragmatic in the antioxidant activity of mint water extract. The highest % inhibition was 65.75 ± 1.5% at 0.5 mg/ml, indicating its considerable scavenging capacity. These findings are in agreement with previous works on the genus *Mentha* (Ahmad et al., 2012; Naqvi et al., 2018). According to relatively high amounts of phenolic compounds in *M. longifolia* and their

antioxidant potential which were confirmed using several *in vitro* assays, this species could be considered for possible applications in food industries (Sun et al., 2014; Abbood et al., 2020).

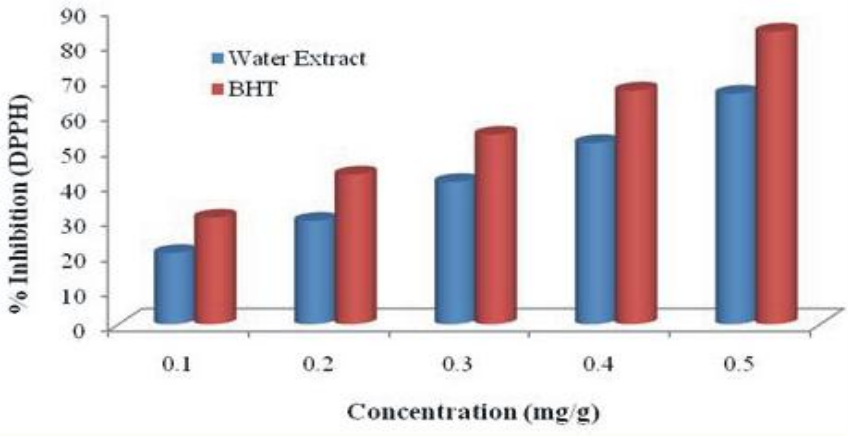

Fig. 2. % inhibition of water extract of *M. longifolia*

Reducing power assay

This assay used to measure the transferring capacity of Fe^{3+} to Fe^{2+}, which then reacts with $FeCl_3$ to form blue colored $(Fe^{3+})_4$ $[Fe^{2+}$ $(CN^{-})_6]^3$ complex that has an absorption maximum at 700 nm. The reducing power was correlated with electron transfer ability of the sample. Increased absorbance of the reaction mixture indicated increased reducing power of the plant extract (Mohamed et al., 2022). Reducibility is usually related to the occurrence of reducing agents. The reducing power effect of reducing agents is based on infringement free radical chains by donating a H°. Figure 3 depicted the reducing power of water extract of mint and it exhibits absorbance ranging from 0.1501 ± 0.010 to 0.5845 ± 0.042 and BHT exhibited 0.3221 ± 0.026 to 0.8197 ± 0.124 at 700 nm (Table 1). This reducing power activity of mint water extract was also depended upon the dose, higher the dose or concentration higher the absorbance (antioxidant activity). These finding suggested that *M. longifolia* water extract possesses significant reducing power due to the presence of secondary metabolites. These results are consistent with prior research on *Mentha* species (Nickavar et al., 2010; Tahseen et al., 2013; Wafa and Sofiane, 2020).

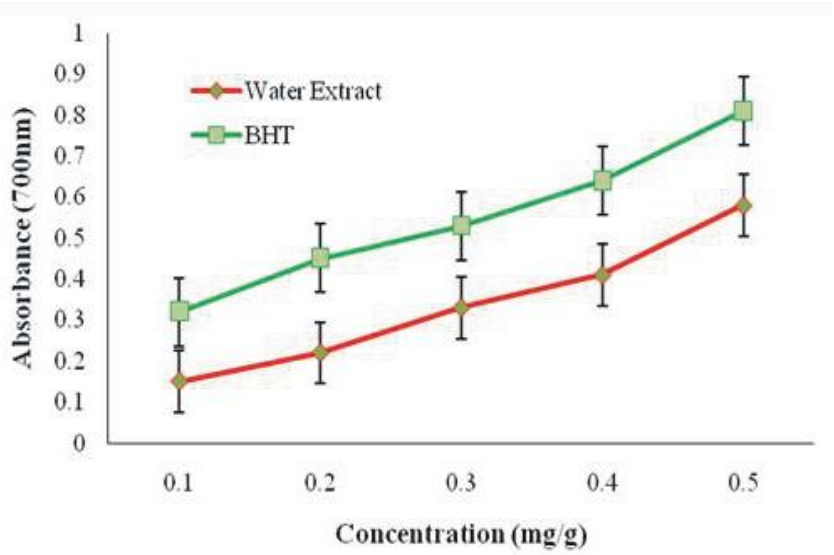

Fig. 3. RPA of water extract of *M. longifolia*

Table 1: Reducing Power Activity of water extract of *M. longifolia*

Sr. No	Concentration (mg/g)	Absorbance (700nm) H_2O Extract of *M. longifolia*	Absorbance (700nm) BHT
1	0.1	0.1503 ± 0. 010	0.3221 ± 0. 026
2	0.2	0.2211 ± 0. 012	0.4526 ± 0. 032
3	0.3	0.3350 ± 0. 015	0.5307 ± 0. 038
4	0.4	0.41233 ± 0. 218	0.6439 ± 0.105
5	0.5	0.5845 ± 0.042	0.8197 ± 0. 124

Data are represented± standard deviation

CONCLUSION

Mentha longifolia is drawn to traditional herbal medicine for health promotion and disease prevention. The main purpose of this study was to assess the antioxidant activity of an aqueous extract of *M. longifolia*. The results of this investigation indicated that *M. longifolia* water extract provides considerable antioxidant activities in these both DPPH & reducing power assays. Moreover, they may be used in pharmaceutical and natural therapies for treatment of oxidative stress.

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Association of Insomnia with Comorbidities among Patients Visiting Tertiary Care Hospitals Lahore

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ABSTRACT: *Insomnia is a condition that affects an individual by inability to get sufficient quality sleep or problem in maintaining sleep that is important for healthy functioning, performance and wellbeing. There is very scarce data regarding insomnia and its relation with different diseases. The present study was aimed to find out the association of insomnia with comorbidities among patients visiting Tertiary Care Hospitals, Lahore. A cross sectional study was conducted from May 2019 to September 2019 in Tertiary Care Hospitals district Lahore. Interviews were conducted by researchers to collect data through Pre-Tested Questionnaire. Informed consent was first taken from all participants. Data were analyzed by using Chi-square to find out the association of insomnia with comorbidities through SPSS version 25.0. Thousand (n=1000) patients suffering from different diseases randomly selected to participate, of which 43.6% were male and 56.4% were female. Results of present study revealed that association of insomnia with the diseases such as cardiovascular diseases (p=0.05), diabetes mellitus (p=0.001), gastrointestinal problems (p=0.034), premenstrual syndrome (p=0.00), joint pains (p=0.01) and prostate cancer (p=0.01) was found to be significant. While an insignificant association of insomnia was noticed with hypertension (p=0.850), hearing impairments (p=1.22), COPD (p=0.288). It was concluded that insomnia was most commonly present in the patients of cardiovascular diseases, diabetes mellitus, premenstrual syndrome, gastro-intestinal problems, joint pains and prostate cancer.*

Keyword: *Insomnia, Comorbidities, Cardiovascular Diseases, Diabetes Mellitus, Premenstrual Syndrome, Prostate Cancer, tertiary care unit.*

INTRODUCTION

Sleep is the essential component to maintain good health of an Individual (Chaput and Dutil, 2016). Insomnia is defined as a condition that affects an individual by inability to get sufficient quality sleep or problem in maintaining sleep that is important for healthy functioning, performance and wellbeing (Bos and Macedo, 2019). Insomnia can exist in healthy individual or may associate with other medical diseases (Araújo et al., 2017). It is described by trouble starting or potentially looking after rest, and is related with critical pain or daytime impedances, regardless of sufficient rest opportunity. Clinical analysis depends on the nearness of these abstract symptoms amid somewhere around three evenings for every week, for no less than three months (Fuller et al., 2016). It is spreading frequently among population and recognized as a serious health problem (Haaramo et al., 2014). Primary insomnia is disturbed sleep at initial stage with unknown cause, last for few days or weeks (Chaput and Dutil et al., 2016; Sake et al., 2019). Secondary insomnia lasts for more than a month and it may be because of any medical condition or it may lead to chronic diseases and other consequences such as CVD, hypertension (Taylor et al., 2005), dyslipidemia, diabetes, mood disturbance, day time impairment, depression (Javaheri and Redline (2017), heart failure, coronary heart disease, anxiety and recurrent acute coronary (Morin et al., 2012). Insomnia can influence at any age and gender (children, adults, women and men) but increasing with age in women more than men (Araújo et al., 2017; EtindeleSosso et al., 2017).

In reality, the majority of the psychological issues are brought about by brokenness in one or numerous physiological adjustment. Due to these dysfunctions, sleep impairments are created and their clinical result might be moderate or interminable. A solid way of life with customary physical action may guarantees a sound mind, and an amazing shield against both central nervous and peripheral disorders, and cognitive deterioration as well (Wickwire et al., 2019).

The prevalence of insomnia spans from 25-40% whereas, it is 9-12% approximately in the overall population. Moreover, there is an increase of insomnia in elderly people of the United States (Maust et al., 2017). There are 45% higher chances of insomniac patients suffering or dying from heart or heart vessel diseases. Approximately, 33% of 3,300 adult Australians have frequent problems falling or staying asleep (Kidwai and Ahmed, 2013). A total of 9% to 15% experience day time

consequences of insomnia and 8% to 18% are not satisfied with the quality of sleep and its duration. Primary insomnia spans at 2% and 4% in general population whereas, 1% to 3% prevalence of insomnia is due to psychological disorders. In Pakistan, according to a survey done at Agha Khan Hospital of Karachi, out of 1488 adults from 5 non rural and semi non rural areas of Karachi, 466 (31.3%) participants had insomnia out of which 141 (30.2%) were taking sleeping pills (Farazdaq et al., 2018).

The present study was aimed to identify the association of insomnia with comorbidities among patients which visited tertiary hospitals and also highlight the secondary factors that cause insomnia. Thus, after identifying the factors, awareness could be created through extensive health education to reduce the financial burden and severity of insomnia in the society.

METHODOLOGY

A cross sectional study was conducted through pre-tested questionnaire at The University of Lahore Teaching Hospital and Sir Ganga Ram Hospital, Lahore. Athens Insomnia Scale (AIS) was utilized to identify and categorized the insomnia among patients. A total of 1000 insomniac patients was selected suffering from different diseaseslike cardiovascular diseases (CVD), diabetes mellitus, prostate cancer, hypertension, chronic obstructive pulmonary disease (COPD), gastrointestinal problems, premenstrual syndrome, joint pains and hearing impairments attending different departments. All the participants wererandomly selected using non-probability convenient sampling technique. Interviewing techniquewith brief of questionnaires was used to collect the data from the participants. Among the participants 43.6% were male and 56.4% were female aged between 12 to 81 years. The study was conducted from May 2019 to September 2019 in Tertiary Care Hospitals district Lahore. Non cooperative and disabled (requiring attendant for the interview) were not selected for the study. Prior written informed consentswere taken from the participant.

Ethical Approval of the study

For this study ethical approval was taken from the institutional review board (IRB) of The University of Lahore, Lahore.

Statistical Analysis

Data were analyzed by using SPSS version 25.0. The qualitative variables were reported using percentages and frequencies. Frequencies were derived and chi-square was applied to find the association of insomnia with

comorbidities. *p*-value less than or equal to 0.05 was considered significant reference for all methodology.

RESULTS

Analysis revealed that 67.6% (n=676) of patients were with mild insomnia, 27.6% (n=276) were with moderate insomnia and 4.8% (n=48) have severe insomnia. The way by which mild and severe categories were identified shown in Table 1. Categorization was done according to the Athens Insomnia Scale.

Table 1: Categories of Insomnia among the participants

Categories	Frequency	Percentage
Mild	676	67.6
Moderate	276	27.6
Severe	48	4.8
Total	1000	100.0

The results showed that chronic diseases like cardiovascular diseases (CVD); p=0.05, diabetes mellitus; p=0.01 and prostate cancer; p=0.01, were having significant associations with insomnia categories. While hypertension; p=0.850 and chronic obstructive pulmonary disease (COPD); p=0.288 were having insignificant association with insomnia categories, as shown in Table 2.

Table 2: Association of insomnia with Chronic Diseases

Chronic Diseases		Categories of Insomnia			p-value
		Mild	Moderate	Severe	
CVD	Yes	93	583	676	0.05
	No	19	257	276	
Diabetes mellitus	Yes	120	21	7	0.01
	No	556	255	41	
COPD	Yes	61	21	7	0.288
	No	615	255	41	
Prostate cancer	Yes	4	6	3	0.01
	No	672	270	45	
Hypertension	Yes	178	76	14	0.850
	No	498	200	34	

As far as the non-chronic diseases were concerned gastrointestinal disorders; p=0.03, premenstrual syndrome; p=0.01, joints pains; p=0.01 were having significant associations with insomnia categories, while hearing impairment; p=0.12 had insignificant association with insomnia categories. Table 3.

Table 3: Association of insomnia with other Diseases

Other Diseases		Insomnia categories			p-value
		Mild	Moderate	Severe	
Hearing impairment	Yes	21	13	4	0.12
	No	655	263	44	
Gastrointestinal	Yes	139	68	17	0.03
	No	537	208	31	
Premenstrual syndrome	Yes	28	28	12	0.01
	No	648	248	36	
Joints pain	Yes	222	107	28	0.01
	No	454	169	20	

DISCUSSION

Insomnia along with comorbidities has and always been a major risk factor affecting the quality of life. Present study showed significant association between insomnia and cardiovascular disease (CVD) (p=0.05). Whereas according to Choi et al. (2018) there was no association between sleep quality and occurrence of CVD; however chronic sleep deprivation was associated with the risk of CVD. The current study revealed that there was significant association between insomnia and diabetes mellitus (p=0.01). Similar results were observed by a study and they investigated the risk of diabetes in patients with insomnia and without insomnia. It is stated that patients with insomnia had a higher risk of diabetes than patients without insomnia (Lin et al., 2018). Present study concluded significant association between insomnia and prostate cancer (p=0.01). These results were in accordance with the findings of Chung et al. (2019) also stated that significant association was

found between prostate cancer patients and sleep disorder patients. Current study showed that 62.6% patients were having mild insomnia among that 178 patients had hypertension, 27.6% patients were having moderate insomnia 76 of that were hypertensive and 4.8% were having severe insomnia out of which only 14 were hypertensive so concluded that association of insomnia with hypertension was insignificant (p=0.850). Whereas contradictory findings were observed from another study, reporting significant association of insomnia with hypertension, in which more than half of the patients were experiencing insomnia and 39% were experiencing daytime sleepiness (Uchmanowicz et al., 2019).

Another significant association was found between insomnia and gastrointestinal disease (p=0.034). Results were similar to the findings of Hyun et al. (2019) that sleep disturbances were associated with digestive symptoms especially with abdominal pains, acid regurgitation, abdominal distension and eructation (Hyun et al., 2019). According to the current study out of 1000 patients, 28 patients with mild insomnia reported premenstrual issues, in moderate category of insomnia 28 patients had premenstrual issues and in severe category of insomnia 12 had premenstrual issues, and a significant association was observed between insomnia and premenstrual syndrome (p=0.001). The findings are in accordance with the study by Jehan et al. (2016) which reported that women with PMS leads to an increase in the state of stress and anxiety, which caused sleep disturbance and affect the quality of life such as tiredness, fatigue and daytime sleepiness. Another important finding from current was that there was significant association between insomnia and having joint pains (p=0.01). Similar findings were observed by another study, that chronic joints and musculoskeletal pain increased the risk of insomnia whereas healthy lifestyle decreased the risk of insomnia and helps to maintain the quality of sleep (Skarpsno et al., 2018).

CONCLUSION

It was concluded that insomnia was most commonly present in the patients of cardiovascular diseases, diabetes mellitus, pre-menstrual syndrome, gastrointestinal problems, joints pain and prostate cancer. Insomnia was not most prevalent among the patients of hypertension, hearing impairments and COPD. However, detail of patients like age, family background, duration of suffering, use or not use of sleeping pills

and many more things must be recommended to investigate in future study.

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Incidence of Psychiatric Disorders in Children affected with Intellectual Disability

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ABSTRACT: *Intellectual disability is caused by environmental influences, genetic disorders and infections including perinatal, postnatal and neonatal infections. Physical disabilities are also associated with intellectual disability (ID) children. Identification of ID patients occurred by test of intellectual disfunctioning. A cross sectional and analytical study was performed from September 2019 to March 2020 on intellectually disabled children on the Pakistan Air Force (PAF) children school Lahore, Fountain house in Sargodha and Shadab institute of special education, Punjab, Pakistan. The level of intellectual disability was based on the intelligence quotient (IQ). In present study, out of 200 children, 80 children were suffered from this disease, 56 were males and 24 were females. Cases related to intellectual disability were identified with in different age groups such as age group 0-5 (N = 13), 6-10 (N = 27), 11-15 (N = 27), 16-20 (N = 10), 21-25 (N = 3) and 26-30 (N = 3). A total of 33.75% were belong to 6-10 years of age group. Out of 80 children 27.50% (N = 22) were affected due to malnutrition in pregnancy, 16.25% (N = 13) children had folic acid deficiency, 15% (N = 12) children were due to exposure to the chemicals or radiations in pregnancy, 21.25% (N = 17) children had hypothyroidism and 16% (N = 20) children had other gene disorder associated with intellectual disability. Proper recognition of disorder can play an important role in dealing with psychiatrists patients.*

Keyword: *Intellectual Disability, Malnutrition, hypothyroidism, Radiation and Physical Disabilities*

INTRODUCTION

Intellectually disability defined as a state of abnormality in mental conditions that is frequently linked to various medical and psychological illnesses including emotional and

behavioral disorders such as Down syndrome, fragile X syndrome, attention defect hyperactivity disorder and autism with cerebral palsy (Lee et al., 2019). It is not rare for persons with ID to have coexisting psychological illnesses. In the field of psychiatry, where the research of intellectual impairment is involved, dual diagnoses have long been prevalent (Lakhan, 2013).

In view of American Association of Intellectual and Developmental Disabilities, ID involved limited adaptive behaviour (AAIDD 2010). Age allowed for diagnosis for autism spectrum disorder was changed by current issue of DSM (Diagnostic and Statistical Manual of Mental Disorder), with precise age of onset at 3 years of age being excluded (Grzadzinski et al., 2013). There is no link between sex and the occurrence of a coexisting disease. Oppositional defiant disorder (ODD) was shown to be more common in children under the age of six (51.9 percent versus 41.1 percent, p=0.007), but no other significant age-related relationships were found (Lecavalier et al., 2019).

Persons with intellectual disability had more chances to develop psychiatric disorders with respect to general population and to have viral or infective diseases (Lakhan, 2013; Kendall and Owen, 2015). While, they have less risk to get cancer and musculoskeletal disorders than general population (Liao et al., 2021). Due to a lack of comparable data, it was impossible to differentiate between mental and behavioral illnesses. Despite this, some percentage of intellectual disabled got a mental health disorders at some point in lives (Hatton and McMahon, 2020).

Signs of mental illness along with intellectual disability do not reflect psychiatric disease in general population. So, identification of psychiatric problems is mainly diagnosed in intellectual disabled children and became field of interest. Furthermore, in children with ID, weak cognitive ability may obscure other psychiatric illnesses, hindering appropriate identification of comorbid diseases (Lakhan, 2013). In past, it was commonly assumed that people having intellectual disability could not have disorders of mental health, but the increased incidence of mental health problems among intellectual disabled are now well known. Despite the fact that claimed prevalence rates fluctuate, population report of intellectual disabled have consistently shown comorbid mental health disorders of 30–40% (Reardon et al., 2015).

The evidences of shared genetic contributions and common phenotypic traits between schizophrenia and intellectual impairment as well as other neuropsychiatric illnesses such as autism and epilepsy are increasing. There has long been evidence of a relationship between schizophrenia and intellectual impairment. Intellectual incapacity, according to Kraepelin, the cause of 3.5 percent of instances with early-onset dementia praecox, also known as Pfropfschizophrenie (Prinzio et al., 2018).

This research determines the children's incidence with psychiatric problems along intellectual disability and also without intellectual disability. Intellectual disabled patients are mostly diagnosed behind the time as our population is unaware about intellectual disability. It must provoke the patients of intellectual disability to manage their daily life affairs by themselves. The study aimed to provides information that can be used for planning of treatment in health department, careful clinical evaluations, government approvals and subsequent development and treatment.

MATERIALS AND METHODS

Intellectually disabled Children were identified by looking in PAF children school, Shadab institute of special education Sargodha and Fountain house in Sargodha, Punjab, Pakistan. The study period was from 2019 to 2020. We have studied approximately 80 children of intellectual disability. We had also studied 5 families with three affected individuals.

Performas were generated to get very important date for the postal address, disease recognition and diseases present condition for children. The questionnaires were used as the design for recognition of serious cognitive, seizure, physical, learning, hearing and vision impairment among children, in visiting diverse population of Sargodha. Some of the questions emphasized on cognitive abnormality while some related to movement disorders including vision, hearing problems too. It was also converted from English to urdu. After that it was given to guardian of child, mainly parents during a face to face meeting. They were also asked to each child to fill out a structural form with demographic information. Performas were filled out by inquiring the patients of intellectual disability in the schools. Schools records of the intellectual disability (ID) children were assessed.

Identification for intellectual disabled children

To get information about children, the institute also organized a meeting

between children's parents and researchers. Information about different variables were asked as age, gender, residence value of IQ, relatedness of any genetic factor, environmental factors and physical factors that can be linked with intellectual disability as shown by symptoms. Children, that was intellectual disabled also identified by teacher in schools.

Clinical Evaluations

The meeting was organized and clinical evaluations of children were performed by group work of local psychologists along with physicians. After independently examining the youngster and discussing their results, a psychologist and a physician gathered to investigate ID. Non verbal assessments and adapted behavioral scales were created for children in Pakistan and used to assess mental impairment. Child developmental history and observation of structural language of child is very important for physical diagnosis.

Tests for intellectual quotient

It was designed to measure intellectual and mental disability. It was named as IQ tests. Factors like experiential learning, abstract thinking, problem solving, academic and mental learning along with reasoning was evaluated. For cut off score criteria, the person with 70 or lower IQ score was adjusted. The main purpose was assessment of intellectual disability.

StatisticsAnalysis

The results were obtained and analyzed using chi square test in computer tool (SPSS 22 version software) designed for analyzing data from multi phase experiments. Thus, the data for these children were weighted in the analysis.

RESULTS

Present study was carried out on different schools of Sargodha. Many of physical disorders were linked with intellectual disability. Some genetic disorders were also associated with the intellectual disability. The most representative age group was 6-10 years which included 32.5% (N=27) children as shown in table 1.

Table 1: Distribution of age in Intellectual Disabled

Age (Years)	No. of males	No. of Females	Total
0-5	5 (8.95%)	8 (33.33%)	13 (16.25%)
6-10	15 (26.78%)	12 (50%)	27 (33.75%)
11-15	23 (41.10%)	3 (12.50%)	26 (32.50%)
16-20	9 (16.10%)	1 (4.17%)	10 (12.50%)
21-25	4 (7.14%)	0 (0.00%)	4 (5.00%)
Total	**56 (100%)**	**24 (100%)**	**80 (100%)**

A large number of children had problems in feeding usually in childhood. 48.75% suffering from moderate intellectual disability. The range for very severe intellectual disability is twenty to thirty five IQ level. The most represented level of intellectual disability was moderate intellectual disability was in 48.75% (N = 39) children. 35% (N = 28) children had microcephaly, 21.25% (N = 17) children had hypothyroidism and 23.75% (N = 19) children had phenylketonuria as single gene disorder associated with intellectual disability as shown in table 2.

Table 2: Incidence of Genetically Disorders in Intellectual Disabled Children

Single gene disorders	Total no. of Individual
Microcephaly	28 (35%)
Phenylketonuria	19 (23.75%)
Hypothyroidism	17 (21.25%)
Other disorders	16 (20.00%)
Total	**80 (100%)**

In table 3 and 4 incidence of physical disorder and disorders in intellectual disabled children were reported.

Table 3: Incidence of Physical Disorders in Intellectual Disabled Children

Symptoms for disorder	Total No. of Children
Floppy limb	12 (18.75%)
Problem in feeding	32 (40.00%)
Cleft lip	11 (13.75%)
Large head	12 (20%)
Weak limbs	28 (35%)
Club feet	14 (17.50%)
Lump on back	3 (3.75%)
Lump at navel	2 (2.5%)

Table 4: Incidence of Symptoms for Intellectual Disability

Symptoms for Disability	No. of Children
Sitting Problem	26 (32.5%)
Standing Problem	29 (36.25%)
Walking Problem	33 (41.25%)
Difficulty in seeing in daytime	18 (22.5%)
Difficulty in seeing at night	19 (23.75%)
Difficulty in hearing	27 (33.75%)
Difficulty in Understanding	41 (51.25%)
Difficulty in moving his arms	25 (31.25%)
Loses consciousness at some times	34 (42.50%)
Child is not learning to do things like other children	45 (56.25%)
Cannot speak at all or make himself understood in word	35 (43.75%)
Child cannot name objects like toys books etc.	32 (40%)
Appear mentally backward and affected	39 (48.75%)

Environment factors influence on the intellectual disability. Out of 80 children 27.50% (N = 22) children were affected from intellectual disability due to malnutrition. 16.25% (N = 13) children were affected from intellectual disability due to iodine/ folic acid deficiency, 15% (N = 12) children were

fromexposure to the chemicals or radiations in pregnancy of their mother, 22.50% (N = 18) children were due to maternal infection in pregnancy of their mother and 18.75% (N = 15) children were affected due to Rh incompatibility as shown in fig. 1.

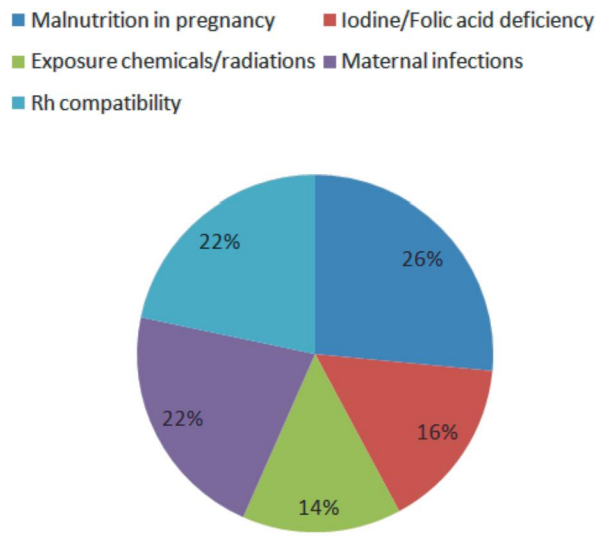

Fig. 1. Prevalence of Environment Influence on the children of Intellectual Disability

Some of the children infections are responsible for the intellectual disability. Out of 80 children 17.50% (N = 14) children had placental dysfunction. 20.00% (N =16) children had severe prematurity. 7.50% (N = 6) children had birth trauma. 30.00% (N = 24) had children complicated delivery. 25.00% (N = 20) children had not perinatal infection as shown in fig. 2.

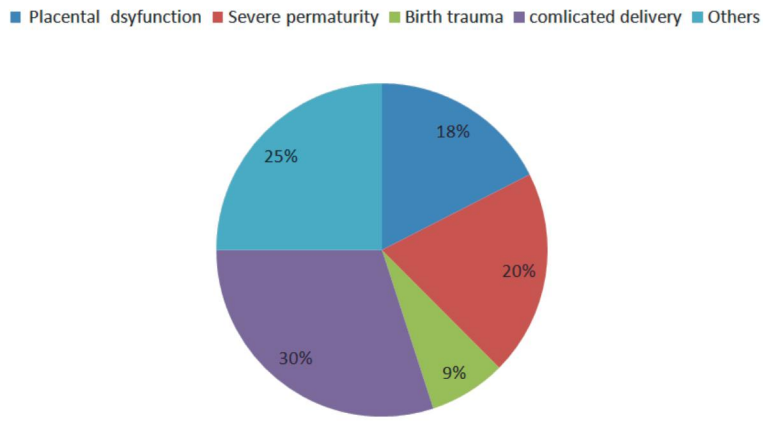

Fig. 2. Prevalence of Perinatal Infections in children of Intellectual Disability

Neonatal infection is also responsible of intellectual disability. Out of 80 children 35.00% (N = 28) children had septicemia. 26.25% (N = 21) children had severe jaundice. 10.00% (N = 8) children had hypoglycemia. 28.75% (N = 23) other children hadneonatal infection as shown in fig. 3.

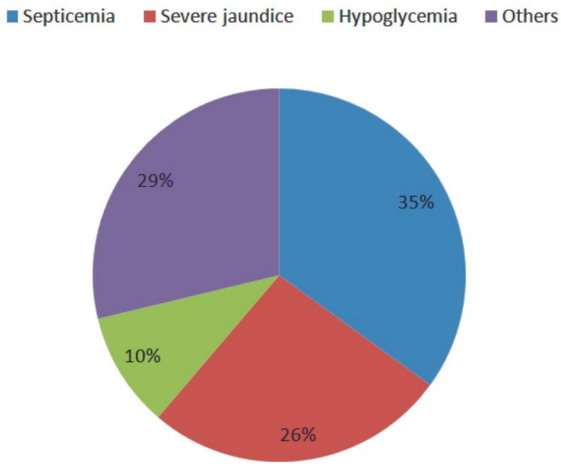

Fig. 3. Prevalence of Neonatal Infections in children of Intellectual Disability

Postnatal infection is also responsible of intellectual disability. Out of 80 children 35.00% (N = 28) children had brain infections. 18.75% (N = 15) children had head injury. 13.75% (N = 11) children had lead exposure. 32.50% (N = 26) children had malnutrition as shown in fig.4.

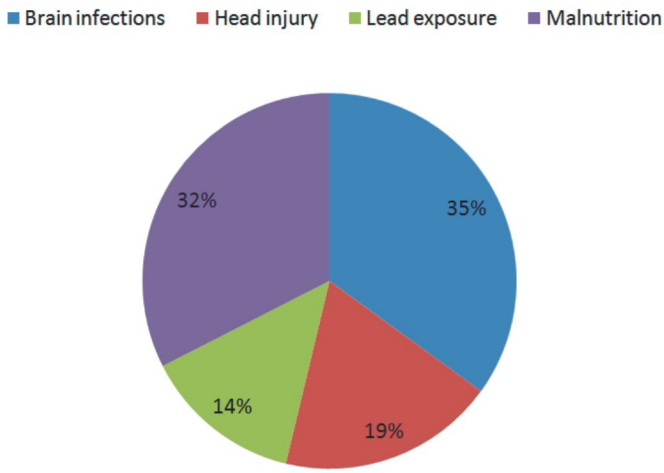

Fig. 4. Prevalence of Postnatal Infections in children of Intellectual Disability

Out of 80 children 40% (N = 32) children had not any other member of their family affected with intellectual disability. 31.25% (N = 25) children had

one other member of their family affected with intellectual disability. 22.50% (N = 18) children had two other member of their family affected with intellectual disability. 6.25% (N = 5) children had more than two other member of their family affected with intellectual disability.

DISCUSSION

A study conducted in Assiut (Egypt), in which total number of cases was 90, 63 males and 27 females, 76% of cases were coming from rural areas while 24% of cases were coming from urban areas (Nemerimana et al., 2018). Frequency of parent's consanguinity was 72.1% (n=227) and non-cousin marriage was 27.9% (n=88). In contrast, a study conducted to investigate the parental consanguinity among mentally retarded children found that (63%) were born to non-consanguineous marriages (Zeldin et al., 2012).

In another study 217 adults of population with intellectual disability and sample of two thousand three hundred and fifty adults without intellectual disability randomly participated. Elder intellectual disablers have greater risk for wellbeing, according to unadjusted comparisons. Results support the idea that intellectual disabled person have greater rates of health related disorders than general and normal population (Hatton and Mcmahon, 2020). The frequency of mild, moderate, severe and profound ID were 46.7% (n=147), 32.0% (n=101), 14.6% (n=46) and 6.7% (n=21) respectively, which is related with a study in which non-genetic ID patients were 97 in numbers that includes 24% mild ID, 40% moderate, 23% severe-profound and 10% unspecified ID (Anitha et al., 2011).

Decreased cultural and geographical diversity (both in urban and rural areas) have increased incidence of psychological problems in ID patients. Overall prevalence rate is from 13.9% to75.2%, the large range is due to greater sample size with different rate of geographical variation. The cultural and geographical diversity lowers psychological problems in ID patients as acting a environmental factor. Hence, particular attention should be given in diagnostics and sampling methodology (Buckles et al., 2013). Children with intellectual disabilities faced more psychological problems than normal having psychological problems. At current time there is no proper instrument that diagnoses psychological problems. Only PAS-ADD Checklist questionnaire and the Mini PAS-ADD interview (two third-party assessments instruments) are

used to diagnose psychological problems (Muller et al., 2022). While in another study the Health of the Nation Outcome Scales for people with Learning Disabilities into French (F-HoNOS-LD) was adopted to examine ID patients and study showed the scale gave valid and reliable results (Straccia et al., 2022).

Social status of class room and social environment that ID patient encompasses may increased liklihood of psychological problems. Social behavior of ID patients tells them whether they were accepted or rejected in environment. It was observed that, likeness and dis-likeness was low at beginning of schooling in ID patients but increase with social and class room behavior. About 10% of behavior change was due to social environment. Behavior problem is a greater risk for social exclusion of ID patients and special attention should be given in special school of ID (Schoop-Kasteler et al., 2022).

CONCLUSION

It was concludedthatin 200 studiedchildren, 80 cases of intellectual disability were identified which included 70% males and 30% females. Among them 32.5% belongs rural areas while 67.5% lived in urban areas. Problems during pregnancy (developmental problem in fetus) and problems at birthmight be lead to intellectual disability including developmental problems (getting not enough oxygen).Some of the children infections werealso responsible for the intellectual disability. Out of 80 children 17.50% children had placental dysfunction. Both neonatal and postnatal infections were also responsible of this problem. Attention of people to intellectual disability was very low. Hence, proper diagnosis of psychiatric disorder can help psychiatrists for dealing with patients as people diagnosed later in life.

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Assessment of Aflatoxins production and its various Control strategies: A Review

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ABSTRACT: *Mycotoxins specifically the aflatoxins contaminate about 25% of the agricultural products in the world. They effect greatly the growth rate, feed intake, and feed conversion ratio. There is a great economical loss to farmers due to loss of food values and effecting human as well as animal health. The different preventive measures are in use to control fungal contamination such as chemicals (acids, bases, and salts), HSCAS ,zeolite polymers, activated charcoal and yeast with variable findings. Fraction of yeast sludge has proved to minimize the adverse effects of aflatoxicosis due to the presence of manno-oligosaccharides in its cell wall. The present review highlights the remedial measures particularly yeast sludge to control aflatoxicosis.*

Keyword: *Aflatoxin, Contamination, Yeast sludge, Glucomannan*

The Historic Background of Mycotoxins

Mycotoxin's effects on humans and animals health has been reported since the 14th century. In 1952, the ingestion of mold-contaminated maize feed from swines in South USA resulted in an epidemic of "mouldy corn toxicosis" (Sean, 2002). AFB1 found to be the main cause for turkey deaths (Agag, 2004).

In 1962, aflatoxins were named due to the presence of *Aspergillus flavus*. This Aflatoxin have shown green and blue UV fluorescence. It can cause toxicity, mutagenicity and teratogenicity and are mainly produced by certain toxigenic fungal strains like *A. paraciticus* and *A. flavus.* Aflatoxins can be further divided into AFB and AFG (Agag, 2004). In 2004, hundred Kenyans became critically ill and nearly 125 victims were registered in 2004

because of acute aflatoxicosis (Wu et al., 2011).

Mycotoxins

Mycotoxins are secondary metabolites derived from mycelial filament frameworks (Dewegowda et al., 1998; Hussein and Brasel, 2001). The most common mycotoxins are the types of *Aspergillus, Penicillium, Fusarium, Zearalenones* and *Ochratoxins* (Binder, 2007).

Grains are more infected by mycotoxins as pathogensare present in fields.Secondary metabolites from moulds enforce harmful effects (Hussein and Brasel, 2001). Different routes including ingestion, absorption through the skin, and ingestion can lead to mycotoxins entering the body. This leads to various lethal effects which can be carcinogenic, immunotoxic, neurotoxic and teratogenic (Casteel and Rottinghouse, 2000; Frisvad et al., 2007). Species, sex, age, nutritional health, etc. are the magnitude of the results recently been reported to be carcinogenic (Yiannikouris and Jouany, 2002).

Cereal products are widely used for incursions into fungi, leading to production of aflatoxins (Zaki et al., 2012). Some 25 to 40% of the total global production of cereals is polluted with mycotoxins, particularly aflatoxins, directly and indirectly. Aflatoxins are manufactured in storage by *Aspergillus flavus* and *Aspergillus parasiticus.* They can be located in hot and humid tropical countries in contrast with moderate regions (Yiannikouris and Jouany, 2002; Maurice, 2002).

Aspergillus flavus is typically present in rice, peanuts and cotton. *Aspergillus parasiticus* is uncommon in South-East Asia, but it does not have *Aspergillus flavus*, which is only threatened by *Aspergillus parasiticus* (Pitt et al., 1997). AFB1 and AFB2 are generally formed by the *Aspergillus flavus*, while AFB1, AFB2, AFG1, and AFG2 are created by *Aspergillus parasiticus* (Agag, 2004).

The study of aflatoxins indicates that AFB1 is up to 77% the largest grain contaminant (Wilson and Payne, 1994). The embryo part of the grains is the key location for the development of *Aspergillus flavus*, which results in further aflatoxin bloom (Ghahri et al., 2010). In regions between 40 °N and 40 °S equator in latitude at temperatures 24-35 °C and humidity content exceeding 10% (Williams et al., 2004).

About 4.5 billion people in developed countries are exposed to aflatoxins, and high levels of aflatoxins in tropical and sub-tropical regions are in deteriorated areas (Verma, 2004).

There can be no place in the world free of aflatoxins due to the transport of agricultural supplies (Maurice, 2002).

Throughout Pakistan, aflatoxins are found to be 91.1% of the most prevalent mycotoxins in survey of poultry diets in 2 decades. The aflatoxin B2 were found to be present in ten commercial feed mills in Punjab. AFB2 was found to be more than 20 µg/kg in layer as well as broiler starter poultry (Shareef, 2010).

AFB1 was most often found to be 26.1 percent of the samples are aflatoxins, in particular meat, cereal as well as oil seeds (Bokhari, 2002). The overall level of exposure of aflatoxins to shell walnuts is 40%, with no shell at 70%, and peanuts with shell at 40 percent, is observed in KPK and northern parts of Pakistan (Luttfullah and Hussain, 2011).

The highest incidence of aflatoxin is in 60% maize, 40% sorghum and 25% in various trade shops (Lutfullah and Arshad, 2012). *Aspergillus flavus* in Pakistan had the highest incidence (Shah et al., 2010).Borutova et al., (2012) reported the occurrence in 2010 in the Asian area of various mycotoxins on various feeds including rice, wheat, soybeans, maize gluten, dried distillery, etc.

Chemical Nature and Structural illustration

300-400 secondary fungal metabolites and mycotoxins have been found in different commodities (Binder, 2007). Aflatoxins are molecular derivatives of bifuranocoumarin. The chemical composition contains a furan ring and the coumarin nucleus with such a pentenone ring (AFB and AFM) and a lactone ring with six members (AFG) (Brase et al., 2009).

Fluorescence under ultraviolet light can distinguish the four most common compounds such as B1, B2, G1, G2, M1 and M2 (Fig. 1) (Agag, 2004). Aflatoxins are soluble in methanol, chloroform and acetone. They are reactive and these contaminants decline at their boiling points, which vary from 237°C to 299°C but do not damage under standard cooking conditions (Jallow, 2015).

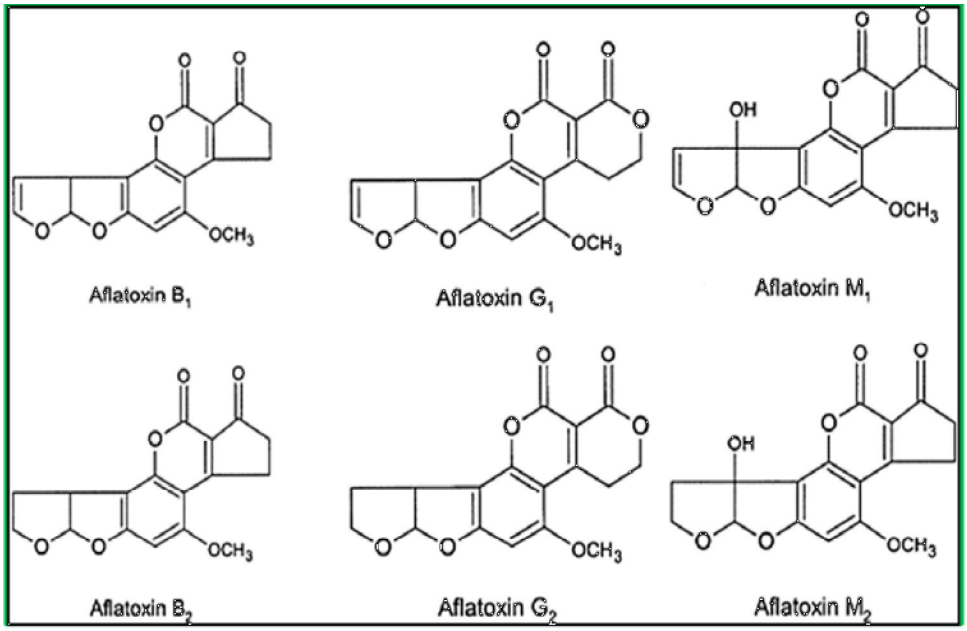

Fig. 1. Chemical structures of aflatoxin B1, B2, G1, G2, M1 and M2 (Musleh et al., 2017)

Factors that affect Toxicity

Physical, biological and chemical factors that influence toxin development are specific. Moisture, heat, humidity as well as mechanical damage are physical influences. Relative oxygen, carbon dioxide, medium structure, contaminants, and fungicides are the chemical influences included. Biological factors include plant variety, pressure, various insects and aggregation of spores that can influence the level of toxicity in development (Wayne, 2012). For the development of fungal mycotoxins, water, oxygen, temperature and pH (3-8) play a key role. Water activity can differ from 0.61 to 0.91, as the majority of fungi are grown at 0.75. Limited temperature varies from 12-41 °C in the processing of aflatoxins by *Aspegillus flavus* and *Asprgillus parasiticus* with maximum productivity at 25-32 °C. However, the synthesis of the aflatoxins increases by upwards of 27 °C and moisture by over 62 per cent (Agag, 2004).

The synthesis of AFB1 is higher as compared to AFG1. AFB1 is best produced at 24-28°C while 23°C is best temperature for the production of AFG1. The output of equal volumes of AFB and AFG is reassured by a low temperature of 8 to 10 °C. However, the production of total aflatoxins is limited and longer is required (Agag, 2004). Fungi are active in food as food spoilers

at higher levels of moisture (Zaki et al., 2012). In comparison, *Aspergillus* species may be at lower moisture levels than *Fusarium* species (Wayne, 2012). Oxygen is a crucial growth driver as it influences the production of mushrooms. Its development is controlled to below 1% oxygen (Zaki et al., 2012).

Aflatoxins pollution is positively impacted and potentially overlapped by high temperature and drought by insect damage to plants (Wayne, 2012). These conditions allow "hot spots" to expand in stored grains. Specific maize grains can be occupied with aflatoxins of 400,000 μg/kg (Richard, 2007). Before and after harvesting the accumulation of mycotoxins meditates primarily on climate factors, such as *Fusarium* species developed cereal toxins under high moisture conditions during harvest periods, and before harvesting crop pollution with aflatoxins, such as peanuts and maize, combined with high temperatures, insect damage and extreme drought conditions (Wayne, 2012). Genetic fungal genes and pathways have been dislocated for the manufacturing of mycotoxins and parameters (Yu and Keller, 2005; Bhatnagar et al., 2008). These genes may aid the development of plants which are difficult to aggregation of toxins (Wu et al., 2004).

Effects of Aflatoxins

Toxicity ofdifferent types of aflatoxins such as B1, G1, B2 and G2, depends on feed exposure period, species, sex, health status, and age of animal (Richard,2007; Denli et al., 2009). The disturbance of well-developed hepatic enzyme processes and break down of toxins potentially makes children less immune than adults (Quist et al., 2000).

The most infected species by aflatoxins are turkey chickens, broilers and layers (Huff et al., 1986). Goslings, pheasants and quails have an intermediate role for susceptibility, whereas chickens seem to be extremely immune. Ducklings are 5-15 times more sensitive than laying hens; but within layers, few varieties can be three generally more concentrated than other strains of layers (Azizollah et al., 2009; Suksombat et al., 2011).

Any mycotoxin after intakehas ability to affect body functions and contributes in diseases. Clinical signs and disease can differ even between species as the Aflatoxins which did not seem to affect the wellbeing of the broiler yet had a detrimental effect on the human community development

(Haschek et al., 2002; Grenier et al., 2011; Wayne, 2012).

Chronic aflatoxicosis, overt or indirect signs take in to account growth depression, lower feed volume and lowered nutritional intake (Humphrey and Klasing, 2004; Klasing, 2007). The decline in growth following the ingestion of aflatoxins is responsible for the low production of pancreatic digestive enzymes.The ingestion of aflatoxins has not been shown to affect apparent metabolizable energy (AME) in foods, but substantially decreases weight gain energy efficiency, AME energy intake and higher heat performance in broilers tissue gain (Wayne, 2012). Aflatoxin in feed may trigger an increase of 5% depression of broiler (Dersjant Li et al., 2003) and the poultry industry to cause a big difference between benefit and loss at each aflatoxin dose rate as mg/kg of feed (Kaki et al., 2012).

Aflatoxins Detoxification

Universally, the feed contaminated by aflatoxins B1 is almost unavoidable (Rawal et al., 2010). Decontamination of mycotoxins is referred to different methods by which their toxicity is removed. They may be physical and chemical methods (Diaz and Smith, 2005).

Physical Methods

Different methods have proven effective in reducing moderate mycotoxins concentrations as cleaning the kernel surface (Huwig et al., 2001; Wayne, 2012). Conversely, this seems relatively lengthy to remove highly contaminated feedstuffs.

Chemical Methods

A group of acidic compounds, alkaline compounds, salts, oxidants, reducing agents etc., are being utilized for degradation of mycotoxins in feedstuff (Jalili et al., 2011). These available methods are ineffective and relatively pricey. To reduce aflatoxins levels, ammoniation has been established but it is not acknowledged in the United States (Park and Price, 2001). Ammoniation abolishes the mycelia and lethal spores of fungi. Moreover, elevated quantities of doses of acetic acid, isobutyric acid, propionic acid, methyl bromide, ethylene oxide, ethylene dibromide, propane or propene, sulfur dioxide and phosphine show fungicidal activity. Although, these chemical compounds decrease dietetic quality and are corrosive to humans and animals.

Any of them will confiscate aflatoxins during the digestive phase as they are added to foods infected with

aflatoxins, causing mycotoxins to travel harmlessly through livestock gastro intestinal regions (Phillips et al., 1990). The more successive and realistic approaches to solving the issue of aflatoxins are the application of adsorbents and the degree of measured adsorption will range from 0% to 87% from a number of different resources of clay minerals (Schiedeler in 1993). Analysis has shown that aflatoxins can be adsorbed by sodium aluminosilicates and determines the type (Mahesh and Devegowda, 1996).

Mineral adsorbents such as zeolites, silica and phylosilicates demonstrate a range of binding potential for aflatoxin and are active, at basal levels, in channels of interlaying, on the surface, pores as well as at particle edges (Daković et al., 2000). It can be done the bentonite adsorption potential varies from 17% to 36%. An additional significant advantages of such adsorbents are the relatively cheap, healthy and easy to apply to animal feedingstuffs (Magnoli et al., 2008).

Soybean bentonites are white, light in weight and formed from volcanic centigrade, which primarily contain montmorillonite and are made up of salt from Na, K, Ca of hydrated aluminosilicates sometimes containing Fe, Mg, Zn and Ni etc (Diaz and Smith, 2005). The layered microphase of these adsorbents makes it possible for aflatoxins to react for adsorption at locations and boundaries surfaces in the interlayer region (Ramos et al., 1996). Solid colloidal compounds of zeolites have the ability to swell and raise water easily, which produces a gelatinous, thixotropic material (Pasha et al., 2008; Safaeikatouli et al., 2011).

The interlayer of montmorillonite that convinces the adsorption of multiple organic molecules including mycotoxins, zeolite and montomorillonite particles. The theory of atmosphere creating the hydration of exchangeable cations. Zeolites surfaces attract polar feature community of aflatoxins (Kolossova and Stroka, 2009).

In comparison to hydro-calcium aluminosilicate (HSCAS), the influence of aflatoxins on baby animals is decreased by 1 percent (Kolossova and Stroka, 2009). The strong negative charges of HSCAS are balanced with cations such as magnesium, potassium and sodium which are present in the cavities and which, by neutral pH or alkaline origin, are not reacted with feedstuffs and behave as an inert matter (Khanedar et al., 2012).

Aluminosilicates are being used as "anti-caking" agents at a frequency of

up to 2% but there are many drawbacks to the decrease in the use of minerals and a limited range in binding ability (Kolossova and Stroka, 2009). Bentonite minerals can affect ca-metabolism, nitrogen cations like NH_4^+ and can be used for the adsorption of AFB1. The development of tibial mineral values in chick feeds that have nutritional deficiencies is not shown to harmful effects (Southern et al. 1994).

There was a competition, at lower levels of toxins between AFB1 and montmorillonite clay supplemented feed of broiler chicken for adsorption sites (Liu et al., 2011). Bio-transformation of mycotoxins into less toxic metabolites, is another alternative by the application of microorganisms like *Corynebacteriumrubrum* (Yiannikouris and Jouany, 2002). This takes action in the intestinal area of animals earlier to absorption of mycotoxins but toxicity of products by enzymes, undesired sound effects of fermentation and with microorganisms on food quality is leftover.

The efficacy of Hydrated Sodium Calcium AluminoSilicate (HSCAS), with a blend of clays and yeast cell wall against Aflatoxins were determined on one day old chicks in 11 treatments with 5 replicates pens and observed mortality rate for control with FCR was not affected by dietetic treatments. So two adsorbents comparison indicated that chicks gained more with HSCAS supplemented feed than yeast cell wall feed (Zhao et al., 2010).

Control and Management

Strategies to control mycotoxins ought to be economically consistent and meet up the standards of FAO, UNEP or WHO on toxicity of mycotoxins. According to international criteria, control strategy should inactivate the toxin with no carcinogenic products, nodestructionof nutritive value of products and no change in quality of products (Liu et al., 2011).

Anokwuru et al. (2011) found the effect of factors like proper irrigation, genetically resistant crops and bio pesticide therapy to remove the toxicity produced by using *Aspergillus* species and determined the ability of ethanolic aqueous extract of *Acacia indicia* bark to lessen hepatotoxicity. Aqueous extracts of a variety of medicinal plants like *Punicagranatum, Cassia alata, Daturastramonium, Polyanthia longifolia* and *Annonasquamosa* were analyzed against *Aspergillus parasiticus* to inhibit production of aflatoxins (Rajani et al., 2012) .

Fruit extracts of *Libidibiaferrea* and *Paulliniacupana*showed significant

antifungal activities (Breda et al., 2016). Production of aflatoxins can be restricted by maintaining cereals without moisture and use of propionic acid to inhibit growth of molds by decreasing pH. Irradiating of UltraViolet rays, X-rays or microwave is also one of the convenient method to detoxify aflatoxins (Yiannikouris and Jouany, 2002).

Yeast Sludge

In Asia and African countries, yeast groups, chiefly *Saccharomycese cerevisiae*, contribute the principal character in fermentation of food, along with lactic acid bacteria (LAB) for food processing as well as preservation (Sari et al., 2008). In Pakistan, yeast sludge can be collected from sugar mills and distilleries (Mumtaz et al., 2000). Hassan et al. (2012) evaluated that 100 ml. of yeast sludge contains 8.96 gm of yeast cells; 0.26 % of mannan oligosaccharide and this is the prime compound that binds the aflatoxins and Ochratoxin A and improves the practical yield and financial side of profitable in poultry fabrication.

Saccharomyces cerevisiae and lactic acid bacteria (LAB) like *propionibacteria*, *bifidobacteria* and *lactobacillus rhamnosus* bind themselves by cell wall components against mycotoxins powerfully without toxic effects on animal health (YiannikourisandJouany, 2002; Kolossova and Stroka, 2009). In early 1990s, *Saccharomyces cerevisiae* (yeast), was used as a growth promoter and found to have valuable effects on weight gain and immune system in broilers exposed to aflatoxins (Rawal et al., 2010).

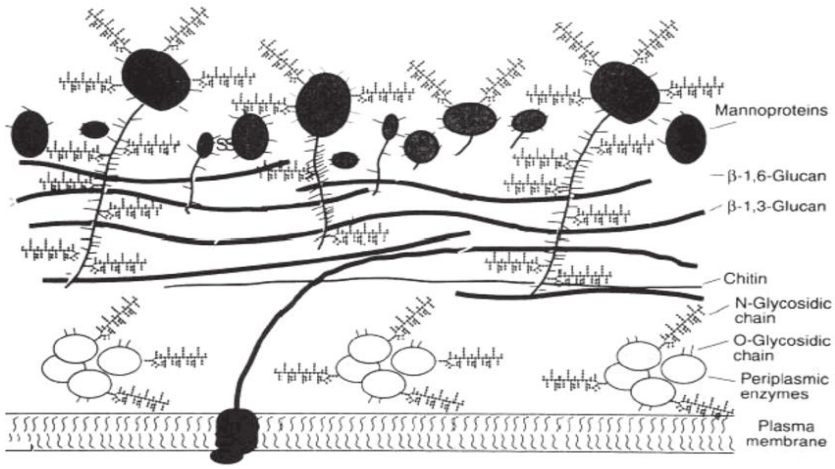

Fig. 2. Composition and structure of the yeast cell wall

CONCLUSION

Aflatoxins are a major source of disease outbreaks due to lack of knowledge and consumption of contaminated food andfeed worldwide. Extreme levels of aflatoxins in food inundeveloped countries are of major concern. Several effective physical, chemical, biological, and genetic engineeringtechniques have been employed for the mitigation, effective control and management of aflatoxins in food. But, developing fungal resistant and insect resistant hybrids/crops to combat pre-harvest infections and their outcome is a major issue of concern. Post-harvest treatments to remove aflatoxins such as alkalization, ammonization, and heat or gammaradiation are not generally used by farmers. However, some of plants have the ability to degrade and reduce the aflatoxin contamination in different types of agricultural products. Therefore, methods of using yeast sludge to reduce aflatoxin are currently being focused. Moreover, application of genetic recombination in *A. flavus* and otherspecies is being investigated for its potential to mitigateaflatoxins to ensure the safety and quality of food.

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Evaluation of Awareness and Preventive Measures among Transgender Regarding Sexually Transmitted Diseases

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ABSTRACT: *Sexually transmitted diseases are infectious disorders spread through unsafe sexual contact among sex workers and transgender community. The present study was conducted to evaluate awareness and preventive measures among male transgender in transmission of sexually transmitted diseases (STD). It was a descriptive cross-sectional survey study that conducted in Lahore, Pakistan. After informed consent the data was collected using the snowball sampling technique for 6 months. 41 transgender including all types of age ranging 18-45 years were included as per convenience. A self-administered survey-based questionnaire was developed using WHO and CDC guidelines. Data were analyzed with the help of the (SPSS) version 22 for the descriptive statistics. The mean age was 29.8 ± 7.09 with the highest age of 45 and lowest 18. Regarding education majority of the transgender were dropped out at Middle 18(43.9%) and 9(22%) were illiterate. The highest frequency among professionals were of dancers 17(41.5%) and sex workers 11(26.8%). A Majority 28(68.3%) has unsafe sexual contact with one or more partners. Some have experienced discharge from urethra 14(20.3%), pain during urination 18(26.1%), ulcers or sores in genital areas 6(8.7%) altogether which can be a symptom of STD. About 24(58%) transgender practices self-medication without the prescription of a medical physician. It was concluded that transgender have a poor understanding of STDs due to low education and sex selling as a profession. They engage in risky sexual behaviour and were found unaware of proper treatment resources.*

Keyword: *Transgender, Sex workers, STD, HIV, Awareness, Prevention*

INTRODUCTION

Sexually transmitted diseases (STDs) are infectious disorders spread through unsafe sexual contact among sex workers and the transgender community (Collumbien et al., 2008). These diseases are caused by the transmission of viruses, bacteria, fungus, parasites, protozoa, or arthropods from one person to another through unsafe sexual means (Khan et al., 2008; Aral et al., 2011). There are about 30 known different pathologies or conditions formed due to sexual contact are Human Papillomavirus (HPV), Genital Herpes, Syphilis, Hepatitis, Trichomoniasis, Gonorrhoea, Chlamydia and Human Immunodeficiency Virus (HIV) (Khan et al., 2013; Van Gerwen et al., 2020). It is common in the transgender community because the majority of them are receptive partners in anal intercourse (Ejaz et al., 2022). Previous researchers have found that HIV and STD incidence is nineteen times higher among transgender sex workers (Hawkes et al., 2009). They are more likely to have their anal lining burst and get infected (Landers et al., 2015; John et al., 2021). STDs are one of the world's most important public health challenges affecting both developed and developing countries (Van Gerwen et al., 2020). According to the World Health Organization (WHO) more than one million individuals globally get a sexually transmitted illness every day and 500 million people each year including Transgender and other identities as the third gender. Sexually transmitted infections (STIs) have a significant influence on sexual and reproductive health around the world (WHO, 2013; Williamson et al., 2020). Every day, almost one million STDs are acquired. WHO estimates that 374 million new infections with commonly four sexually transmitted diseases (STDs) were expected to be occur in 2020 as chlamydia (129 million), gonorrhea (82 million), syphilis (7.1 million), and trichomoniasis (7.1 million) (Barrow et al., 2020; Hsieh et al., 2022). The most serious viral STD is human immunodeficiency virus (HIV), which leads to AIDS and incurable (Mayer et al., 2011). Human papillomavirus (HPV), hepatitis B, and genital herpes are among the other incurable viral STDs which is affecting Transgender health (Khan et al., 2011; Ejaz et al., 2022). A study in Karachi Pakistan showed the prevalence of STDs among Pakistani males was determined to be 4.4 percent and Sexually-acquired infections (SAIs)

were prevalent in 60 percent of Transgender and 36 percent of commercial sex workers (Aijaz and Mehraj, 2020).

Due to a lack of legal rights, prejudice, and intolerance in Pakistani society, transgender people are forced to work as sex workers, dancers at weddings and celebrations, blessing babies, and begging on the streets (Tabassum and Jamil 2014; Manzoor et al., 2022). Transgender receive more money and customers to engage in unprotected intercourse increases the risk of contracting sexually transmitted disease (Ramanathan et al., 2014; Minget al., 2016). As a result the consideration of money and customer pleasure makes it difficult to persuade them to use condoms (Abbas et al., 2014; Budhwani et al., 2017). Transgender face stigma and discrimination in health care, educational and housing facilities which are significantly linked to a rise in increased sexual behaviour and actions towards sex work (John et al., 2021; Manzoor et al., 2022). They lack knowledge about awareness and prevention of sexually transmitted diseases which eventually raise the risk of contracting the human immunodeficiency virus (HIV) and sexually transmitted diseases (STDs) (Sahastrabuddhe et al., 2012).

This study was conducted to evaluate the awareness and prevention knowledge about sexually transmitted diseases. The Transgender involved in sex work described the transmission of diseases associated with their sexual behaviour. This Survey has highlighted the effects of sex selling, lack of knowledge, unavailability of health resources among the transgender population in Pakistan. This research have also importance in the development of successful transgender prevention and treatment programs.

MATERIALS AND METHODS

It is a Descriptive Cross-sectional Survey conducted in Lahore, Pakistan. A sample size of 41 was considered as per convenience. The data was collected using the Snowball Sampling technique for 6 months. The transgender included all type a gender, androgynous, bigender, pangender, gender fluid etc. The age group of 18 to 50 years were included after taking informed consent. A self-administered survey-based questionnaire was developed using WHO and CDC guidelines followed and pilot tested from 10 health experts.

Statistical Analysis

Data were analysed with the help of the (SPSS) version 22. Descriptive statistics about age were calculated. The frequencies and percentages were

mentioned as the response of the question.

RESULTS

Transgender is the socially unprivileged population in Pakistan and lacks basic facilities and necessities of life. This study was planned to visualize a lack of knowledge and awareness about sexual transmitted diseases. The data obtained from the formal questionnaire was based on important variables i.e. age, profession and qualification of Transgender. The inclusion age was 18 to 45 in which the mean age was 29.8±7.09 with the highest age 45 and lowest 18.

Professions of transgender were included as sex workers, dancer and blessing birth and beggars as mentioned in table 2 the educational qualifications were categorized into Illiterate, Middle, Matric, Intermediate and bachelors. The majority of the transgender were school and college dropped outs as Middle 18(43.9%) Matric 10(24.4%), 9(22%) were illiterate had no basic education and very few of them as 3(7.3%) and 1(2.4%) had intermediate and bachelors (table 1). Table 1 showed the highest frequency of dancers as 17(41.5%), following sex workers 11(26.8) with lowest as blessing birth 9(22%) and begging 4(9.8%).

Table 1: Education level and Profession of Transgender

Education of transgender	Frequency	Percentage	Valid percentage	Cumulative percentage
Bachelors	01	2.4	2.4	2.4
Intermediate	03	7.3	7.3	9.8
Matric	10	24.4	24.4	34.1
Middle	18	43.9	43.9	78.0
Illiterate	09	22.0	22.0	100.0
Total	41	100.0	100.0	
Professions of transgender				
Sex workers	11	26.8	26.8	26.8
Dancers	17	41.5	41.5	68.3
Blessing births	09	22.0	22.0	90.2
Begging	04	9.8	9.8	100.0
Total	41	100.0	100.0	

The response towards knowledge and awareness of STDs as the majority of them were totally unaware of all types of STD's following 30(73.2%) HPV, 38(92.7%), 38(92.7%) genital herpes, 35(85.4%) syphilis, 33(80.5%) trichomoniasis, 34(82.9%) gonorrhoea, 27(65.9%) chlamydia whereas very few of them were aware of hepatitis as 24(58.5%) and HIV as 18(43.9%).

Table 2: Awareness of transgender regarding STDs

Which of the following sexually transmitted disease you are aware of?	**Yes N (%)**	**No N (%)**
1. Human Papillomavirus (HPV)	11(26.8)	30(73.2)
2. Genital Herpes	3(7.3)	38(92.7)
3. Syphilis	6(14.6)	35(85.4)
4. Hepatitis	24(58.5)	17(41.5)
5. Trichomoniasis	8(19.5)	33(80.5)
6. Gonorrhea	7 (17.1)	34(82.9)
7. Chlamydia	14 (34.1)	27(65.9)
8. Human Immunodeficiency Virus (HIV)	18(43.9)	23(56.1)

The sexual practice of transgender shown 28(68.3%) have unsafe sexual contact with one or more partners and avoid using condoms because of customer pleasure and satisfaction (table 3).

Table 3: Response of Transgender regarding sexual practice, signs and symptoms and treatment approach

	Frequency	Percent	Valid Percent	Cumulative Percent
Do you wear a condom and practice safe sex for the prevention of STDs?				
Yes	13	31.7	31.7	31.7
No	28	68.3	68.3	100.0
Total	41	100.0	100.0	
Do you have any signs and symptoms Regarding sexually transmitted diseases?				
Discharge from urethra	14	20.3	20.3	20.3
Pain during urination	18	26.1	26.1	46.4
Ulcers/sores in the genital area	6	8.7	8.7	55.1
Never Experienced	31	44.9	44.9	100.0
Total	69	100.0	100.0	
If you or your fellow transgender needed treatment for sexually transmitted disease where they could approach it?				
Local pharmacy	24	58.5	58.5	58.5
Govt. Hospital/Health-care facility	12	29.3	29.3	87.8
Private doctor / nurse/ clinic	5	12.2	12.2	100.0
Total	41	100.0	100.0	
Do you believe that STD awareness and Prevention programs could improve the quality of life in Transgender?				
Yes	34	82.9	82.9	82.9
No	7	17.1	17.1	100.0
Total	41	100.0	100.0	

The questions were asked from transgender about their experience having any of these symptoms some of them had experienced as discharge from

urethra 14(20.3%), pain during urination 18(26.1%), ulcers or sores in genital areas 6(8.7%), some had all symptoms together and considered in frequency. About 31 (44.9%) have no symptoms and have never experienced it (Table 3). The response of transgender towards their treatment regarding symptoms about STDs was calculated as 24(58%) visited the pharmacy and asked for medicine from the salesman and use them without prescription of medical physician, 12(29.3%) visited Government Hospital while 5(12%) visited private doctor or nurse in a clinic (Table 3). The response from the transgender population was considered for the future interventions that did they believe awareness and prevention programs can improve their quality of life 34(82.9%) were sure and responded in favour of more awareness and prevention programs towards the marginalized and socially discriminated population (Table 3).

DISCUSSION

The current study was conducted to evaluate awareness and prevention regarding STD transmission among transgender in Lahore, Pakistan. Moreover, the researcher was interested in exploring the important factors like profession and qualifications contributing towards transgender health status. The researcher has evaluated the factors with the help of self-administered survey-based questionnaires based on evaluation regarding knowledge of STDs including symptoms and medication. STDs is transmitted through sexual contact with an infected person and is caused due to HPV, HIV, Hepatitis B virus etc. Sexually transmitted disease occurs usually because of the ruptured lining of the penis and rectum. The transgender have less knowledge about transmission, causes, signs and symptoms and do not know accurate treatment plans.

The current study found that the maximum number of transgender is totally unaware of all types of STD's. The respondents had negative responses as 30(73.2) HPV, 38(92.7%), 38 (92.7) Genital herpes, 35(85.4%) Syphilis, 33(80.5%) Trichomoniasis, 34(82.9%) Gonorrhoea, 27(65.9%) Chlamydia, whereas very few of them were aware of hepatitis as 24(58.5) and HIV as 18(43.9%). A study by Ejaz et al. (2022) conducted to study male and transgender sex workers who had genital warts and anal cancer due to sexually transmitted human papillomavirus infection. The previous study evaluated knowledge, risk perceptions about STDs including HPV beliefs and attitudes towards HPV

prevention, and recommendation of participants for HPV vaccination and anal pap screening. The current study has also evaluated that transgender were unaware of the human Papilloma virus and may have a higher risk of genital warts and anal cancer. The participants described lack of knowledge of HPV and its health consequences. The current study was planned to visualize lack of knowledge and awareness about sexual transmitting diseases and found similar results. The preventive measures such as condom use are fewer in both studies. They preferred to have unsafe sexual contact without a condom for customer satisfaction.

A systemic review by Van Gerwen et al. (2020) and accessed the prevalence of HIV and STIs among both transgender and reviewed 25 studies which represented 11 countries. HIV was the most often investigated STD, with prevalence rates ranging from 0% to 49.6% in transgender women and 0% to 8.3 percent in transgender men. The review had comparable perspectives and conclusions were studied about the indications and symptoms, as well as their frequency of getting infection while having unsafe sex. The current study has focused on the symptoms that some of them experienced discharge from urethra 14(20.3%), pain during urination 18(26.1%), ulcers or sores in genital areas. Some had all symptoms together and considered in frequency. About 31 (44.9%) have no symptoms and never experience it. The review also mentioned that the transgender suffered discharge from the urethra and pain during urination, which is a sign of sexually transmitted disease. The current study also asked for symptoms and the frequency of symptoms of occurrence as 31(75%) have never experienced any symptoms while 7(17.1%) experienced once and only 3 (7.3%) had more than once, which was related with the systematic review and shows the frequency of first and repeated infection.

In the current scenario, the transgender have faced sexual abuse, social discrimination and regional marginalization which are forcing them towards sex selling. The current study also explains the sexual practice of transgender which is shown as 28(68.3%) have unsafe sexual contact with one or more partners and avoid using condoms because of customer pleasure and satisfaction, Whereas Khan et al. (2013) looked at the role of Hijras (Male transgender) and found that (58%) had sexually transmitted diseases (STIs) and (38% had multiple infections). Syphilis (50 percent) and

gonorrhoea (18 percent), which were the most common infections, corresponded to current survey findings that they were oblivious of knowledge and transmission. Both the studies found that transmission of an STD can be due to multiple partners and frequent use of condoms.

Transgender from Lahore, Pakistan were also studied by Manzoor et al. (2022) and used a snow-ball sampling technique to include 214 samples. They represented major health issues such as depression (56 percent), anxiety (59 percent), and genital tract ulcers (45 percent). About 70% of transsexual people seek treatment at government hospitals. Whereas a current study found that 24(58%) visited the local pharmacy and got medicine without a prescription from a medical physician and 12(29.3%) visited Government Hospital and 5(12.7%) visited a private doctor or nurse in a clinic. Previous and current studies related to find that the transgender community faces physical, mental, social and reproductive health issues. About 70% of transgender receive poor quality health care services. Non-acceptance, feeling ashamed and non-affordability has been reported as major barriers to getting desired health care. The transgender community was examined for future interventions in response to their opinions, and they feel that awareness and preventive initiatives can improve their quality of life, and they say to enrich them with prevention and awareness programmes for effective STD control.

CONCLUSION

It was concluded that lack of education among transgender people causes a poor understanding of Sexually Transmitted Diseases. According to the findings, they earn money by begging, dancing, selling sex and blessing births. They engage in unsafe sexual contracts while being aware of the available protection for condom use. Their understanding of indications and symptoms, as well as the availability of treatment clinics is very limited depending on self-medication. Transgender people have urged that health issues such as STDs, including HIV, should be addressed through information and awareness campaigns.

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