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A J S M U

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A J S M U

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EDITORIAL

Anti-vaccine Campaign; Ethical Concerns

Shabih H. Zaidi

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'And whosoever saveth the life of one it shall be as if he had saved the life of all mankind'[1].

The Noble Quran has clearly mandated that every life is precious. And yet, the recent stories of loss of life at the hands of a nanoparticle is a blatant reminder of the vulnerability and mortality of mankind—A virus whose weight was measured at 0.85 attograms or about one millionth of a trillion grams. It appears that the entire planet full of 7 billion human beings is at the feet of just 1 gram of viral load. And yet man is arrogant! Johns Hopkins Coronavirus resource centre announced 3,596,142 confirmed cases on 5 May 2020. With total 251,718 deaths, in 187 countries, and rising.[2] The virus did not differentiate between the rich and the poor, the powerful and the powerless, developed or developing, as it hit the British Prime Minister Boris Johnson with near enough the same ferocity as Dr Furqan of Karachi. Boris survived due to timely intervention, Furqan succumbed due to lack of it. The blame game has already started. More than 100 health professionals, primarily of Asian and African descent have already succumbed in the U.K. The delay or inefficient supply of PPE has been a matter of much debate here. What a tragedy indeed!

Rationing despite ample resources in the wealthy countries can hardly be justified.

Earlier on, we saw crops after crops of people dying, in each country, right from Wuhan to the outposts of Northern Canadian territories. Some cities like London, Rome, New York could not even find appropriate burial places for their dead.

Pandemics are known to change the direction of human history. The world does not remain the same once the pandemics hit Circa 5000 BC, the first known pandemic annihilated a prehistoric city in China. Around 439 BC, following the war between Athens and Sparta, 100,000 people died in Ancient Greece. In Antonine plague of 165-180 AD, great physicians like Galen had to escape Rome[3].

The Black Death of 1346-1353 down to Spanish flu of 1918, and then Asian flu of 1957-58, AIDS epidemic of 1981, H1N1 of 2009 -2019, Ebola of 2014-16, the Zika of 2015, SARS and MERS of recent memory to the present COVID-19 are recorded in human history as game changers.[4]

Have we learnt any lessons at all?

Politicians are globally known for their selfish attitude. Generally, they are greedy, power hungry, self-centred and dishonest. What happened during the COVID-19 crisis can only be labelled as the failure of politicians, governments, and powerful people. They had been too busy expanding their powers, imposing wars on poor nations, or simply ignorant of their priorities.

Ethics is the mother of morality. Medical ethics deals with applied ethics in medicine. Bioethics is the study of moral values applied in medicine and allied fields.

It appears that states failed at all levels of ethics. NHS, UK, was introduced after the Second World War by Ernest Bevin. a miner's son from South Wales near Rhonda valley, famous for coal mines, TB, Typhoid, Cholera, Meningitis and many other killers. Bevin revolutionized healthcare in this country. Based upon egalitarian values and social justice, it opened its gates to one and all. Healthcare at the first point of contact based upon needs not greed, affordability, or status, it provided the same service to the duke as to the dust man.

I joined the NHS, when it was only 20 years old. Some of the pre-war isolation hospitals and sanatoria were our training centres. A consultant, a matron and a hospital secretary ran the service smoothly, as you may have seen in the old films. NHS produced such heroes as Adeeb Rizvi, Rab, Saleh Memon, Fazle Elahi, Khwaja Sadiq, Shafi Qureshi, Saeed Qureshi, and numerous pioneers of health and education in Pakistan. But then things changed.

NHS expanded overtime. Knowledge and skills have expanded exponentially over the last several decades. What began as a service to the sick has now been

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merged with social care. It has literally broken its back. Ageing population means co-morbidities duly augmented in the West by lack of family support. Western culture is like a ragged piece of Napoleonic tapestry, with unmendable holes and fibre which is threadbare. Lack of family support means no one is there to take care of the elderly. So, Mother's Day is important as is a Christmas card, as a polite reminder that we care but we can't live with you.

In contrast to that, look at the Eastern culture, which is like a finely woven, intricately patterned Persian gleem. Every fibre intertwined with the other, called *tana bana* giving it strength and durability. To have an elderly in the house is a blessing for Eastern people.

So the COVID-19 demolished the elderly folks living in care homes, like the Australian bush fire. Could a vaccine save loss of human life? Possibly yes. But here is what is happening on the vaccine front. Anti-vaccine campaign has already started even as the scientists are busy finding one.

Pakistan is one of the two or three countries in the world where Polio continues to remain a nightmare. The whole world knows who is behind this. The faultline seems to lie in certain areas where ignorance continues to remain a bliss and the clergy rules. On 6 May 2020, a Pakistan TV channel duly highlighted a worrying situation that many children have failed to be vaccinated in Pakistan due to the lockdown. A few cases of measles and mumps have been reported in Karachi in April 2020. Sad but true.

A few years ago, similar opposition was faced by the volunteers who went to the mountainous terrains of northern Pakistan to introduce iodised salt as goitre is pandemic in that region. Strong resistance including the false news that anyone who consumed iodised salt would become either impotent or sterile, led to near failure of the project. Some religious scholars were against to build a dam due to ignorance that water from dam is still water and would not be suitable to make ablution.

A paediatrician published a report a few years ago, linking up some cases of autism with the MMR. Many parents in the U.K. as indeed in several Eastern European countries thereafter refused to get their children vaccinated. Numerous cases of measles were reported in the last few years worrying public health authorities.

And now comes the latest story. COVID-19, a nanoparticle which is no more than a fat covered genetic material, has brought the world to its knees in the spring of 2020.

Intense search for drugs or vaccines is going ahead. So, last week it was announced that an Oxford University lab is on the verge of finding a vaccine. However, it was developed from the cell line of a human foetus aborted in 1972, but preserved for scientific research. Immediately, the ethicists jumped into the ring. A report published by the renowned Bioethics group, raised their concern on the morality of the use of an aborted foetus.[5]

All monotheistic faiths condemn abortion unless it is to save the mother's life. Much debate has gone on to lower the age of allowable abortion from 22 weeks to 14 weeks of gestation, as the neural crest develops immediately after the 14th week. And the neural crest is the precursor of the brain which means conscience which means the heart is already developed and the foetus is alive though vegetative in nature.

The bone of contention is the use of aborted foetal tissue for developing germ cell line. Until recently, spare embryos after an IVF cycle were saved for such research; but now the Islamic countries in particular have mandated that such spare embryos should be allowed to perish naturally.

Anti-vaccine campaigners argue that if an anti COVID-19 vaccine is developed from a human embryo, they will boycott it.[5] What is worse is that a world champion of a very famous sport of a huge following has emerged as the anti-vaccine campaigner. What can one say except repeat the same old saying that you can debate with a wise person but shouldn't argue with an ignorant one?

Scientists have solved at least one dilemma. That the embryonic tissue may not be needed as other sources may be available. But the point is that with thousands of deaths across the world are you looking for a messiah to come save you?

For God's sake, be humane, be positive and save mankind scientifically. Can any faith based upon rationality and reason justify loss of human life or disabilities? And the same message goes out to the opponents of Polio immunization.

Let knowledge and reason prosper! Let sense prevail!

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ORIGINAL ARTICLE

In Vitro Assessment of Antimicrobial Potential of Ethanolic and Aqueous Extract of *Phlomis Umbrosa* Against Some Highly Resistant Pathogens

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ABSTRACT

Objective: To find out the antibacterial potential of ethanolic and aqueous roots extract of *Phlomis umbrosa L.* against both Gram positive and Gram negative isolates

Methodology: Disk diffusion method according to Clinical Laboratory and Standards Institute (CLSI) standard was used to examine the in vitro antibacterial activity of *P. umbrosa* extract while minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using broth dilution technique. Miles and Misra technique was also utilized to count the number of colonies CFU/mL of bacteria at different concentrations of extract.

Results: All the studied strains showed a diverse range of vulnerability against both ethanolic and aqueous plant extract. Among all tested isolates, ethanolic extract of *P. umbrosa* showed highly significant activity against Gram positive isolates i.e. *S. aureus* (20.1 mm) and *B. subtilis* (22.9 mm) with least MIC (12.5 mg/mL) and MBC (12.5 mg/mL) as compared to Gram negative isolates. A progressive decline in bacterial colonies (CFU/mL) was observed in Miles and Misra technique. One way ANOVA followed by postHoc Tukey test showed the significant differences in antimicrobial activities of plant extract with two tested antibiotics i.e. Amoxicillin and Erythromycin (10 µg/disc) as positive control at p-value of 0.05. The antimicrobial activity of this plant exhibit may be due to the presence of such chemical constituents namely monoterpenoids and sesquiterpenoids compounds.

Conclusion: It is concluded that roots ethanolic extract of *P. umbrosa* has a promising antibacterial potential so it can also be used as an alternative medicine to treat different infections for reducing bacterial resistance and side effects associated with antibiotics.

Key words: *Phlomis umbrosa;* amoxicillin; erythromycin; antimicrobial activity; Miles and Misra method

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INTRODUCTION

For centuries, the use of medicinal plants as an essential source of medicinal agents for maintaining good health is well known and a number of novel compounds have been isolated from such plants[1]. Around 80% of population relies on traditional medicines primarily from herbal origins. Secondary metabolites as tannins, flavonoids, terpenoids, and alkaloids extracted from plants with high structure diversity possess strong antimicrobial significance[2].

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Moreover, rising credence is being given to the extraction and augmentation of distinct medicines from plants in modern culture[3]. Studies show that large populations heavily rely on medicinal plants to fulfill their primary health care needs[4].

Antimicrobial resistance (AMR) against pathogenic bacteria or multidrug resistant bacteria (MDR or superbugs), is a serious global threat for humans, animals, environmental health, and one of the major causes for endangering the worth of antibiotics. Reduction in financial inducement, inadequacy of newer drugs, over the counter availability, poor hygiene and sanitation, and misuse of antibiotics are attributable to the crisis of antimicrobial resistance. Comprehensive efforts are needed to minimize the pace of resistance by studying emergent microorganisms, resistance mechanisms, and antimicrobial agents[5].

This dilemma features the immediate demand for advance strategies and latest classes of antibiotics. In developing countries, plants are widely used as a source of medicine where traditional medicine plays a major role in primary healthcare. About 80% of individuals from these countries still use plants as remedies for many diseases, using their own personal recipes which have been passed down through generations. Clinical plant utilization provides a steady inspiration of bioactive antimicrobial agents with less toxicity, broad microbial coverage and good pharmacokinetics without chemical modification[6,7].

The genus *Phlomis* belonging to family *Lamiaceae* is a perennial herb. More than 100 species are reported from this genus widely distributed throughout Asia, Europe and Africa. Number of species are well known for their aromatic and medicinal function. Many species of this genus have usage for medicinal and aromatic purposes. Folk uses of different species of this plant include as stimulant, carminative, tonic, appetizer, antidiuretic, and in herbal tea. A number of biological activities are reported such as antimicrobial, anti-ulcerogenic, immunosuppressive and free radical scavenging, and anti-inflammatory[8,9].

Previously, several studies reported on screening of antimicrobial activities of several medicinal and traditional plants[10-12]. The antimicrobial activity of essential oil of flowers extract of *Phlomis umbrosa* was also evaluated[13]. However, according to the literature survey, no studies have been conducted on screening of antimicrobial potential of root extract of *P. umbrosa*. Therefore the aim of this study was to evaluate the antimicrobial activity of aqueous and ethanolic roots extract of *P. umbrosa* in comparison with standard marketed antibiotics.

METHODOLOGY

Collection of antimicrobial agents and chemicals
Dried powder roots extract of *Phlomis umbrosa* were recieved from a manufacturer and supplier M/S. HUNAN NUTRAMAX INC. (Changsha, China) with a batch number of PUE-160419 and was identified by a pharmacognosist and meritorious professor of University of Karachi, Pakistan. The standard antibiotics disc such as erythromycin and amoxicillin were purchased from distributor Musaji Adam and Sons (Karachi, Pakistan). Methanol was purchased from Sigma-Aldrich (St. Louis, USA). DMSO was procured from Merck (Darmstadt, Germany), nutrient agar and Mueller Hinton broth (MHB) were collected from Oxoid LTD (Hemisphere, England). Both antibiotics discs were of 10 µg.

Collection of different clinical and standard ATCC strains
Gram positive and Gram negative highly resistant clinical strains including *Bacillus subtilis* (MT 0250), *Staphylococcus aureus* (MT 0484), *Streptococcus pyogenes* (MT 0258), *Salmonella enterica* (MT 0691) were obtained from pathological laboratories of Dr. Essa's Laboratory and Kutiyana Memon Hospital in Karachi, Pakistan. ATCC standard cultures strains used in this study were *Bacillus subtilis* (ATCC 04262), *Staphylococcus aureus* (ATCC 08854), *Streptococcus pyogenes* (ATCC 10258), *and Salmonella enterica* (ATCC 10691). The received bacterial strains from different laboratories were identified by pathologists based on their cultural, morphological, and biochemical reactions.

Antibacterial activity
Inoculation of bacterial strain: Antibacterial activity against collected isolates was performed using well reputed disc diffusion method which was first reported in 1940. The Clinical Laboratory and Standards Institute (CLSI) have approved standards for testing of different clinical and highly resistant pathological strains[14]. Two different antibiotics (Amoxicillin 10 µg and Erythromycin 10 µg/disc) as positive control and *Phlomis umbrosa* ethanolic and aqueous extract disc (6 mm in diameter) were used to evaluate their antibacterial activity. The discs of plant extract were prepared using concentrations of (20 mg/mL, 30 mg/mL, 40 mg/mL and 50 mg/mL) dried powder of *P. umbrosa* obtained by both aqueous and alcoholic solvents. Whereas, Dimethyl sulfoxide (DMSO) was used as negative control in whole study because, it is considered as a non-toxic solvent[15]. These prepared discs were placed on the pre-inoculated Mueller Hinton Agar (MHA) plates with different collected bacterial cultures and were placed in incubator for 24 h at 37^{o}C.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC): The concentration of extract at which the growth of clinical isolates was inhibited is refered to as minimum inhibitory concentration (MIC) while the concentration used for complete killing of clinical isolate is known as minimum bactericidal concentration (MBC). The MIC and MBC of ethanolic and aqueous root extract of *P. umbrosa* were determined using broth dilution method[10]. As much as 100 µL crude root extract of plant was taken to prepare 50 mg/mL concentration, then further dilution was made using serial dilution method up to 0.5 mg/mL. Tween 20 was used to solubilize the different concentrations of plant extract in nutrient broth. Each bacterial strain was adjusted to the concentration of 1×10^{8}cfu/mL in

respective media. The plates of different bacterial strains were incubated at 37°C for 24 h. The growth of bacterial strains was examined by the turbidity found in their respective test tubes. The prepared broth culture was incubated at 37°C for 24 h in Tyramide Signal Amplification (TSA) system.

Miles and Misra method: The technique of Miles and Misra was used to examine the antibacterial potential of ethanolic and aqueous root extract of *P. umbrosa* at different concentrations by counting the number of colonies forming units (CFU) of bacteria after exposure to extract[16]. The test isolates suspension was prepared in pH 7 PBS buffer and the inoculums of tested organisms defined at 1.5×10^8 cells/ml using the 0.5 McFarland index.

Statistical analysis

All the above defined experiments were performed in triplicates. The obtained results of all experiments were presented as mean ± standard deviation (SD). Statistical software (SPSS version 23) was used to analyze the obtained data by applying one way ANOVA with Tukey's post Hoc Test at minimal level of significance of $p < 0.05$. Furthermore, Pearson correlation was also applied to evaluate the relationship between the concentrations of plant extract with their antimicrobial effects.

RESULTS

The extract of *Phlomis umbrosa* roots with ethanol and also in water was light brown in colour and exhibited great antibacterial activity against some highly resistant clinical pathogens including *B. subtilis, S. aureus, S. pyogenes* and *S. enterica*. The obtained antibacterial activities of ethanolic and aqueous extract of plant are dose dependent and found within ranges of (5.9 ± 1.074 mm to 22.6 ± 1.195 mm) in ethanolic and (4.0 ± 0.821 mm to 17.9 ± 0.995 mm) in aqueous extract at different concentration as presented in Table 1 and 2 respectively. Among all the tested clinical isolates, Gram positive isolates i.e. *S. aureus* and *B. subtilis* were highly susceptible to the ethanolic plant extract at 50 mg/mL i.e. 25.6 ± 2.009 and 24.8 ± 1.371 respectively. However, the obtained zones of inhibitions from plant extract, primarily ethanolic extract, were much greater and significant than two tested antibiotics. The susceptibility of ethanolic and aqueous extract of plant also tested antibiotics against some ATCC cultures presented in Table 3 and 4. The obtained values of ZIs were significantly different at different concentrations of ethanolic plant extract against clinical isolates i.e. *B. subtilis (P = 0.016); S. aureus (P = 0.021); S. pyogenes (P = 0.027)* and *S. enterica (P = 0.015)*. Moreover, highly significant differences (*P>0.005*) were found in ZIs of amoxicillin against *B. subtilis* and *S. enterica* while significant differences (*P>0.05*) were found in antibiotics against tested isolates compared to extract at 30 mg/mL concentration. The values of MIC and MBC of ethanolic root extract of *P. umbrosa* indicated the positive results in screening test of different clinical highly resistant microbes (Table 5). The lowest and same MIC and MBC values were observed against *B. subtilis* i.e. 12.5 mg/mL while the highest were against *S. enterica* as 20.0 mg/mL. The Miles and Misra test showed a gradual decrease in log of CFU/mL of bacteria with increasing concentration of both aqueous and ethanolic root extract of *P. umbrosa* as indicated in Figure 1. Among all tested organisms, log of CFU/mL of *S. aureus* and *B. subtilis* were decrease up to 3.7 and 3.9 respectively in ethanolic extract.

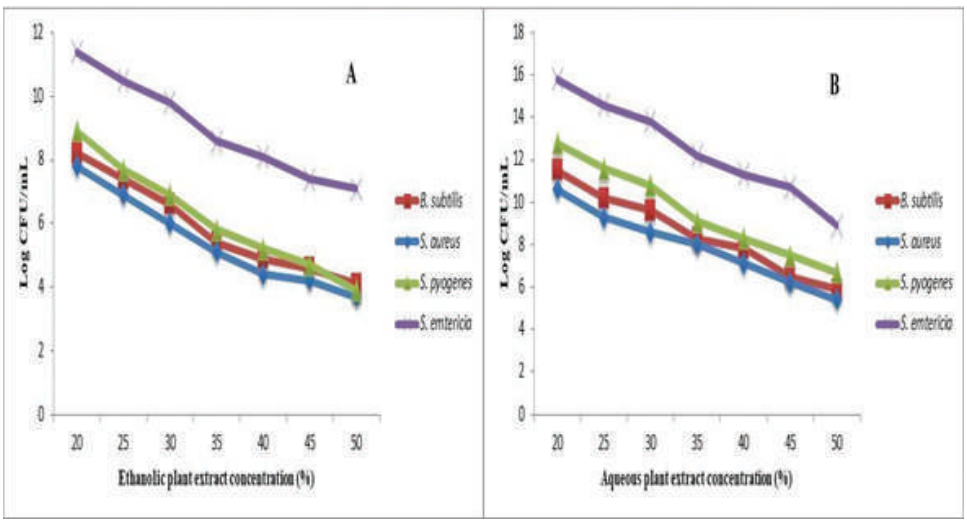

Figure 1: (A) Log of CFU/ml at different concentrations of ethanolic extract (B) Log of CFU/ml at different concentrations of aqueous extract

Table 1: Antibacterial Activity of Ethanolic Plant Extract and Different Antibiotics Against Clinical Isolates

Clinical isolates	Zone of inhibitions in mm						Standard drugs	
	Plant extract (Ethanolic)						Amoxicillin	Erythromycin
	20 mg/mL	30 mg/mL	40 mg/mL	50 mg/mL	Pearson correlation	P-value	10 µg/discs	10 µg/discs
B. subtilis	13.8 ± 1.326	15.5 ± 1.461	18.1 ± 0.513	20.1 ± 1.254	0.998	0.016	9.1 ± 1.224**	12.1 ± 1.724*
S. aureus	14.5 ± 0.862	17.0 ± 0.472	21.7 ± 1.316	22.9 ± 1.614	0.985	0.021	12.6 ± 1.375*	11.5 ± 1.215*
S. pyogenes	10.8 ± 1.246	14.2 ± 1.725	17.6 ± 1.258	20.7 ± 1.290	0.994	0.027	11.6 ± 1.224*	12.0 ± 1.552*
S. enterica	5.9 ± 1.514	8.5 ± 0.914	12.6 ± 1.413	15.7 ± 1.309	0.985	0.015	7.9 ± 1.256**	11.6 ± 0.646*

n=10, Average values ± SD
*p = 0.05 significant as compared to control
**p = 0.005 highly significant as compared to plant extract at concentrations of 50 mg/mL

Table 2: Antibacterial Activity of Aqueous Plant Extract and Different Antibiotics Against Clinical Isolates

Clinical isolates	Zone of inhibitions in mm							
	Plant extract (Aqueous)						Amoxicillin	Erythromycin
	20 mg/mL	30 mg/mL	40 mg/mL	50 mg/mL	Pearson correlation	P-value	10 µg/discs	10 µg/discs
B. subtilis	10.0 ± 1.248	12.2 ± 1.215	14.2 ± 1.214	15.6 ± 0.825	0.992	0.024	9.1 ± 0.813*	12.1 ± 1.226
S. aureus	9.4 ± 0.925	12.6 ± 1.425	15.0 ± 1.242	17.9 ± 0.635	0.993	0.014	12.6 ± 1.074	11.5 ± 0.673*
S. pyogenes	7.2 ± 0.725	8.7 ± 1.346	11.6 ± 1.220	13.0 ± 0.971	0.987	0.021	11.6 ± 1.577	12.0 ± 0.804
S. enterica	4.0 ± 0.901	5.9 ± 1.250	7.3 ± 1.457	9.1 ± 0.729	0.994	0.047	7.9 ± 0.703	11.6 ± 0.750*

n=10, Average values ± S.D
*p = 0.05 significant as compared to control
**p = 0.005 highly significant as compared to plant extract at concentrations of 50 mg/mL

Table 3: Antibacterial Activity of Ethanolic Plant Extract and Different Antibiotics Against Standard ATCC Strains

Clinical isolates	Zone of inhibitions in mm							
	Plant extract (Ethanolic)						Amoxicillin	Erythromycin
	20 mg/mL	30 mg/mL	40 mg/mL	50 mg/mL	Pearson correlation	P-value	10 µg/discs	10 µg/discs
B. subtilis (ATCC 04262)	16.3 ± 1.451	19.3 ± 1.126	21.6 ± 1.341	24.8 ± 1.157	0.996	0.014	16.8 ± 0.742*	20.4 ± 1.265
S. aureus (ATCC 08854)	18.2 ± 1.625	20.2 ± 1.134	22.4 ± 1.327	25.6 ± 1.709	0.994	0.021	19.4 ± 0.912*	22.3 ± 1.524
S. pyogenes (ATCC 10258)	13.2 ± 0.936	16.3 ± 1.615	19.7 ± 1.184	23.4 ± 1.525	0.991	0.018	17.3 ± 0.742*	22.0 ± 0.814
S. enterica (ATCC 10691)	8.4 ± 1.604	11.5 ± 1.260	14.0 ± 1.610	18.2 ± 0.723	0.988	0.009	14.7 ± 1.459	15.3 ± 1.230

n=10, Average values ± S.D
*p = 0.05 significant as compared to control
**p = 0.005 highly significant as compared to plant extract at concentrations of 50 mg/mL

Table 4: Antibacterial Activity of Aqueous Plant Extract and Different Antibiotics Against Standard ATCC Strains

Clinical isolates	Zone of inhibitions in mm							
	Plant extract(Aqueous)						Amoxicillin	Erythromycin
	20 mg/mL	30 mg/mL	40 mg/mL	50 mg/mL	Pearson correlation	P-value	10 µg/discs	10 µg/discs
B. subtilis (ATCC 04262)	12.3 ± 0.743	14.1 ± 1.424	16.8 ± 1.472	18.7 ± 1.273	0.995	0.012	16.8 ± 0.736	20.4 ± 1.363*
S. aureus (ATCC 08854)	10.5 ± 0.642	13.8 ± 0.757	16.8 ± 1.635	18.0 ± 1.356	0.988	0.026	19.4 ± 1.324	22.3 ± 1.642*
S. pyogenes (ATCC 10258)	8.6 ± 0.853	10.9 ± 0.681	13.0 ± 1.635	16.7 ± 1.635	0.986	0.024	17.3 ± 1.206	22.0 ± 0.752*
S. enterica (ATCC 10691)	6.2 ± 0.692	8.4 ± 1.624	9.9 ± 1.344	12.3 ± 0.843	0.992	0.031	14.7 ± 1.526	15.3 ± 1.532

n=10, Average values ± S.D
*p = 0.05 significant as compared to control
**p = 0.005 highly significant as compared to plant extract at concentrations of 50 mg/mL

Table 5: MIC and MBC of Ethanolic and Aqueous Extract Against Clinical Isolates

Clinical Isolates	Ethanolic Extract		Aqueous Extract	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
B. subtilis	12.5 ± 1.381	12.5 ± 1.513	15.0 ± 1.424	15.0 ± 1.753
S. aureus	12.5 ± 1.514	15.0 ± 1.361	17.5 ± 1.467	17.5 ± 1.646
S. pyogenes	15.0 ± 0.724	15.0 ± 1.525	20.0 ± 1.624	20.0 ± 1.632
S. enterica	20.0 ± 0.510	20.0 ± 1.424	25.0 ± 0.585	30.0 ± 0.745

n=10, Average values ± S.D

DISCUSSION

One of the most pressing issues around the world is anti-microbial resistance. In order to overcome this problem, there is an immediate need of new antimicrobial agents with novel systems of activity. Scientific strategies are being adopted based on continuous planning and processing by researchers to find out new antimicrobial agents with minimum side effects and maximum efficacy. In developing countries, traditional medicines are gaining importance persistently. A number of plant and herb species are available in the market with strong antimicrobial potential[17].

In the present study, the antibacterial potential of *Phlomis umbrosa* was determined using ethanolic and aqueous crude root extract at different concentrations (Table 1 and 2). It was observed that with the increase in concentration, sensitivity of organism towards sample also increased. Pearsons correlations was used to show dose dependent effect of plant extract on their antibacterial efficacy. The correlation values of Pearson correlations analysis also indicated the dose dependent antibacterial activities of plant extract in both ethanolic and aqueous medium.

However, ethanolic extract was found to be more effective compared to aqueous extract against Gram positive isolates than Gram negative isolates. Miscibility of ethanol with water is well known because of its low polarity compared to water and active microbial compounds present in plants are mostly saturated and non-polar. Lipophilic compounds are difficult to extract in water, while ethanol and methanol are good choices of solvents in such cases[18,19]. Earlier, Guang-hui et al studied the phytochemical components accounted for 91.53% of the all peak area in *P. umbrosa* flowers extract. They reported the antimicrobial activity of this plant occuring due to the presence of such chemical constituents as monoterpenoids and sesquiterpenoids compounds, the major components were toluene, phthalic acid, diisobutyl ester, á-linalool, diphenylamine, and 1-octen-3-ol[13].

A number of antibiotics reported resistance against these collected pathogenic strains. Literature illustrates that Penicillin G, Gentamycin, lincosamide and tetracycline show resistance pattern against these organisms. In case of both aqueous and ethanolic extracts, maximum activity was found against *Staphylococcus aureus* followed by *Bacillus subtilis* with zone of inhibition (22.9 ± 1.195) and (20.1 ± 0.755) at concentration of 50 mg/mL respectively. These findings are in line with the study performed by Hui et al., in 2008 on antimicrobial activity[20]. Moreover, Morteza-Semnani et al., in 2006, reported the antibacterial activity of *Phlomis* species against some highly resistant pathogen including *E. coli, K. pneumonia, S. aureus, S. sanguis*, and *P. aeruginosa*[21]. However, limited data is available on antimicrobial studies of this plant in Pakistan, but according to our study, this plant would be a promising future candidate against microbes.

S. enterica was least sensitive isolate against test sample except at 50 mg/mL with zone of inhibition (15.7 ± 1.419). Erythromycin (10 µg/discs) was more effective compared to amoxicillin (10 µg/discs) used as positive control. Results were also noticed against ATCC cultures and it was observed that standard cultures were more sensitive towards plant extract in comparison to clinical isolates. Mounting graph of resistance among clinical isolates may be contributed to by a number of reasons playing their role in lack of antimicrobial potential. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were also determined (Table 5). Least value of MIC was analyzed against *S. aureus* (12.5 ± 1.011 mg/ml) with MBC value of 15.0 ± 1.254 mg/mL. ANOVA followed by post Tukey showed significant differences in antimicrobial activities of plant extract with two tested antibiotics.

The results of Miles and Misra technique also showed the inhibitory effect of ethanolic as well as aqueous extract of *P. umbrosa* against both tested clinical and standard ATCC cultures by lowering down the log of CFU/ml at increasing concentrations of plant extract. Inhibitory effect of this plant even at low doses also supports its use in folk medicine for not only the treatment of infectious diseases but also for other ailments. Therefore, it appears to be promising for treatment of bacterial infections. Activity directed bioassay along with extensive activity of bioactive compounds is required for further investigation of compounds actually responsible for antimicrobial potential.

CONCLUSION

Study showed that crude root extract of *P. umbrosa* have potent antimicrobial potential and produce both bacteriostatic and bactericidal effects. The low values of MIC and MBC reflected the antibacterial potency of this studied plant. It is recommended that systematic and focused researches are required to get new chemical, biological and pharmacological findings.

Authors' contributions: Dr Yousra Shafiq designed the initial study, searched related literature, collected data and conducted the study. Dr. Muhammad Arif Asghar designed the initial draft of manuscript, reviewed and made corrections. Dr Huma Ali worked on literature search, review and finalized results and discussion. Dr Saima Abedien reviewed the literature, and contributed to the discussion. Dr Ahad Abdul Rehman reviewed the study outcomes and conclusion. All authors contributed to the final manuscript.

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ORIGINAL ARTICLE

Prevalence of Smartphone Addiction Among College and University Students in Saudi Arabia: A Multicenter Study

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ABSTRACT

Objective: To find out the prevalence of smartphones addiction among college and university students in the Kingdom of Saudi Arabia (KSA) and its association with demographic variables

Methodology: A study was conducted in all provinces of KSA between December 2015 to June 2016. An electronic survey was sent to students' clubs of various universities. Smartphone Addiction Scale (SAS) was used which had 33 closed-ended questions. Total score of SAS was calculated and three categories of addiction was made: low, moderate, high. Questionnaire also included sociodemographic questions, and smartphone usage patterns and addiction behaviour.

Results: The total number of participants was 1941 (response rate of 80.9%) students representing most of the provinces of Saudi Arabia. The prevalence of smartphones addiction was found to be 19.1%. Female participants were more addicted than males ($p < 0.001$). Medical students were less smartphone-addicted than non-medical college students ($p = 0.007$). Inverse and significant relation between age onset of mobile usage with its addiction was found ($p = 0.02$).

Conclusion: Prevalence of smartphone addiction found in the present study was not high. However, female and non-medical colleges students were found to be more addicted to smartphones. Those who started using smartphones at an early age were also found to be more addicted. Less awareness about harms of smartphones addiction could be one of the reasons of having high prevalence of addiction among non-medical students and those who started using at a younger age.

Key words: Smartphones, addiction, epidemiology, Saudi Arabia, students

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INTRODUCTION

The smartphone appears to facilitate learning by increasing interactivity and providing easy access to resources and information. Enhancing communication with colleagues and teachers and sharing information, are among the key advantages or factors of smartphones[1,2]. However, apart from its advantages, there are some disadvantages as well. Its overuse can cause memory and concentration problems, physical abnormalities, changes in eating behaviour and sleep disturbances[3-5]. Smartphone usage may be associated with low academic performance because it diverts from studying, exam preparation, fulfilling requirements and following planned schedules etc.[6]

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Smartphone addiction has been defined as the overuse of smartphones to the extent that it disturbs the users' daily lives[7]. Psychological impairment could be related to smartphone usage[8,9]. Many factors play a significant role in increasing or decreasing the probability of its addiction. These factors can be age, gender, socioeconomic status, physical fitness, self-esteem, education and hobbies. Technical factors such as the availability and speed of the internet have a role in the rate of use[9,10].

Addictions can be classified as either substance or non-substance. Substance addiction includes drugs and alcohol, whereas non-substance abuse includes activities likes gambling, games, internet, shopping[11]. The prevalence of smartphone addiction is higher than internet addiction due to difference between mobile phone and the internet. Furthermore, if the features of smartphones will increase, the likelihood of addiction will also increase.[12,13]

The percentage of smartphones users is rapidly increasing in the Saudi population. Hejab et al, reported that Australia, United Kingdom, Sweden, Norway,

Saudi Arabia and the United Arab Emirates all boast of smartphone adoption rates above 50%[13]. However, among the recent studies which were published from Saudi Arabia, all were based on a single center[13-16] and reported data from Riyadh, Jeddah, Najran etc. To the authors' best knowledge, this study is the first of its kind which was conducted in multiple universities across the Kingdom of Saudi Arabia. Hence, the purpose of the present study was to find the prevalence of smartphones addiction among college and university students across the Kingdom of Saudi Arabia (KSA). Furthermore, the association of demographic variables with smartphones addiction was also studied.

METHOD

This cross-sectional study was conducted in various provinces of KSA between December 2015 and June 2016 after receiving approval from Research Committee at the Saudi Council for Health Specialties of the joint programme of family medicine at the eastern province of Saudi Arabia. The study was conducted in various colleges and universities across KSA. All undergraduate students (male or female) aged from 17 to 27 years were eligible to participate in the study. Students who were either diagnosed or received any treatment for smartphone addiction were not eligible to participate. Participation was voluntary, and participants could leave the survey at any point.

The sample size was calculated by using Raosoft calculator for sample size. After allowing only a 2% chance of error, the obtained sample size was 2399. Then the survey was sent to student clubs at all colleges and universities. Simple random sampling was used to recruit study participants.

The prepared questionnaire had three sections: (1) socio-demographics, (2) smartphone usage patterns and addiction behaviour, and (3) Smartphone addiction scale (7). Sections one and two were to include background data as a determinant of smartphone addictions. The questionnaire was translated first by a certified translation office specialized in translating official governmental papers and college researches from English to Arabic, then reviewed by two consultants of family medicine to check that the words used in each question were giving the nearest meaning to the original English question. Finally, the Arabic version of the scale was retranslated to English. The 'Cronbach's Alpha was 0.967.

The survey was designed electronically, then authors started contacting the leaders of student clubs in the universities explaining about the study, objectives, approval, how to share, and ensuring confidentiality. After the leaders received official approvals from their respective universities, the weblink was emailed to students who were participating in the study with instruction on how to fill the self-administered questionnaire after giving online consent.

Smartphone addiction scale (SAS) is a scale to analyze smartphone addiction having 33 questions with six-point Likert scale ranging from '1=strongly disagree' to '6=strongly agree'. Higher scores indicate the higher risk of smartphone addiction. A total score for each participant was calculated by adding scores of all questions. Smartphone addiction was categorized as low if total score of a participant was between 33 and 87, intermediate if it was between 88 and 142, and high if score was between 143 and 198. Those who were under the third category were considered as smartphone addicts.

Statistical Package of Social Sciences (SPSS V.23) was used for data entry and analysis. Independent variables, which included age, gender, marital status, GPA, medical and non-medical students, years since using smartphones and its daily usage, were used for descriptive and inferential analysis. Due to the categorical nature of independent (gender, marital status, type of college--medical or non-medical, etc) and dependent variables, Chi-square test was used. However, only significant results were presented in the tables. Level of significance used for p-values was 0.05.

RESULTS

After inviting 2399 students from twenty different universities in KSA, 1941, students sent their feedback by filling the questionnaire. Hence the response rate was 80.9 percent. Evaluation of the responses revealed that there were 874 (55.3%) male and 707 (44.7%) of female participants in the study. Majority of these were single (n=1360, 86%) and only 14% (n=221) were married. Minimum age of a respondent was 17 years, and the oldest ones were 27 years old, the average age was 21.5(±2.68). It was found from further assessment of demographic variables that the number of medical college students in the study was 467 (29.5 %) and the rest were from non-medical colleges (n=1114, 70.5%). Total 58 percent of the participants belonged to the 1st to the 3rd year of college, and 42 percent were from 4th to 7th-year students. Approximately 70 percent of the participants started using smartphones when they were less than 18 years old.

Evaluation of SAS score revealed that prevalence of smartphone addiction among university students in the Kingdom of Saudi Arabia was 19.1%, low-risk group

Table 1: Smartphone addiction in relation to gender and students

		Score Group SAS			P-value
		Low	Intermediate	High	
Gender	Male	170(19.5)	565(64.9)	136(15.6)	<0.001*
	Female	108(15.3)	433(61.3)	165(23.4)	
I am a student at medical school	Yes	95(20.5)	301(64.9)	68(14.7)	0.007*
	No	183(16.4)	697(62.9)	233(20.9)	

*Statistically significant at 0.05 level of significance

Table 2: Participants' perception and age onset of usage of smartphones in relation with SAS score

		Score Group SAS			P-value
		Low	Intermediate	High	
Do you feel addicted to your smartphone?	No	115(52.5)	99(45.2)	5(2.3)	<0.001*
	Mild	98(21.2)	334(72.3)	30(6.5)	
	Moderate	58(9.6)	438(72.5)	108(17.9)	
	Severe	7(2.3)	133(44.5)	159(53.2)	
When did you start using smartphone? (age of onset)	< 15 years	49(14.0)	214(61.0)	88(25.1)	0.02*
	15-18 years	121(17.1)	463(65.3)	125(17.6)	
	18-22 years	82(20.4)	249(61.9)	71(17.7)	
	22-24 years	13(18.8)	45(65.2)	11(15.9)	
	> 24 years	13(29.5)	25(56.8)	6(13.6)	

*Statistically significant at 0.05 level of significance

Table 3: Smartphone addiction concerning its usage

		Score Group SAS			P-value
		Low	Intermediate	High	
How many hours per day are you using smartphones (hours/day)?	< 1 hour	11(57.9)	7(36.8)	1(5.3)	<0.001*
	1 hour	23(45.1)	23(45.1)	5(9.8)	
	1-3 hours	97(34.5)	166(59.1)	18(6.4)	
	3-5 hours	93(19.0)	332(67.8)	65(13.3)	
	> 5 hours	54(7.4)	468(63.8)	212(28.9)	

of smartphones addiction included 17.6% of students out of whom the majority of students (63.4%) were in moderate risk group. Apart from SAS score, students were also asked 'whether they feel they are addicted to smartphones'. Only 220 (13.9%) stated 'not addicted', the rest agreed that they were addicted and their addiction level was varying from mild to severe.

Through the comparison of demographic variables with SAS score, it was found that female students were highly addicted to smartphones compared to male students (Table 1). In addition, students from non-medical colleges (20.9%) were more addicted to the smartphone than the medical students (14.7%) with p-value 0.007 (Table 1).

When students were asked 'do you feel addicted to smartphones?', their responses were following the SAS score (Table 2). As many as 115 (52.5%) out of 219 said that they were not addicted to low SAS score, 334 (72.5) and 438 (72.5) thought addiction level was mild and moderate respectively while their SAS score was intermediate and 159 (53.2) said addiction level was severe while having high SAS scores.

Furthermore, daily usage of smartphones also got significant p-value when tested with SAS score. The trend was increasing and statistically significant, with p-value <0.001 (Table 3).

DISCUSSION

The current study measured and classified smartphone addiction into three categories: high, moderate and low. The high-risk group was considered as smartphone-addicted. The prevalence of smartphone addiction in the present study was found at 19.1 %. However, variation in prevalence was observed when the literature was reviewed. Some studies reported high while some reported average or low prevalence of mobile phone addiction among university students. A study from Riyadh (KSA) got 48% prevalence of mobile phones addiction among university students[17]. An Indian study published in 2014 stated the smartphone addiction was ranging from 39-44% among adolescents[18]. In contrast, studies from Korea, the United Kingdom, Spain, and Switzerland reported prevalence of less than 20% among the studied population[19-22].

Female students were found to be more prone to mobile phone addiction than male students, and it was statistically significant too. This finding is in line with a study published from Turkey where authors found the prevalence was 21% in boys and 39% in girls[23]. Similarly, in Korea, it was 10% in male and 39% in females[24]. Hegazy AA et al. found that girls were 16-18% more prone to be addicted than boys[15]. While many studies did not find any correlation between the gender of the participants and prevalence of smartphones addiction[16,25]. Females use phones to sustain social relationships and to express their emotions[26]. Level of dependency was found to be related to many factors like personality differences between males and females, phone-related behaviours, types of applications favoured by males or females, impulsiveness, cultural and usage pattern or purpose[27,28].

In our study, no relationship between academic grades and the level of risk of smartphone overuse was found. In Riyadh, study showed the academic performance affected by using smartphones[16] and it was supported by a lot of studies[29-31]. High frequency of smartphone usage can cause distractions, impulsivity, passivity and little intellectual effort[32]. Smartphone usage may divert student from studying, exam preparation, completion of assignments, follow the planned schedules and lead them to spend more time in amusement[6].

Daily usage of smartphones was found to be an essential factor which had a significant association with addiction, and this association can be studied repeatedly in the literature. Studies from Riyadh[16], Najran[13], Jeddah[15], South Africa[33], and Korea[24] also showed the same relation between the two variables. In our study, high-risk group spent around 5 hours on their smartphones daily. The Najran[21] and South African[33] study found the same result. In Korea[24], it was noticed that 7 hours was the average time of daily use while James et al, found the students spent nearly 9 hours per day on their mobile phones[34].

Generally, there was an increasing trend of the prevalence of smartphone addiction towards the youngest ages. This is a well-established phenomenon of smartphones addiction and can be found in many previous studies[16,21,22]. In this study, the prevalence of smartphone addiction was the highest among the youngest students and followed the downward trend as age increased. Hence, the early start of smartphone usage could lead to addiction.

In the present study, smartphone addiction was higher among non-medical student compared to medical students. In a Jordanian study[35], they found a significant difference relative to the specialty: humanities students had a double risk of addiction (16%) than natural sciences students (9%). These findings were corroborated by Iranian[36] and Australian[37] studies, and the speciality was found to be playing a major role on other addiction types besides the smartphones addiction, for example gambling addiction in science students or shopping addiction in Art students[38].

Furthermore, there was a significant relation of smartphone addictions objectively to subjectively measuring by SAS survey. Students' perception about addiction was entirely in line with their SAS score. Table 2 shows that those who categorized themselves as not addicted had low SAS scores. Similarly, those who thought they were severely addicted to smartphones had high SAS scores. This showed that students were aware that they were suffering from smartphone addiction. It was important to study how close the relationship between conception and reality is. Realization is the first step towards solving any problem, hence it was a positive indicator that students were admitting their smartphone addiction.

Study limitations: Lifestyle might affect the addiction as the study was conducted in various cities of Saudi Arabia. Possibility of reporting bias cannot be ignored because data was self-reported. The study was done on a specific age group. This study did not include factors related to lifestyle and daily activities which could be affected due to smartphones addiction.

CONCLUSION

Although the prevalence of smartphone addiction was 19.1% which was not as high as reported in literature. However, a large proportion of students had moderate level of addiction and that was the point of concern

because that moderate level could increase to high in future which can increase the prevalence of addiction. Furthermore, female and non-medical college students were found to be more addicted to smartphones. However, students were accepting that they were overusing smartphones, and it was a positive attitude. Steps need to be taken in order to minimize and to prevent its increase. It is required to educate them about the consequences of addiction and how to overcome this problem. Furthermore, smartphones should not be given at a younger age, and only be given when a child can differentiate its healthy and productive use from addiction. Less awareness about harms of smartphones addiction could be one of the reasons of having high prevalence of addiction among non-medical students and those who start using at a younger age.

Authors' Contribution:

Name	Contribution
Rashed Al Boali, Abdulrahman Fouad ALkhateeb	Methodology, Data collection and Analysis
Dr. Waleed Alharbi Dr. Olfat Saleh	Article writing

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ORIGINAL ARTICLE

Diverse Histopathological Changes Due to Cholelithiasis Seen in Surgically Removed Gall Bladder Specimens

Khalid Rashid[1], Bilal Burki[2], Ghansham Ratwani[3], and Saleem Ahmed Somro[4]

ABSTRACT

Objective: To review the diverse histopathological changes found in the cholecystectomy specimens operated for gallstones

Methodology: The study was conducted at surgical ward No. 26 of JPMC for 5 years from January 2012 to December 2017. As per policy, specimens from all patients with symptomatic gall stones who underwent cholecystectomy, were sent for histopathology, the reports were reviewed and frequency of different pathological changes were noted.

Results: We observed not merely chronic cholecystitis but a plethora of different histopathological changes including tuberculosis, premalignant conditions and carcinomas, with almost no suspicion of existing pathology pre-operatively.

Conclusion: This strengthens our belief that every gall bladder specimen should be subjected to histopathological examination.

Key words: Gallbladder Specimens, Cholelithiasis, Histopathological changes

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INTRODUCTION

Cholelithiasis is the commonest disorder of gall bladder, It is prevalent both in the east and the west, although the pathogenesis may be different. Common in females above the age of forty, stones are basically of three types: pigment stones, composed of bile pigments; cholesterol stones; and mixed stones composed of cholesterol, bile pigments and bile salts in various combinations. In the Asian population, 80% cases are pigment stones while 80% of stones found in Europe and USA are cholesterol stones and mixed stones[1].

Chronic cholecystitis is associated with gallstones in over 90% of cases. Supersaturation and infection of bile are the factors that lead to stone formation and also contribute to the onset of inflammatory changes in the gall bladder wall.

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A high molecular weight glycoprotein called Mucin plays an important role in protecting the gall bladder mucosa from the detergent effects of bile, while on the other hand, when secreted in large amounts, acts as a pronucleating factor and has been implicated in gallstone disease[2].

With the passage of time, stones grow in size and number within the gall bladder, causing continued inflammation, which brings about various pathological changes in its wall including malignancy.

The aim of our study is to review the diverse histopathological changes found in the gall bladder specimens operated for gallstones in the local population.

METHODOLOGY

This is an observational study of 680 cases of symptomatic gallstone disease which were operated on in the Department of Surgery, ward 26, JPMC. Both open and laparoscopic procedures were performed. The duration of study was five years, from January 2012 to December 2017.

All patients were adults above the age of 12 years and the removed specimens were sent for histopathology,

transported in 10% formalin solution. The specimens were dehydrated, fixed and sectioned. Standard hematoxolin eosin staining methods were applied. Histological slides were prepared and examined by postgraduate students, supervised by the consultant and reported. Histopathology reports were reviewed and results were drawn.

RESULTS

A total of 680 cholecystectomies were done in the duration of study.

	Histopathology of Gall Bladder Associated with Cholelithiasis	Number of Patients
1	Chronic cholecystitis	660
2	Cholestrolosis	2
3	Tuberculosis	2
4	Xanthogranuloma	9
5	Porcelain gall bladder	1
6	Intestinal metaplasia	1
7	Adenocarcinoma	4
8	Hetrotropic pancreas	1

DISCUSSION

In this study, 660 out of 680, the pathology in cholecystectomy specimen clearly showed chronic inflammatory changes, and in more severe cases, reactive proliferation of mucosa and eventually outpouching into the wall, forming Rokytinski-Aschoff sinuses. Although Rokytinski-Aschoff sinuses are a common finding in a chronically inflamed gall bladder, they may sometimes be mistaken for adeno carcinoma. Dorantes-Heredia R et al reported 8 cases of gall bladder specimen with Rokitansky-Aschoff sinuses which were misinterpreted as adeno carcinoma. They explained differentiating features of carcinoma and shared experience of having foci of adeno carcinoma within the Rokitansky-Aschoff sinuses in some specimens. They further suggested that pathologists should be well aware of these incidental lesions[3].

Studying the effects of gallstones on the gall bladder mucosa, light microscopy examination of sections of the surgically removed gall bladder specimens, showed epithelial damage, and large number of mucous secreting cells. Gall bladder dyskinesia leads to gall bladder epithelium and smooth muscle layer is constantly exposed to concentrated biliary solutes, including cholesterol and potentially toxic bile salts[1]. Moreover abundant mucin may provide a favourable environment for nucleation of cholesterol crystals from supersaturated bile[4].

Xenthogranulomatous cholecystitis develops when Aschoff sinuses rupture into the wall of the gall bladder, followed by accumulation of phospholipid filled macrophages. Such aggregation of macrophages is called xenthoma.

In our study, we found two cases of cholestrolosis associated with cholelithiasis. In most cases of cholesterolosis, the gross description shows yellow, punctuate deposits in a diffuse distribution, they look like the surface of a strawberry hence, the term strawberry gall bladder. Supersaturation of the bile with cholesterol, and abnormal lipid transport across the mucosa causes the formation of the lipid deposition[5].

Epithelial hyperplasia is the most frequent change we found in 60% of our gall bladder specimens. Rahul et al found epithelial hyperplasia in 83 (69%) gall bladder specimens[6].

Discussing the significance of hyperplasia, Albores-Saavedra J, suggest that a small number of hyperplasia of the gall bladder may convert into atypical hyperplasia that progresses to in-situ carcinoma which eventually develops into invasive carcinoma[7].

In rare cases of chronic cholecystitis, dystrophic calcification ensues, yielding to porcelain gall bladder, which may be a precursor to carcinoma gall bladder. It is rare and seen in 0.06 to 0.8% of cholecystectomy specimens[8]. In our study, we found just one case of porcelain gall bladder out of 680 cases.

The most important risk factor for carcinoma of gall bladder is gallstones which are present in 95% of cases, evidence suggests that only 1-2% of patients of gallstones develop carcinoma. It is reasonable to conclude that the common thread tying gallstones to cancer is chronic inflammation[9].

Autopsy studies indicate that 1-4% of all patients with cholelithiasis develop cancer compared to less than 0.2% of those not containing stones[10].

The incidence of carcinoma varies in different studies, for example, in 290 consecutive cholecystectomies for gallstones, only 2 cases of carcinoma were found[11]. Whereas in a study from Pakistan of 188 cases of symptomatic gall bladder disease, 13 (6.9%) cases turned out to be carcinomas, 11 of which were associated with gallstones. All cases were in the sixth decade of life which is in conformity with our observation[12]. Similarly, another local study of 260 cases of cholelithiasis, carcinoma was found in the range of 6%[13].

Preoperative diagnosis of carcinoma gall bladder is difficult. Commenting on the role of ultrasonography, Samad reported 1396 cholecystectomies performed during six years, out of whom 16 patients (1.15%) were diagnosed as gall bladder carcinoma on histopathology, whereas only three patients had pre-operative ultrasonographic features to raise suspicion for malignancy. The author concludes that ultrasonography which is the most common investigation for gallstones, can miss a significant number of malignant lesions of the gall bladder and every cholecystectomy specimen should be sent for histopathology[14].

In our study, we found four cases (0.6%) of adenocarcinoma out of 680 gall bladder specimens, whereas no squamous cell ca was found. There was no preoperative suspicion of malignancy and diagnosis was made on histopathology. All patients were in the sixth decade of life.

An interesting finding in our study was the presence of heterotopic pancreatic mucosa in one cholecystectomy specimen. Heterotopic pancreas is defined as presence of pancreatic tissue in an anatomical place not related to pancreas. Most frequent locations are stomach and small bowel. Gall bladder is rare. About 30 cases have been reported so far[15]. The preoperative diagnosis of heterotopic pancreas is difficult and the significance of incidental finding is unclear and requires a systemic review of the subject[16].

Intestinal metaplasia was found in one specimen in our study, it is considered a premalignant condition therefore needs some scrutiny. Jorge Albrose showed intestinal metaplasia in 49 specimens removed for cholelithiasis. A whole range of changes were observed from the presence of mature goblet cells to those containing argentafin cells, peneth cells and gland like structures. He supports the hypothesis that cholelithiasis induces the formation of stem endodermal cells which may differentiate into mature intestinal or gastric mucosa[17].

Khan et al reported 114 cholecystectomy patients out of 293 (39%) with intestinal metaplasia, this itself shows high frequency of metaplasia in Pakistan. Significant association with age more than 60 years, borderline association with moderate to high red chili pepper consumption and North Indian origins were noted[18].

Tuberculosis of gall bladder with gallstones is very rarely mentioned in world literature. Around 150 cases have been described since 1870. Out of 680 cases operated for gallstone disease in five years, only two cases of tuberculosis were found in our study. We could not diagnose them preoperatively, as they appeared as usual cholelithiasis on ultrasonic examination.

After the diagnosis on histopathology, patients were treated with anti-tuberculous chemotherapy and recovered uneventfully.

Usman Ismat Butt et al reported a case in a Pakistani man who had cholecystectomy for gall bladder mass with stones. They were suspecting carcinoma but on histopathology, it turned out to be tuberculosis. Patient was treated with anti-tuberculous drugs and made an uneventful recovery. The authors suggested that tuberculosis is very common in Pakistan. It is therefore, likely that the incidence of tuberculosis of gall bladder is more than that reported in the Western literature[19].

CONCLUSION

This is a descriptive study of 680 cases, operated for cholelithiasis, during a period of 5 years in our department, where histopathology specimens were later reviewed to observe not merely chronic cholecystitis but a plethora of histopathological changes including tuberculosis, premalignant conditions and carcinomas. This strengthens our belief that every gall bladder specimen should be subjected to histopathological examination.

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ORIGINAL ARTICLE

Measuring Carbon Monoxide levels of Hookah Cafés in Karachi, Pakistan

Rabia Baloch[1], and Mehak Pervaiz[1]

ABSTRACT

Objective: This study aimed to assess the levels of carbon monoxide (CO) in the air inside water-pipe cafés in Karachi, Pakistan.
Methodology: During June 2015, three water-pipe cafés in the city of Karachi, Pakistan were selected through convenience sampling. CO air samples were collected from the selected cafés using Carbon Monoxide USB Data logger. The graphs were automatically generated through the USB Data logger and the collected data was analyzed using Microsoft Excel.
Results: The results showed that the overall readings of CO levels were within/lower than threshold limit value (TLV) of 25 ppm. However, there was an increase of CO levels in indoor air of all included water-pipe café during peak hours when the cafés were open and had a regular customer flow compared to the CO levels overnight.
Conclusion: The findings of this study provide evidence that the air quality in water-pipe cafés is potentially hazardous to the health of its employees, which is critical to inform tobacco control policies and regulations for such venues. The study findings also indicate a clear need to extend research to not only focus on the indoor air quality of water-pipe cafés, but also the biological monitoring of employees in water-pipe cafés.

Key words: Water pipe smoking, second hand smoke, carbon monoxide, indoor air quality, hookah cafés

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INTRODUCTION

Tobacco-related research and tobacco control regulations generally remain limited to cigarettes, while other forms of tobacco uses are common worldwide[1]. Furthermore, some cities and states have specific exemptions that allow water-pipe smoking to remain in operation[2] which may be due to the ubiquitous but incorrect perception that water pipe smoking is less harmful compared to cigarette smoking because of the belief that dangerous tobacco components are filtered by the water[3,4].

In a water-pipe smoking session, the smoker inhales through the hose drawing smoke from the charcoal and tobacco combustion through the water as a result of which there is incomplete combustion of the tobacco. Both the charcoal briquette and the incomplete tobacco combustion contribute to the levels of carbon monoxide (CO) and particulate matter (PM) in the air[5,6]. Several toxicological studies have shown that a typical water-pipe tobacco smoke session has almost 25 cigarettes worth of tar, 11 cigarettes worth of CO and 2 cigarettes worth of nicotine[7].

Water-pipe tobacco smoke have shown to cause damaging effects on cell function in lung epithelial cells and vascular endothelial cells, which potentially leads to development of chronic obstructive pulmonary disease (COPD) and vascular disease[8]. Water-pipe smoke inhalers absorb a significant amount of toxic chemicals similar to those in cigarette smoke that are identified as a causal factor for lung disease, vascular damage, cancer, and dependence[8]. Therefore, the increase in water-pipe smoking popularity raises a public health concern.

Though, the dangers of water-pipe smoking have been documented, but data from water-pipe cafés is lacking[9,10]. Water-pipe smoke produces sweet smelling noxious fumes that the employees of a water-pipe café inhale along with the toxins from the charcoal that is used to heat the tobacco[2]. Assessing air quality through measurement of CO levels in closed room cafés may aid the understanding of the toxins and exposure of

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the employees. This study aimed to assess the level of carbon monoxide (CO) in the air inside water-pipe cafes in Karachi. The results can provide novel information regarding water-pipe smoke's effect on air quality which may help generate regulatory efforts to protect the café employees against water-pipe smoke. To the best of the authors' knowledge, this is the first study of its kind addressing carbon monoxide levels in water pipe cafes in Karachi, Pakistan.

METHODOLOGY

A cross sectional study was conducted in water-pipe cafés in Karachi, Pakistan during June 2015. Water-pipe cafés were identified through an internet search. Out of total 9 waterpipe cafes, 3 were selected through non-probability convenience sampling. All cafés were indoor, air-conditioned and had a dark interior structure. An initial visit to the selected venues was done to introduce the researcher and study objectives to the owners of the selected cafés and to seek their consent for the study. CO air samples were collected from the included cafés using Carbon Monoxide USB Data logger. The data logger measures and stores readings over a 0 to 1000 ppm measurement range and the data can be easily viewed by plugging it into a computer's USB port.

The sampling device was left with café managers. It was left for 30 hours at venue #1, for almost 7 hours at venue #2 and for 70 hours at venue #3 to measure the CO levels in the respective cafés. Different duration for recording CO levels in different cafes was to compare the variation in data among the cafés. The graphs were automatically generated through the USB Data logger and the collected data was analyzed using Microsoft Excel. All the graphs were compared for peak levels at various intervals. Ethical approval for this study was obtained from Dow University of Health Sciences (IRB-564/DUHS/-15/51).

RESULTS

All venues were poorly ventilated and had closed windows and doors that only opened occasionally. Almost 60% of the café employees were less than 25 years old whereas the others were 25 years old or above. The average sampling duration of CO inside the cafés was around 36 hours. The date from the USB data logger was graphed which was then analyzed. Figure 1 depicts the readings of CO levels in indoor air of water-pipe café venue #1. The levels showed that CO levels increased up to 15 ppm from 4 PM to 1:30 AM and then again started to peak, going up to 20 ppm around 1 PM the following day.

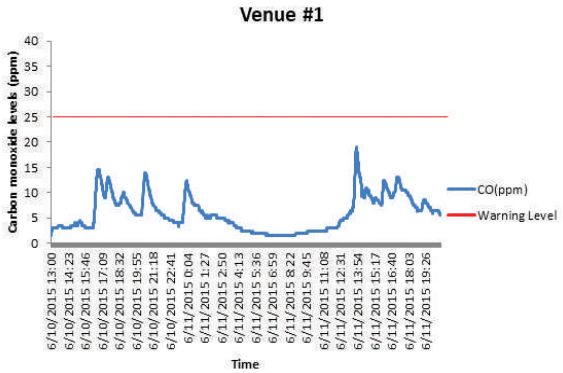

Figure 1: Carbon Monoxide levels at Venue #1

Figure 2 illustrates CO monitoring samples from venue #2, which were taken for 7 hours from 1 PM to 8 PM. The CO levels were observed to increase above 5 ppm from 4 PM onwards, reaching the highest level of 21 ppm between peak hours (6 PM – 7 PM).

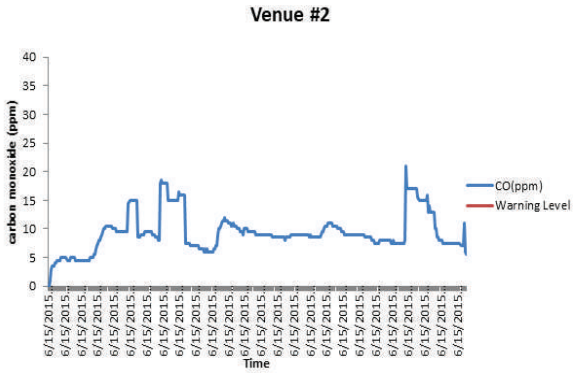

Figure 2: Carbon Monoxide levels at Venue #2

Figure 3 shows CO levels at venue #3 which were measured for three days. Each day CO level started to increase at around 12 PM and remained increased till 12 AM. They reached the highest peak at almost 20 ppm at day 3 around 12 PM.

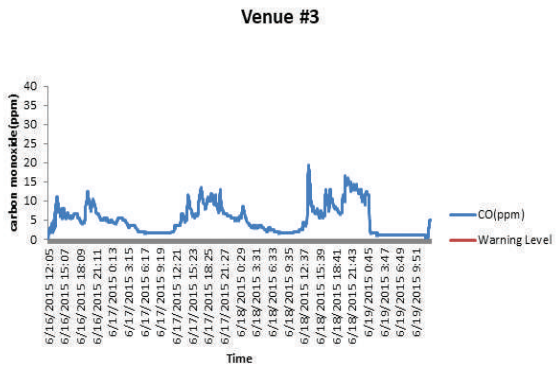

Figure 3: Carbon Monoxide levels at Venue #3

Overall, readings of the CO monitoring samples from all included venues peaked at an average of 20 ppm which was within threshold limit value (TLV) of 25 ppm and MAK maximum workplace value of 30 ppm.

DISCUSSION

Indoor airborne concentrations of CO were noticeably elevated in the sampled water-pipe cafés from Karachi city; supporting previous evidence that water-pipe affects indoor air quality by producing a significant quantity of CO[11]. The results showed an increase of CO levels during the hours when the cafés were open for business and had a regular customer flow. Whereas from 12 midnight to 12 noon, readings from all venues showed a decrease level of CO in the air. Thus, indicating that CO levels increase during hours of high number of active water-pipes and customers[12], as majority of water-pipe users in Karachi prefer smoking it at the café[13]. In addition to CO, particulate matter (PM2.5), particle-bound polycyclic aromatic hydrocarbons (p-PAHs) and air nicotine are also seen to be increased in indoor air of water-pipe venues[11] however, these indicators of air quality were not measured in the present study.

The charcoal used to heat the water-pipe tobacco was likely the major source of CO measured in the present study as the venues did not have an alternate source of combustion byproducts such as cooking and open fires. However, cigarette smoking can be considered as a confounder for the level of CO found in the cafés which calls for additional research to assess this correlation.

According to the National Ambient Air Quality Standards set by United States Environmental Protection Agency (EPA), the overall average CO concentration at venue #1 and venue #3 were within the EPA 8-hours CO level of 9 ppm[14]. Whereas, the average CO level at venue #2 exceeded 9 ppm, however as the measurement was taken over a period of 7 hours, the EPA 8-hours CO air quality standard cannot be identified. Similarly, the measured CO levels from all venues were within the EPA 1-hour CO level of 35 ppm[14].

Thus, the results showed that the CO levels in the included venues were below the warning level and appeared to be safe for employees working in that environment. This contraindicates with finding from previous studies in which CO was seen to be remarkably increased in indoor air of cafés[15,16]. A possible explanation of our findings can be that the concentration of CO in air depends on multiple factors, including the number of smokers in the room, the rate at which they smoke, the volume into which the smoke is distributed, the rate at which the air in the space exchanges with uncontaminated air and the rate at which the smoke is removed from the air. Significant correlations have also been observed between mean CO and the number of water-pipe smokers, number of water-pipes and water-pipe smoking density[17].

Additionally, evidence suggests that CO levels are higher in patrons of water-pipe cafés, for both current and non-cigarette smokers, compared to cafés where water-pipes are not available as people who did not use any cigarette in the past month but visited a water-pipe café demonstrated to have significantly higher CO values (mean=28.5ppm)[18]. As a smoker can inhale 10 times the number of "puffs" in a single hookah session and each "puff" can have 10 times the volume of CO of that in a conventional cigarette, it leads to an increased CO level in hookah smokers[19]. In spite of this, it remains an under researched topic and an overlooked hazard among Pakistani health planners[20]. It is crucial to test CO levels of employees of water-pipe cafés to further investigate the effects of second hand smoking of water-pipe on a person's health.

Limitations in the present study should be kept under consideration when generalizing the results to other water-pipe venues and cities. Study limitations include the small sample size and the failure to assess the air exchange rates or ventilation within the venues, which made it difficult to explain the variability in CO levels within and between venues. Additionally, all concentrations reported in the paper may not necessarily signify actual personal exposures as they are based on area sampling only. Also, as the sampling device was left with the café managers, this could have introduced measurement bias during data collection. Thus, further studies should be conducted on larger sample size including personal air sampling and direct ventilation assessment to more accurately assess exposures.

CONCLUSION

The findings of this study showed that the overall readings of CO levels were within/lower than threshold limit value (TLV) of 25 ppm. However, the aforementioned limitations of this study indicate a clear need to extend research to not only focus on the indoor air quality of water-pipe cafés, but also the biological monitoring of employees in water-pipe cafés.

Author's contributions: RB designed the study proposal, did the data collection and analysis with MP's support. MP did the study write-up and developed the manuscript with the help of RB.

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ORIGINAL ARTICLE

Morphological Spectrum of Gall Bladder Diseases at a Tertiary Care Hospital of Karachi

Asma Shabbir[1], Zareen Irshad[1], Saba Javed[2], Fakiha Nadeem[2], Nazish Jaffar[1], and Syed Mehmood Hasan[3]

ABSTRACT

Objectives: To determine morphological spectrum of gall bladder diseases at a tertiary care centre of Karachi and to correlate with age and gender.
Methodology: Data was collected from histopathological records between June 2017 to June 2019. Relevant data including registration number, age, gender of the patients and diagnosis were recorded. Data was entered and analyzed using SPSS version 21. P-value < 0.05 was considered as significant.
Results: In our study, patients 26-35 years of age were predominantly found to be susceptible to gall bladder diseases and male to female ratio was 1:3.7. Out of the total 150 cases, 75 were identified as chronic cholecystitis with cholelithiasis whereas 32 cases of chronic cholecystitis were without stones or any other associated pathology. Out of 150 cases, 40 were seen in combination with different pathologies. Our series also included three cases of adenocarcinoma and all of these were diagnosed in females. Significant association of diagnosis was seen with gender and type of stones (p-value = 0.03) (p-value = 0.01) respectively.
Conclusion: In our study, chronic cholecystitis with cholelithiasis was the most common finding which was most predominantly seen in females. Younger age group was mainly affected in both genders. Mixed type of gall stones were the most common stones found in our series.

Key words: Cholelithiasis, cholecystitis, adenocarcinoma

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INTRODUCTION

One of the most common diseases among gastrointestinal disorders are those arising from gall bladder. The disease entity varies from cholecystitis (acute, chronic, follicular and xanthogranulomatous), cholelithiasis, cholesterolosis, adenomatous hyperplasia, metaplasia and carcinoma[1]. According to recent epidemiologic study, there is evidence of strong association of Helicobacter pylori species with chronic cholecystitis, gall bladder carcinoma and cholesterol stones.[2] Cholelithiasis has been the most common gastrointestinal disorder which presents with acute abdominal pain that may require hospitalization in otherwise healthy people, however, more than half of the cases are asymptomatic.

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Prevalence of gallstones is the highest in the West, particularly in elderly women.[3,4] Increased incidence of gallstones in western countries is most likely due to an ageing population and obesity.[5] In western countries, 70-80% are pure cholesterol gallstones whereas in Asian countries, mixed stones are predominant.[6,7] The common risk factors of gallstones formation in Asia are middle age, fertility, female gender, and flatulence.[8] According to Globocan 2018 data, gall bladder cancer accounts for 1.2% of all global cancer diagnosis and 1.7% of all cancer death.[9] Though gall bladder carcinoma is rare but it is an aggressive disease with poor prognosis. Less than 5% of the patients survive for up to 5 years, but if diagnosed and treated at early stage, 75% of the patients can survive for 5 years.[10] The presenting symptoms of gall bladder carcinoma are mostly related to invasion of adjacent organs involving liver as the most common site, other organs might be involved through distant metastasis. Hence, extensive tumour sampling is required for accurate staging of invasive cancers which is essential to determine the prognosis and treatment.

Acute pancreatitis is the other severe complication of cholelithiasis.[11] Moreover, literature shows that gall bladder diseases are the major risk factor for developing hepatocellular carcinoma independent of cirrhosis and viral hepatitis.[12] The study of gall bladder diseases has gained importance because of its associated co-morbidities. Females with rheumatoid arthritis older than 60 years of age were found to be associated with significantly higher prevalence of gall stones compared with general population and the most probable reason is hyperlipidemia and chronic inflammation.[13] The other co-morbidities with cholelithiasis include Crohn's disease, ileal resection or other diseases of ilium, hence it is important to know the frequency of gall bladder diseases which might provide the clue about co-morbidities as well.

The aim of this study is to identify the morphological spectrum of different gall bladder diseases at a tertiary care hospital of Karachi.

METHODOLOGY

This descriptive study was conducted after ethical approval from Institutional Review Board of Jinnah Sindh Medical University (Reference no: JSMU/IRB/2019-244) and Dr. Tahir laboratory, Hamdard University and Hospital, Karachi. Data was collected from the records available between June 2017 to June 2019. All histopathologically diagnosed cases of gall bladder diseases reported during two years were included in the study. Relevant data including registration number, age, gender of the patients and diagnosis were recorded. Cases with incomplete data were excluded from the study. Data was entered and analysis was done using SPSS version 21. The frequency of gall bladder diseases was studied. Furthermore, association of diagnosis with age and gender of the patients were calculated using chi-square test. P-value < 0.05 was considered as significant.

RESULTS

Over the period of two years, 150 cases of gall bladder diseases were diagnosed at a tertiary care centre of Karachi, out of which 32 (21.3%) were males. The mean age of the group was 42.36±1.26. We found significant association between diagnosis and age (p=0.001). We observed that patients in 26-35 years age group were more susceptible to gall bladder diseases closely followed by 36-45 years age group as compared to older age group (65-75 years). From the total 150 cases, 75 cases were identified as chronic cholecystitis with cholelithiasis, whereas 32 cases of chronic cholecystitis were without stones or any other associated pathology. Out of 150 cases, 40 cases were seen in combination with different pathologies. Our series also included 3 cases of adenocarcinoma and all of them were diagnosed in females. We observed significant association between diagnosis and gender (p=0.03), where chronic cholecystitis with cholelithiasis was most common in females. (Table 1) (Figure 1 and Figure 2)

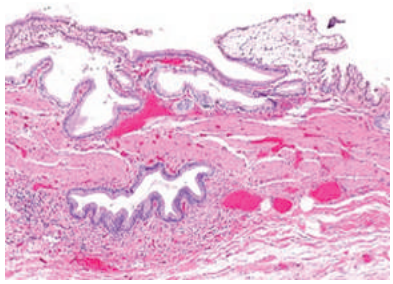

Figure 1: Chronic Cholecystitis

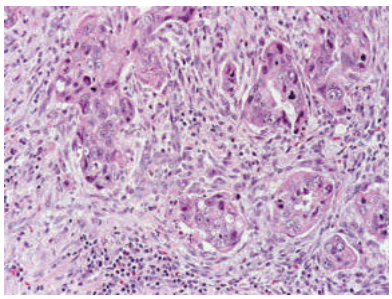

Figure 2: Adenocarcinoma gall bladder

Out of total 150 cases, 102 cases were associated with stones and 48 were without stones. From 102 cases reported with gallstones, 67 were mixed stones, 18 were pure cholesterol stones and 17 were pigmented stones. Significant association was seen between types of stones and diagnosis, where mixed type of stones were most commonly seen in chronic cholecystitis without any other associated pathology (p= 0.001). (Table 2)

DISCUSSION

Gall bladder diseases seriously affect the quality of life of the patients. In this retrospective study, we investigated different histopathological diseases of gall bladder at a tertiary care hospital of Karachi**.** Our study observed gall bladder diseases to be more common in females as compared to males with a ratio of 3.7:1. This is consistent with other studies globally which also reported females to be more susceptible to gall bladder diseases particularly cholecystitis.[14,15] The most probable reason for female predominance of cholecystitis might be assumed by the role of estrogen secretion in females. Literature shows that, estrogen

Table 1: Association Between Diagnosis and Gender

Diagnosis	Gender		Total	P-value
	Male	Female		
Acute Necrotizing Cholecystitis	1	2	3	0.03
Acute Necrotizing Cholecystitis with Cholelithiasis	1	5	6	
Chronic Cholecystitis	4	28	32	
Chronic Cholecystitis with Cholelithiasis	15	60	75	
Chronic Cholecystitis with Cholelithiasis with Cholesterolosis	2	14	16	
Chronic Cholecystitis with Cholelithiasis with Reactive Hyperplasia	2	3	5	
Adenocarcinoma	3	0	3	
Follicular Cholecystitis	4	6	10	
Total	32	118	150	

Table 2. Association Between Diagnosis and Type of Stones

Types of Gallstones	Chronic Cholecystitis	Acute Necrotizing Cholecystitis	Chronic Cholecystitis with Cholesterolosis	Chronic Cholecystitis with Reactive Hyperplasia	P- value
Pigmented	15	1	0	1	0.001
Cholesterol	0	2	16	0	
Mixed	60	3	0	4	
Total	75	6	16	5	

influences gallstone formation as it appears to increase saturation index of biliary cholesterol.[16] Iron deficiency anaemia appears to be another risk factor for cholecystitis and past studies have observed high prevalence of iron deficiency anaemia in Pakistani females which could be one of the most likely causes of cholecystitis in females of our region.[17] Other factors contributing to increased frequency of cholecystitis among females include multiparity, high Body Mass Index (BMI), frequent use of oral contraceptives and hormone replacement therapy.[18]

In our study, we found that the peak incidence for occurrence of gall bladder diseases was 26-35 years in both genders, closely followed by 36-45 years which is consistent with other studies conducted in Asian countries, including Pakistan.[19,20] Whereas this finding is contrary to the studies conducted in the West where gall bladder diseases are more common among elderly people aged above 60 years.[15] This difference might be due to the majority of the population in our region being young individuals who consume less vegetarian and high saturated fatty diet.[22] The other reason could be majority of obese individuals in elderly ages in the West. However, the exact reason for this discrepancy remains unanswered.

In our study, chronic cholecystitis with cholelithiasis is the most commonly encountered gall bladder disease which is in line with other studies done in Pakistan and neighbouring countries.[21,22] However, in developed countries, chronic cholecystitis is a common finding as compared to association with cholelithiasis.[23]

Our study is also in accordance with other studies from Asian countries where we found mixed type of stones as the most common gall stones.[24] On the contrary, in the West, pure cholesterol stones appear to be the more common type.[7] However, pure cholesterol stones are also commonly seen in different regions of Pakistan.[25,26] We assume that most probable reason for variation in types of gallstones in our country is delayed presentation and diagnosis of cholelithiasis which results in conversion of cholesterol stones into mixed type of stones. With time, gallstones may be colonized with bacteria which triggers gall bladder mucosal inflammation and cystic enzymes from bacteria and leukocytes hydrolyze bilirubin conjugates and fatty acids. As a result, cholesterol stones may accumulate the substantial proportion of calcium bilirubinate and calcium salts producing mixed stones over time. In agreement with the West, adenocarcinoma of gall bladder is less frequently seen in our region.[27] Our

study reveals that gall bladder carcinoma is common among females as compared to males which is in correspondence with literature from other regions of the world.[28] As mentioned earlier, estrogen increases the risk of gallstones thus estrogen might be the primary cause of increased incidence of gall bladder cancer in females.[28] Moreover, long standing gall stones is a probable risk factor for adenocarcinoma. Hence, symptomatic patients with cholelithiasis should be promptly treated and every specimen after cholecystectomy must be sent for histopathological reporting to confirm the diagnosis and also to reveal incidental findings.

CONCLUSION

We conclude that chronic cholecystitis with cholelithiasis was the most common finding in our study which was most predominantly seen in females. Young age was mainly affected in both genders. Mixed type of gall stones were found to be the most common stones.

With the best of our efforts, we have collected and compiled all the common and rare diseases of gall bladder broadly which have been reported during one year at a tertiary care centre of Karachi. This might help us to know the disease burden in our region and might help in future to plan the appropriate precautionary diagnostic and treatment strategies. Further studies with larger sample size are needed to focus on the gall bladder diseases associated with co morbidities.

Author's Contributions: Dr. Syed Mehmood Hasan & Dr. Asma Shabbir conceived the idea. Saba Javed and Fakeha Nadeem contributed in data collection & literature search. Saba Javed worked on introduction & methodology. Dr. Asma Shabbir did statistical analysis. Fakeha Nadeem worked on results & discussion. Dr. Zareen Irshad & Dr. Nazish Jaffar did critical review. All the authors approved the manuscript.

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CASE REPORT

Squamous Cell Carcinoma Kidney in a Patient of Renal Calculi: Rare and Aggressive Variant of Renal Cancer

Syed Mehmood Hasan[1], Talat Zehra[1], Salma Parween[1], and Sadaf Razzak[1]

ABSTRACT

Squamous Cell Carcinoma (SCC) of kidney is a rare presentation. It behaves aggressively in case of kidney. It is usually diagnosed at an advance stage and has a poor prognosis. Renal squamous cell carcinoma is usually associated with stones. Chronic irritation due to stone causes metaplasia of lining epithelium resulting in transformation of squamous cell carcinoma which is rare but the most fatal complication. Here we are presenting a case of renal squamous cell carcinoma associated with stones.

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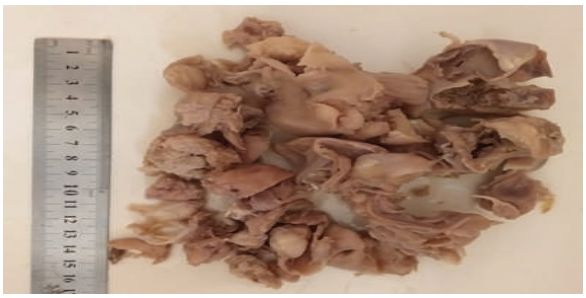
INTRODUCTION

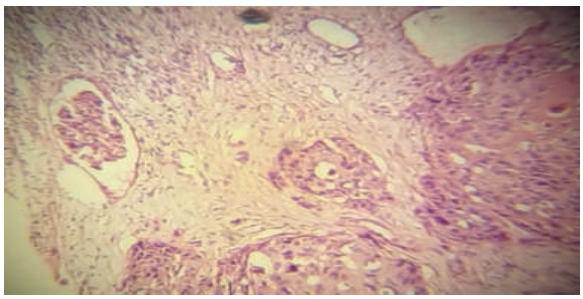
Squamous cell carcinoma is a very rare tumour which makes up under 1% of all malignant tumours of kidney. This tumour is very aggressive in nature and prognosis is poor because it is usually diagnosed in advance stage[1]. Risk factors leading to renal squamous cell carcinoma include renal calculi, infection, endogenous and exogenous chemicals, vitamin A deficiency, hormonal imbalance, and radiotherapy[2].

Case presentation:

A 63-years-old female presented to the urology ward of a tertiary care unit with the complaint of renal stones for seven years with flank pain. Ultrasound kidney showed kidney with features of hydronephrosis and multiple renal stones. She underwent nephrectomy. The specimen was sent for histopathological examination in 10% buffered formalin. On gross examination, the specimen was previously (surgically) distorted and fragmented. On further slicing, there were features of hydronephrosis, thinned out cortico-medullary areas and most of the other areas revealed gray white cut surface with friable to cut areas as shown in photograph 1. The collective dimensions of the fragment are 15 x 10 x 4 cms. Histological examination revealed a malignant neoplastic lesion comprising of a nest of squamous carcinomatous components with keratin pearl formation. Few glomeruli and tubules were also observed at the periphery intermixed with neoplastic lesions as showed in the photomicrograph 2 and 3. There was a dense to diffuse mixed inflammatory infiltration and areas of necrosis were also seen in the interstitium.



Photograph 1: The gross examination of received sample exhibiting fragments of kidney with solid to cystic areas



Photograph 2: The histopathological image showing with an invasive squamous cell carcinoma on the right side of this field distorting the renal architecture with a single glomerulus of the left side of the field is identified. X 100

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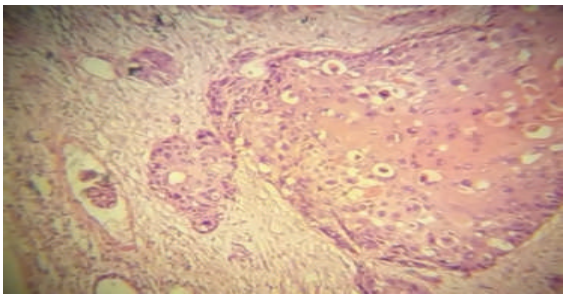

Photomicrograph 3: The histopathological image showing a fibrosed glomerulus of the left side of the field surrounded by scattered inflammatory cells in the interstitium with invasive squamous cell carcinoma on the right side. X 100

DISCUSSION

Renal squamous cell is extremely rare. Bladder and male urethra are its more common sites than the renal pelvis[3]. Histologic hallmarks of renal SCC include intercellular bridges, pearl formation, and keratotic cellular debris[4]. Possible carcinogenic mechanisms for SCC of the renal pelvis have been proposed. Chronic irritation of urothelium may result in squamous metaplasia, which if persistent, may later develop into SCC. SCCs of the kidney are frequently associated with renal stones, hydronephrosis and chronic infection, all of which contribute to chronic irritation and subsequent development of squamous metaplasia in the neighboring epithelium[5]. Renal SCC usually presents at an advanced stage with extensive local infiltration and has a poor prognosis[6]. Primary SCC has a slight female preponderance occurring most commonly in the age group of 50-70 years[7]. Studies show that SSC generally spreads locally with associated symptoms of regional lymphadenopathy. Cases have been reported with metastasis to the lungs, liver, and bone. SCC is a highly aggressive tumour. It has the worst prognosis among histological subtypes of renal pelvis tumours. Its median survival rate is 3.5 months[9].

CONCLUSION

Renal SCC is a very rare entity. Its insidious onset and nonspecific radiological findings permit late diagnosis resulting in poor prognosis. These tumours are usually treated with aggressive surgery and chemo radiation in case of metastatic disease. The possibility of primary renal SCC should be considered in cases of nonfunctioning kidney with renal stones after ruling out metastatic disease with the help of clinical and radiological findings.

Authors' contribution: Syed Mehmood Hasan worked on histopathological diagnosis and details. Talat Zehra conceived the idea and wrote the manuscript. Salma Parween and Sadaf Razzaq carried out the literature search.

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SHORT COMMUNICATION

Next Generation Sequencing and its Role in Clinical Microbiology and Molecular Epidemiology

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INTRODUCTION

The microbiological diagnosis of infectious diseases and its related molecular epidemiology has come a long way owing to the advent of newer molecular diagnostic techniques. There was a time when specification of pathogens was determined by phenotypic methods only. Later on, the implementation of Polymerase Chain Reaction (PCR) based assays came in the limelight. Although these methodologies are less time consuming and highly sensitive, they require etiological hypothesis of a clinician and require presumptive predefined targeted sites[1]. Therefore, exploration of newer methods has been direly needed. The next generation sequencing (NGS) is now becoming the hallmark in detecting pathogenic and non pathogenic micro organisms from a given sample. The older sequencing techniques including Sanger sequencing and Maxam-Gilbert sequencing are indisputably gold standard techniques but their role in clinical microbiology, field and molecular epidemiology is somewhat restricted.

The first generation sequencing techniques were able to produce high output data but evolution of Genome analyzers in 2005 was a game changer, which took sequencing runs from 84 kilo base(kb) per run to 1 gigabase (Gb) per run and NGS staggeringly increased the data output from gigabites to terabites[2]. Also NGS reduced the time span, the cost and enabled parallel running of many samples. The NGS techniques primarily work upon one of the two strategies: sequencing by hybridization or sequencing by synthesis[3]. On the basis of these strategies, the sequencing platform can be broadly represented by Ion torrent, Roche/454, illumina/Solexa, and ABI/SOLid sequencing[3]. These NGS techniques have possibly made the tracking of unidentified and resistant micro organisms which are associated with hospital and community associated break outs. The NGS is now being used worldwide in tertiary care units for epidemiological studies and trailing chain of infection. The University Medical Centre Groningen (UMCG) in Netherlands is using Illumina Miseq? and Life Technologies Ion PGM^{TM} sequencing for detection of resistant micro bugs and investigations for disease outbreaks. On average, the molecular laboratory of UMCG receives 5750 samples per year, out of which, 1500 samples are proceeded for NGS techniques and the results are commendable[4].

NGS is a breakthrough in whole genome sequencing (WGS) particularly in bacterial genomics. The NGS begins by obtaining good quality purified DNA using flourometric quantification method. Purified DNA is fragmented into short sequences and run through DNA sequencing process following the protocol of sequencing platform. The data is analyzed by utilizing bioinformatics tools[5]. Currently NGS is used in outbreak management, detection of genes in antimicrobial resistance, microbiological surveillance, transmission of zoonosis, molecular characterization of bugs and metagenomics. The NGS has high standard discrimination power between pathogenic and non pathogenic clones. The molecular characterization and phylogenetic analysis of *E. coli* (STEC) 0154:H4 and shiga toxin-producing Enteroaggregative *E. coli* (EAEC Stx2a+) O104:H4 is made possible because of NGS[5]. The polymicrobial detection from a single and multiple samples at the same time along with genomic analysis is an additional perk of NGS. The sensitivity and reduced bias is one of the factors that emphasizes the utility of NGS over other molecular and phenotypic methods[6].

CONCLUSION

The Next Generation Sequencing of a bacterial genome is a benchmark in Molecular diagnostics and

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Microbiology. It eases clinical diagnoses of various lethal infections, the causative agents behind them which in turn collectively aid patient's treatment and eventually a safer community buildup. The future is waiting for the new developments in the field of microbiological genomics. Therefore, clinicians and microbiologists should buckle themselves up for future challenges and new advancements.

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LETTER TO THE EDITOR

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Pandemic: A Dilemma for Dental Health Care Professionals

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Sir,

The outbreak of Corona Virus Disease-2019 (COVID-19) has affected humankind in the worst way imaginable. The rapid and unmanageable spread of disease has affected millions around the globe[1]. The mortality rate is on the rise despite active control measures and awareness campaigns amongst populations in hotspots. Several drugs, remedies and vaccine trials are ongoing in the research laboratories with a hope to confront the pandemic[2]. So far, the battle has been won by a few countries with strict post recovery measures to avoid re infection[1].

In the light of current research, COVID-19 can be avoided and cured with self-immunity boost, adoption of preventive measures and symptomatic management of sufferers[3]. The first line of defense in this battle are health care workers deployed in the COVID-19 red zones who are working tirelessly to overcome the health perspective of this deadly pandemic. Similarly, amongst the frontline doctors and paramedics, dental care professionals are also in service and are among the most vulnerable to transmission of virus from the host due to working in close contact with patients and involvement in continuous aerosol and microdroplet producing dental procedures[4]. Dental clinics, along with other potential risky fields, were closed in the beginning of the pandemic when it spread from mainland China to other geographical regions[1].

In spite of potential hazards, dentists are performing emergency dental procedures and essential dental services to overcome the patient burden. The dentists are limiting transmission of SARS-CoV-2 mostly by preventive measures like regular thermal check, thorough history of patients, mouth rinses before and after procedures and judicious application of personal protective equipment with strict infection control procedures[3]. In spite of this, the dentists are not only at risk of getting infected but also could be transmitting infection to their family, colleagues, and patients. Moreover, their mental health levels are in stress and gradual anxiety[2,4].

Under the current situation, it is natural to develop the fear of getting infected from patients in close contact. As a preventive measure, dental care professionals must either close down their practice, defer non-essential dental services or provide limited dental services according to the recommended local or American Dental Association Guidelines (ADA) and World Health Organization (WHO) for an indefinite period. This would be an appropriate step in curtailing the spread of COVID-19.

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