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**Editorial**

Antibiotic Resistance in Present Era

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The antibiotics are an essential group of therapeutic drugs used to kill bacteria on various levels in the human body. These antibiotics had played a significant role for the treatment as well as the prevention of bacterial infections. The effectiveness of antibiotics against bacterial infections cannot be denied. However, an overuse and misuse of antibiotics, the current poor hygiene and contamination control mechanisms have led to the improvement of antibiotic resistance.

Anti-microbial or antibiotic resistance is an international public health issue, greatly dominant in the developing countries. Antibiotic resistance is a bacterial adaptation, which allows bacteria to persist regardless of the presence of antibiotics. Antibiotic resistance is a significant risk to human health and is being seen as a global environmental and economic risk. The relationship between bacterial resistance and misuse of antibiotics had been well documented, and was considered to be a major public health problem.

Antibiotics are important to treat the bacterial infections but inaccurately prescription, misuse and overuse of antibiotics are elevating the antibiotic resistance. It is still a significant health problem in developing countries where not many hospitals have facilities for microbiology of clinical isolates which can assign to blind treatment. Change in the bacteriological profile due indiscriminate use of antibiotics has been associated with the appearance of multiple drug resistance strains. Information regarding the antibiotic susceptibility profile is essential in the selection of the most appropriate treatment and can minimize the antibiotic resistance.

Preventive measures should be implemented in true sense to control antibiotic resistance. "Cleanliness is next to Godliness" and hence, clean surroundings as well as clean hands should be ensured. Soaps should be used for hand washing frequently. Awareness campaigns should be promoted and self-medication should be avoided. Antibiotic susceptibility investigations should be carried out for suspected bacterial infections.



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Guest Editorial

Human Papilloma Virus

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Human papillomavirus (HPV) is a small DNA virus composed of an icosahedral viral particle. It has a genome of 8000 base pairs approximately surrounded by a protein capsid [1]. In sexually transmitted infections and venereal diseases including vaginal intercourse, oral sex and anal sex, HPV has become more common in adults and accounts for 11% of cancer incidence in women worldwide [2]. It has become more common in sexually transmitted diseases. There are many types of HPV that are specific for specific diseases, but HPV type-16 is associated with HNSCC [2]. HPV has genomic DNA and almost 15-20% of HNSCC has the genomic DNA which is present in HPV.

The distribution of HPV positive oropharyngeal cancer is highest in the tonsils, not frequently in the hypopharynx and very rare in the oral cavity. In tonsils the HPV DNA is found in 45%-67% of the cases, in hypopharynx the HPV DNA is found in 13%-25% of the cases and in the oral cavity and larynx the rate is 12%-18% and 3%-7% respectively. This shows the variation of HPV infection with the site of the tumor [3]. Detection of HPV in head and neck squamous cell carcinoma depends on the methodology used and type of tissue examined. Yield of HPV DNA extracted from oral samples is usually low and hence, it is very important to adopt sensitive and accurate techniques.

In many studies HPV is thought to cause infection in stem cells within the basal layer of mucosa [4]. HPV replicates in the basal cells of the stratified epithelium [5]. If we compare tobacco users and never-tobacco users, it is concluded from a study that increased risk of diseases are found in tobacco users with advanced HPV+OSCC than never-tobacco users [6].

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Review Article

Adverse Effects of Heavy Metals on Aquatic life

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ABSTRACT

The metals that are highly dense and toxic at low quantities are termed as heavy metals. These metals include Lead, Mercury, Cadmium, Copper, Zinc, Manganese, Nickel, Arsenic and Silver etc. Among these metals Arsenic, Cadmium, Chromium and Lead are considered most toxic to humans, animals and fish. Heavy metals enter the aquatic ecosystem through disposal of industrial, agricultural and municipal wastes and bio-accumulates in the food chain. Chromium is generally present in aquatic ecosystems between the range 1-10 µg/L which is highly toxic. Higher concentration of Chromium in aquatic ecosystem can result in accumulation in fish and have adverse effects on consumers' health. Cadmium and Lead deposit on the gills of the fish and cause suffocation which may lead to death of the fish. Accumulation of Cadmium is generally associated with organ damage and anemia that results in death of the fish and its consumer. In the presence of Cadmium, the toxicity of Lead generally increases the toxicity drastically in *Gambusia affinis*.

INTRODUCTION

The term heavy metal is used for such metals that have high density and toxicity even at very low quantities. Such metals include Arsenic (As), Mercury (Hg), Cadmium (Cd), Thallium (Tl), Lead (Pb) and Chromium (Cr). Other than these metals, some trace elements such as Copper (Cu), Zinc (Zn) and Selenium (Se) are required by the human body for metabolic processes but high concentrations of these elements can be very toxic. These elements are also known as trace elements and considered heavy metals due to their toxicity. Such elements are usually ingested through food or inhaled through air [1]. The heavy metals that are dealt in environmental sciences usually include Pb, Cd, Hg, Cr, Cu, Zn, Manganese (Mn), Silver (Ag) and Nickel (Ni) etc. Some heavy metals such as Lead (Pb) and Beryllium can be very toxic at low concentrations. These heavy metals can also affect the action of essential elements such as Iron (Fe) by interfering with the metabolic processes which lead to increased toxicity of such metals. Other than the chemical toxicity of metals, some metals also have radiological toxicities. Other than these properties, the oxidation state may contribute to the toxicity of some metals, e.g., Cr(III) is

a trace element and is required in metabolic processes but Cr(VI) is carcinogenic [2]. Heavy metals are present in environment in form of biomass as well as freely. In cases of aquatic environment, heavy metals are present in fish species depending on their growth, development and other physiological factors. These fish are studied to evaluate the effect of heavy metals on aquatic environment and health of aquatic animals. Therefore, consuming sea food can also effect human health if it contains large quantities of heavy metals [3]. Since metals are soluble in water, these are readily absorbed by aquatic animals but even small concentrations can be very harmful as metals undergo bio-concentration which makes their concentration higher in the organism than that of the external environment. Metal toxicity does not directly kill an organism but cause sub-lethal effect by affecting the growth, metabolism, development and reproduction of organisms [4]. Although sea food is considered nutritious and therapeutic as it contains minerals, vitamins and unsaturated fatty acids such as omega-3 fatty acids [5] but since fish are situated at the top of aquatic food chain so they can accumulate

heavy metals from water, food and sediments which may have adverse effects on human health if consumed [6] and [7]. High consumption of sea food contaminated with heavy metal may lead to liver failure, renal damage and cardiovascular disorders which may lead to death of the consumer [8,9]. Many studies have emphasized on the concentration of metals in edible parts of the fish but some researches have also studied the effect of these metals organs such as liver, kidneys, heart, brain and gonads. These studies have suggested that there are many factors that influence the metal accumulation in fish which include reproductive cycle, swimming patterns, living environment and feeding behavior [7,10].

Chromium

History of Chromium: Chromium was discovered in the form of a red crystalline mineral named crocoite ($PbCrO_4$) in 1761 and was used as a pigment. This is why it was derived from Greek word "chroma" that means color. Now Chromium is extracted in the form of an ore chromite ($FeCr_2O_4$) which is the source of chromium used for pigment extraction [11].

Properties of Chromium Metal: According to the periodic table, Chromium is one of the transition elements and is considered the 24th most abundant element in earth's crust. There are three isotopes of this metal that are present in nature with atomic numbers of 52, 53 and 54. Among these isotopes, ^{52}Cr is the most abundant one. Chromium III is an essential nutrient and is required for the metabolism of carbohydrates but Cr (VI) is toxic and carcinogenic [12]. Cr (VI) is more toxic but it can be reduced to Cr (III) to decrease the toxic effect as it will not be able to enter the cell [13].

Sources of Chromium: Various chromium compounds such as sodium chromate and dichromates are available for commercial use and enter the aquatic ecosystem through effluents of various industries. These industries commonly include metal finishing, mining, textiles and tanneries, ceramic and pharmaceutical industries [14,15].

Effects on Aquatic Life: The overall effect of chromium on environment is adverse but in case of aquatic animals, it accumulates in the fish and may affect the consumer's health [3]. It is estimated that the chromium concentration in lakes and rivers ranges between 1-10 $\mu g/L$. However, the recommended or safe level, according to United States Environmental Protection Agency, ranges between 50-100 $\mu g/L$. Chromium does not readily accumulate in the bodies of fish but is taken up through gills. If chromium is present in high quantities in the area near to fish habitat, then chromium is ingested or taken up by gills and damage the tissues [16] and [17]. There are various toxic effects of Chromium reported in fish that include morphological and hematological changes, tissue damage, growth retardation, damaged immune functions and immune

system impairment [18] and [19]. Since it enters the bodies of fish through gills, excessive accumulation may lead to acute poisoning leading to damage in respiratory epithelium resulting in death of fish due to suffocation [20]. The overall consumption and accumulation is highly dependent on the site of fish habitat with respect to the disposal of industrial effluents containing chromium. Fish are at higher levels of food chain among other aquatic animals so accumulation of chromium also depends on the food intake of the fish [21].

Cadmium

Discovery of Cadmium: Cadmium was first discovered as an impurity in zinc carbonate by Friedrich Stromeyer and Karl Leberecht Hermann in Germany in 1817 [22].

Properties of Cadmium: Cadmium is an element with symbol Cd and atomic number of 48. It is soft and bluish white in color and is chemically similar to zinc and mercury. Cadmium is a trace element but its ability to accumulate in living bodies is currently an environmental concern [23] and [24]. Usually, metals are prone to corrosion but cadmium is corrosion resistant and is often used as an outer protective layer on other metals to prevent corrosion. Another interesting property of chromium is that it is not flammable but it may release toxic fumes if burned in powdered form [25].

Sources of Cadmium: Cadmium is released to the aquatic environments through multiple setups including various industries such as batteries, pigment and electroplating industries. This leads to the contamination of the aquatic environments to alarming levels [26]. Other than industrial settings, agricultural wastes, chemicals, pesticides and fertilizers also contain cadmium in minute quantities that may alter the aquatic ecosystem if continuously dumped in the rivers and oceans [27]. It is also evident that fossil fuels and municipal waste are also among largest sources of release of cadmium in the environment that may enter the water bodies [28].

Effects of Cadmium on Aquatic Life: The most toxic form of cadmium is also the one that occurs most abundantly in the form of divalent ion. This divalent metal is known to damage kidneys, hearts, livers and gills of freshwater fish by accumulating in these organs and causing pathological changes [29,30]. When present in low concentrations in fish and humans, this metal induces the synthesis of metallothionein, a low molecular weight protein that decreases its toxic effect by binding to it. But if the concentration of cadmium is very high then metallothionein produced is not enough to detoxify the cadmium which in turn leads to the destruction of erythrocyte that eventually leads to anemia due to hemoglobin deficiency [31,32]. Other toxic properties of cadmium include chronic toxicity leading to impaired reproductive, excretory and hepatic

functions. It is also evident that high concentrations of cadmium may interfere with certain metabolic pathways and lead to hyperglycemia in fish [33].

Lead

History of Lead: The history of lead dates back to 7000-6500 BC in Asia as the initial example of metal smelting. Lead has been extensively linked with extraction of silver from some lead minerals and ores. It is evident Egyptians were the first one to use lead in cosmetics, glasses, enamels and ornaments [34].

Properties of Lead: Lead has been assigned the symbol Pb and has atomic number 82. It is relatively denser than other metals and has low melting point. It is soft and when freshly cut appears in bluish white color that changes to dull gray when exposed to air. It is also used in paints as it has the ability to adhere to wood and imparts brightness to the color. It is considered one of the hazardous metals. There are two forms of lead, Lead(II) and Lead(IV) [35,24].

Sources of Lead: Though lead is a naturally occurring metal, but it is discharged in water bodies through smelter and industries by dissolution of pesticides, precipitations, lead plumbing and municipal waste. It was not considered toxic until the late 19th century and since then it is considered a global issue. It is still a great concern due to its use in battery manufacturing industries. It enters the body of living organisms through inhalation, ingestion and absorption [36].

Effects of Lead on Aquatic Life: Lead deposits in the soft tissues, organs, digestive tract and gills of the fish and may lead to permanent damage to any of the organs and systems. This leads to various disabilities and disorders in the fish bodies [37]. Lead in water deposits on the gills of fish and lead to suffocation. If present in low concentration for long periods of time, it may lead to chronic toxicity and act as a neurotoxin [38]. It is also evident from the recent researches that exposure of lead in combination with cadmium have synergistic effect and the toxicity is increased drastically in mosquito fish *Gambusia affinis* [39].

CONCLUSIONS

High concentrations of heavy metals in environment are threat to all ecosystems but its severity is very high for aquatic ecosystems because of the disposal industrial, agricultural and municipal waste in freshwater and oceans. This had led to chronic toxicity in fish and bioaccumulation of metals in fish and ultimately humans who consume seafood. Though the effect of metals highly depends on the age, environment, habitat, exposure time and species of the fish but overall heavy metals have adverse effects on aquatic animals.

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Review Article

Effect of Environmental Pollutants on Neurological Disorders

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ABSTRACT

Environmental pollution is one of the leading causes towards the rise of the neurological disorders. All types of pollutants whether biodegradable and non-biodegradable both contributes towards different neurological issues and affects human life badly. The effect of these pollutants even affects the baby in the womb. Other than this environmental pollutant is also responsible for causing other complex disorders such as cancer, diabetes, heart diseases and many more. Different types of environmental pollution such as air, water, land, thermal, sound, radiation etc. have different impact on the neurological health of the individuals. Disastrous conditions as well as the areas with poor hygiene and cleaning facilities are also linked with the presence of or growth of harmful microorganisms and these are the sites that are accumulated with heavy metals such as lead, arsenic, mercury etc. Along with this noise pollution also affects our brain and nervous system and can cause complications even can cause depression among people. Similarly, the polluted water contains a number of pollutants and toxic metals which accelerates the process of aging and in longer term to cause neurodegenerative diseases. The role of nutrition is highly recommended in neurological disorders and complications as eating healthy diet and taking safety precautions in food preparations and proper handling of food in industries can decrease the chances of the occurring of such diseases. Concluding the facts of our study it has been seen that environmental pollutants triggers the oxidative stress and cause neurotoxicity and inflammation of the neuron cells which in longer term causes different neurodegenerative diseases.

INTRODUCTION

Environmental pollution is emission of any substance or energy into environment that cause short and long-term damage to ecological stability of earth or may down the standard of living [1]. Human Well-being influence by all kind of environmental impurities. The pollutant comprises the Wide-range of xenobiotic substances and discharge into environment in greater quantity compare to the normal range due to human activities. Environmental pollutants are categorized as biodegradable which are waste water, feces discharge and that can break down in normal situation and non-biodegradable which cannot break down easily such as heavy alloy, cleansing agents etc. Increase in industrial development and migration in cities became the major reason of toxic substances being immensely release into nature [2]. The complex interchange of genetic susceptibility and life incidents

develop cognitive disorders for example chronic illness like schizophrenia that effect the way how the person thinks, or high in emotions or abrupt change in mood which is bipolar. Recent genetic studies show that 145 genomes linked to schizophrenia. But genes alone are not an only factor for onset of cognitive disorders but different factors like environmental, genetics and neurochemical together contribute in onset or seriousness of these diseases. The main neurological and psychiatric diseases cover a wide span of genetic values, allowing environmental impacts to play a part [3]. Environment play a major role in child well-being, with rising proofs that few chemicals are harmful for the brain of individual. From the year of 1950, about 1lac and forty thousand new chemicals have been manufactured [4]. Environmental pollution is a worldwide issue with various and considerable public well-being effects. There

are so many factors which recognized as environmental factors which increases the chances of illnesses like schizophrenia and all psychopathic disease, relatively there has been less evident about that pollution can also be a contributing factor in these diseases. In spite this is the reality that interaction between genotype and environment are now accepted as aspect of schizophrenia pathophysiology, and pollution can possibly bring out connection between cities birth and high risk [5]. Disease pathophysiology become complicated due to different components including gene related factors, chemicals, way of living, pollution in environment. The most dangerous chemicals are polycyclic aromatic hydrocarbons, heavy alloys, nitrogen oxides, particle pollution, and polychlorinated biphenyls, pesticides, and dioxins, additives use in food, antibiotics and hormones. There are uncountable pollutants and great number of them have never been estimated with regards to how toxic they are or how risky they could be for health, particularly those new chemicals who emerge every time as a result of connection to previous one. It is nearly not possible to determine the consequences of these new chemicals on human health. Earlier researches have showed a wide range of pollution related diseases. High prevalence of some cancers, elevated rate of diseases, cognitive impairment in children, occurrence of diabetes mellitus type 2, immune and respiratory system disorders and degenerative nerve diseases all of these has been linked with environmental pollution [6].

Types of Environmental Pollution

The categories of environmental pollution is succeeding: Air, Water, Land, Thermal, Radiation and Marine Pollution.

Prevalence

Environmental pollution is a major factor of illnesses and death rate globally, more dangerous to humans and animals which automatically increase the health care budget [6]. Neurological developmental diseases, neurological impairments and neuro-degenerative disease continue to increase around world dramatically. From the year of 1990-2010, Behavioral or mental related disorders increase more than 37%, Parkinson's disease (PD) increased to 75%, 30% upsurge in developmental disabilities called autism, a neuro-developmental disorder called attention deficit hyperactivity disorder raised by 16%. Dangerous exogenic chemicals are considered to be the reason of increases in different common or deadly and neurological related diseases [7]. Alzheimer's disease (AD) patients are probably increase by three time in coming decades as the population of world is becoming unending [8]. Different scientific researches show that environmental pollution account around 60% for depression, 32% for bipolar diseases and 23% for schizophrenia. Quality of air was

analyst for the findings of bipolar disorders. Pollution of land was the analyst for the personality disorders. Results shows that environment specifically quality of air require more researches to make environmental helper aware about the risk of psychiatric and neurological disorders [3]. Health conditions associated with ageing, like dementia, developed as serious public health issues due to continuously increase in population of world. It is estimated that in 2001, about five million of European facing AD and it was expected to double in 2040. Prevalence of PD among the age over 85 is ten percent and AD prevalence is thirty percent. Factors related to environment play an important role in the pathophysiology of AD and PD [9]. Different cognitive and brain related diseases fall under the category of dementia which is a major term for different diseases. Studies show that dementia divide into AD or non-AD's dementia [10]. PD is a disease related to movement, AD is related to dementia including memory destruction or cognitive failure, when movement is not as much as it should be called hypokinesia, when movement is slow known as bradycardia, when movement is totally absent known as akinesia. Major risk is ageing. Neurodegenerative diseases severity increases due to interaction with metals, pollutants, solvents and toxin substance [11].

Etiology/Risk Factors

Neurodegenerative diseases happen in later life when environmental related factors effects pre or postnatal period. Pesticides, toxic metals including aluminum, arsenic, mercury, and lead, nanoparticles have been associated in developing of AD because it can increase the production of beta amyloid and tau protein attachment to phosphate group causing age related amyloid (protein) plaques and neurofibrillary tangles are biomarkers of AD [12]. Exposure related encephalopathy risk is determined by age, sex and the microenvironment generated by the disaster related factors, in which different cultural, economic and social indicators of health are important [13]. Autism spectrum disorder (ASD) is a complex neurodevelopmental condition described by impairments in three key behavioral domains: social deficits, impaired communication, and repetitive behaviors again. Various risk factors, including genetic, infectious, metabolic, and immunological, environmental, nutritional, and diabetes-related risk factors have been associated with the outcome [14]. The vicious cycle establishes so-called environmental enteropathy, a predominantly subclinical condition (even in the absence of diarrhea) caused by varying degrees of intestinal barrier dysfunction, the low-grade intestinal bacterial migration, low-grade local and systemic inflammation, and disrupted innate immune responses in the gut. can affect growth and cognition and eventually

lead to neurodegeneration as well as liver and metabolic disease later in life [13].

Mechanism involved in Neuro-degeneration

Environmental related factors cause biological related effects, main thing is to check the exposure routes. Specifically, in the case where particles affecting different systems and causing different kind of effects. To check the effects inhalable particles on nervous system or whether the compound reach to the brain it is necessary to check the exposure route of particles. After accumulation, a large number of particles goes to respiratory tract after eliminated through biliary drainage, or through absorption in macrophages which stay in the region of alveoli. Moreover, different engineered data shows that small number of particles which accumulate in airway can move to epithelial barriers with olfactory bulb and sensory to secondary pathways [9].

Effect of environmental Pollutants on Neurological Disorders

Disturbed functioning of neurons results in neuro-degenerative disorders that involves PD and the AD. Air pollution and its detrimental effects on human health has now been recognized in many clinical and experimental studies [15]. It has been shown that Industrial fuels have affected millions of people by causing damage to their brain. And ultimately this brain damage will result in many cognitive disorders such as autism, attention deficit hyperactivity disorder (ADHD) and dyslexia. These impairments are increasing day by day [16]. Recently, it has been observed a link among the air pollution and issues of CNS involving the AD as well as PD, stroke, and hemorrhage. Various toxic components present in air pollutants known as nano-sized or precise particles enter in our central nervous system and stimulate innate immunity [17].

Noise Pollution

Noise pollution is an undesirable source of sound, which may result in the unpleasant impacts on human capacity and also generate many kinds of psychopathies. One of the unauritory systems that is impacted by pollution mainly noise is the central nervous system and the brain. From revealed to auditory voices can activate the autonomic nervous system and endocrine system. This kind of noise can result in the cognitive disorder, including, attention deficit, communication difficulties, learning defects, as well as depression [18].

Water Pollution

Polluting of water supplies seldom involves intense proof of poisoning yet rather includes a continuous and moderate impedance of health. This is because of constant low-dose manifestation that prompts bioaccumulation of water soluble amphiphilic natural poisons that can likewise be intense in lipid stores in body. When a threshold is reached,

cell dysfunction may result. In this context, exposure to minute amount of water pollutants might represent a danger for expanded occurrence of slow advancing diseases that are by and large connected with aging or disabled development. A considerable lot of these problems include the central nervous system which is particularly defenseless to damage during development and afterward with increase of neurodegenerative changes during aging [19].

Industrial Pollution

The organic toxic molecular compounds came into existence due to five major industries; petroleum refining, organic chemical and synthetic industries, steel mining and coal conversion, textile processing, and pulp and paper milling. However, industries alone are not totally accountable for exposure of the chemicals to the environment, Consumers also play a major role. Use of gasoline, aerosol sprays, pesticides, and fertilizers by humans are one of the leading causes of environmental pollution. Remnants from sewage treatment plants are another cause of xenobiotic pollution. Accidental spillage, illegal dumping and unrestrained hazardous waste sites are also polluting the environment. Poor waste disposal techniques are causing the contamination of soil and its biota, which ultimately leads to many diseases caused by microorganisms. Heavy metal pollution, especially in industrialized countries is common to be seen and it is contributing to the environmental pollution [2]. High concentrations of organic pollutants are toxic but moderately low concentrations of organic pollutants are also potential to cause long term health complications in the human body and this toxicity can even pass to next generations. EDCs are associated with the causation of neurological disorders. Recently, public concern has been focused on the effects of EDCs on brain's function, along with an increase in neuropsychiatric disorders, including autism, attention deficit and hyperactivity disorder, learning disabilities and assertiveness. Several researchers suggest that exposure to EDCs can cause depression long with neural deterioration [20]. Perfluoroalkyl acids (PFAAs) are synthetic organic pollutants, found in the environment and may impact human health. PFAA impacts on neuro-biological, neuro-endocrine, and neuro-behavioral outcomes. There are many mechanisms through which PFAAs may enter the brain and interrelate with biochemical endpoints to affect neurological function [21]. Exceptional weakness of the brain in the embryo isn't very much out of danger against modern synthetic substances. Industrial chemicals known or suspected to be neuro-toxic to adults can also affect the developing cerebrum. The placenta doesn't hinder the entry of numerous ecological toxins from the maternal to

the fetal blood circulation have been identified in umbilical cord. Blood and in excess of 200 unfamiliar synthetics furthermore, numerous ecological synthetic compounds are moved to the newborn child through human breast milk. During fetal life and early infancy, the blood-cerebrum boundary gives just halfway insurance against the section of synthetic substances into the CNS. In addition, the human brain is incredibly delicate to injury brought about by harmful synthetic chemicals. Vitro studies show that neural stem cells are very sensitive to neurotoxic materials including methyl mercury. Some pesticides inhibit cholinesterase feature within the developing brain, role of acetylcholine before synapse formation. Thereby affecting the crucial regulatory Early-lifestyle epigenetic changes are also regarded to have an effect on next gene expression inside the mind [16,22]. Mercury (Hg) is extensively recognized as a neurotoxic steel; besides it is able to act as a proinflammatory agent and immunostimulant, relying on man or woman publicity and susceptibility. Mercury exposure might also stand up from inner body pathways, consisting of via dental amalgams, preservatives in pills and vaccines, and seafood intake, or even from external pathways like, occupation, environmental pollution, and handling of steel items and cosmetics containing Hg. In susceptible individuals, continual low Hg publicity may additionally cause systemic inflammation, even exacerbating the already present autoimmune reaction in sufferers with autoimmunity. Mercury exposure can cause disorder of the autoimmune responses and worsen immunotoxic effects related to expanded serum autoantibodies titers. The cause of the existing record is to provide an important evaluation of the many troubles associated with Hg exposure and autoimmunity [23]. Microplastics are global environmental contaminants main to inevitable human publicity. Then again, little is known about the effects of microplastics in human fitness. Exposure may additionally occur by using ingestion, inhalation and dermal contact because of the presence of microplastics in merchandise, meals and air. In all organic systems, micro plastic publicity might also cause particle toxicity, with oxidative stress, inflammatory lesions and expanded uptake or translocation. The inability of the immune system to get rid of artificial particles may also cause chronic infection and growth threat of neoplasia. Moreover, microplastics might also release their components, adsorbed contaminants and pathogenic organisms. Nonetheless, understanding on microplastics toxicity continues to be confined and in large part influenced by using publicity concentration, particle houses, adsorbed contaminants, tissues concerned and personal susceptibility, requiring in addition studies [24].

Role of Nutrients in Neurodegenerative Disease

Neurodegenerative disease isn't curable, but progression of this disease can be slowed down by treating and managing the symptoms in certain ways. The drugs for treating those illnesses only reduce the cognitive impairment and behavioral problems, however they no longer prevent the progression of neurodegeneration. Healthy diet, life-style development and nutraceuticals focused on of oxidative strain, irritation, abnormal mitochondrial dynamics and the mitochondrial interaction with peculiar ailment-associated proteins and evaluation of impact of environmental contaminants such as occupational exposures to pesticides, can be a promising method inside the treatment of neurodegenerative diseases. Those improvements can be basis on company understanding of nutrigenomics and the customized management of individuals at danger [11].

CONCLUSIONS

To finish, environmental pollutants, especially air pollution, contributing to reason the various neurological issues and ill impact on human health. These environmental pollution motive harm to the critical anxious gadget, as they can enter in our frame either through ear(noise), or nasal cavity (air pollutants), cause disruption to blood brain barrier, main to harm of neurons, ensuing in neurological disorders.

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Review Article

Coronavirus Combating Facilities in Pakistan and its Comparison with World

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ABSTRACT

Severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) became a pandemic by the start of 2020. Mortalities went on rise due to lack of understanding of disease as the symptoms varied from population to population and even within the same population. Furthermore, there were lack of facilities in hospitals to accommodate the patients as well as to treat them. Pakistan is no exception and the situation here was also worsened. Government of Pakistan started to take emergency steps and devised new policies to provide facilities to patients regarding quarantine and treatment. In this review, there is an overview of Province-wise situation in Pakistan regarding the hospitals and facilities for COVID-19 patients. Flaws in the system and a comparison with world is also discussed.

INTRODUCTION

Coronavirus disease is a pandemic of recent times, it was termed as COVID-10 by WHO on Feb 11, 2020 [1]. Elderly people and people with comorbidities such as cardiac complications, hypertension and diabetes were at high risk [2,3]. It has high infection spreading rate and transmit from person to person by coughing, sneezing [4]. In Pakistan, first COVID-19 patient was diagnosed in March, 2020. Government of Pakistan devised new policies to combat this health emergency and established quarantine centers all over the Pakistan and other facilities as well [5-7]. Here in this review, situation in Pakistan is discussed according to different regions and provinces as follows:

Regional Response

Punjab: To accommodate the pilgrims returning from Iran, quarantine center was established in the Dera Ghazi Khan district. Several other centers had been established at different locations. The largest quarantine center of Pakistan was established in Multan, city of Punjab province. Punjab Chief Minister advised people to not come out of houses unnecessarily, avoid social gatherings and unnecessary travelling [8,9]. Five hospitals were designated throughout the province for treatment of corona virus patients. A hospital of 1000 beds was established at Expo Center, Lahore in just 9 days. The local authorities were assigned to start disinfecting the areas. The students of universities are playing pivotal role in this

state of emergency. Corona virus protection kits, hand sanitizers and disinfectants have been made by Punjab University and provided to public [8,9]. The Chief Minister announced a relief package of Rs.10 billion for daily wages laborer. An additional pay was given to all the medical workers to encourage them. The CM declared that daily 3200 people would be tested by the laboratories all over the province. Almost 10,000 new doctors and paramedic staff were recruited in Punjab [8,9].

Sindh: The Chief Minister of Sindh ordered the shutting down of all educational institutions till 31st May, 2020 and also imposed a temporary ban on shopping malls, marriage halls, and shrines etc. On 21 March, 14 days lock down was announced by the Government of Sindh and ordered the closure of all public transport, markets, offices, and hotels etc. [10]. A 1000 bed hospital was being setup at Expo center Karachi with military assistance. On request of Sindh Government, the Pakistan Cricket Board has allowed the conversion of Hanif Mohammad High Performance Centre into a transitory living wage for paramedic staff who are working at newly established hospital at Expo center. Facilities have been provided to collect samples from homes. A hospital of capacity of 2000 beds is formed at Labor colony flats in Sukkur. The local government has also launched a mobile service to provide rations to the needy people across the province. All the workers have been given paid leave [11]. To make the lockdown further better, the Sindh Government ordered the closure of grocery shops by 5pm, release of about 4000 prisoners from jails to prevent the spread of COVID-19. A mobile application "Sindh Relief Initiative" was launched for efficient coordination between welfare organizations and provincial government for ration distribution [8].

Balochistan: In late January a 14-member committee was established by Balochistan Government to fight the coronavirus pandemic. Educational institutions have been closed till 31st May 2020 and exams have been postponed. A complete lockdown has been imposed on 24th March in the province. The lockdown implies to all except for medical officials, medical stores, hospitals, laboratories and grocery shops. The media teams were also allowed to move freely [12]. On 24th March, a state of emergency was announced in those districts which bordered attached to Iran. Balochistan is at a higher risk due to its land connection with Iran where the cases of Corona virus has increased exponentially. The jails have been fumigated and disinfectants have been distributed throughout province. The Government of Balochistan has given the relief of Rs. 500 million for establishing quarantine centers to accommodate people returning from Iran [13].

Khyber Pakhtunkhwa: Khyber Pakhtunkhwa province is also under lockdown, most of the institutes are closed and

transport is banned. Screening teams are present at the entry and exit of all towns and cities and are screening the people for COVID-19. About 1330 doctors have been recruited to help the province to overcome the pandemic. 500 new diagnostics kits have been purchased by the provincial government. About 750 liters of sanitizers and 500 protective kits have been given to health department [8].

Gilgit-Baltistan: In this region of Pakistan, medical emergency has already been declared as more cases have been reported and lockdown is observed at present. The military forces have been giving the task to enforce the lockdown. China, the friendliest neighbor of Pakistan has sent medical equipment's and 10 tons of goods to Gilgit Baltistan to fight the COVID-19 pandemic. The WHO has provided aid in form of data management at districts level [8].

Azad Kashmir: The health emergency is declared in this region on 14th March. All institutes are closed, and exams have been postponed. Screening systems have been present at all entry points of the region. Movement of people is restricted to greater extent and only media officials and medical officials having the special passes are allowed [8].

Islamabad: In Islamabad, the Bhara Kahu region is sealed after several cases were reported from this region. The OPDs of hospitals have been closed to avoid the spread of disease. The lockdown in Islamabad is till 14th April, 2020 [8].

Common Factors of All Regions To enforce the lockdown efficiently, the police and military help is extended to all regions. Forces are trying their best to keep people in homes. The military forces are at front line. People not following rules are punished. The quarantine centers are guarded so that people can't escape from there.

1. Flaws in Pakistan System against Corona virus: Pakistan is a developing country, so it is economically weak. Moreover, the literacy rate of country is also very low so there are a lot of hurdles in combating the present pandemic. Pakistan can't go for a complete lockdown because in such a situation, the people may be saved from COVID-19 but they will die of hunger and poverty because a large portion of our population are daily wages laborer and are living below line of poverty. This increases the risk of spread of disease [14]. People of Pakistan have been warned again and again to take the precautionary measures and awareness campaigns have been run by media and government. However, these efforts seem to be fruitless as public is not following the government instructions [14]. The quarantine facilities also have a lot of flaws. The cleanliness is not properly maintained, and medical facilities are also deficient. The major quarantine center established at Taftan city of Baluchistan is lacking

the basic facilities like there is shortage of medical equipment. People with obvious symptoms of COVID-19 couldn't be tested. The security of these centers is also not strictly managed. People continued to escape from there and wander in local areas and markets and put the lives of healthy people in danger [16].

2. Comparison of Pakistan with World: The major difference between countries in their response to Corona virus pandemic is due to difference in their economic conditions. The people of less developed countries are at a higher risk to COVID-19 because of lack of facilities and infrastructure [17]. As a response to COVID-19 most countries are going for lockdown. This lockdown will have drastic effects on low economy countries like Pakistan. The developed countries should extend their help to less developed countries [17]. Almost all the countries have found the lockdown as the only solution at present for controlling the pandemic. USA, UK, Spain, Italy, China and many other first world countries has taken strict measures regarding lockdown and their citizens are civilized enough to play their role in this need of hour [18]. On the other hand, in Pakistan the government has to fight Corona virus on one side and ignorance and illiteracy on other side. The people are creating hurdles for authorities by not following the rules. Most of the countries including our neighbor India has taken measures for the safe return of their citizens from places where COVID-19 has higher rate but Pakistan has not taken any measures in this regard. Thousands of our citizens are still present in severely affected areas [18]. The International Economic organizations like IMF and World Bank are extending their help to emerging business community which has faced the greatest loss as a result of corona virus pandemic. They are giving loans and have made their loan obligations quite flexible. World Bank is providing billions of funds to developing countries in order to make them able to respond to pandemic effectively [18]. The international community has become united like never before to work on vaccine for corona virus. Many laboratories of Germany, China and USA are working for this purpose. Although the developing countries lack the infrastructure for fighting the COVID-19 but they can take lessons from the developed countries response and apply it to own community [18]. COVID-19 has become a major challenge especially in underdeveloped countries and becomes a major pandemic challenge for healthcare professional, as well as the limited financial availability in the country make this a big problem [19]. Due to its present economic condition, health care resources, and the event of preceding outbreaks, the Centers for Disease Control and Prevention (CDC) had already issued a level 3 warning for worldwide travelers to Pakistan [20,21].

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Original Article

Survey of Vitamin D Deficiency and Associated Comorbidities in Lahore

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ABSTRACT

Vitamin D is recognized for its importance in bone health along with the reduced risk of cardiovascular, autoimmune and several chronic diseases. **Objective:** Present study was designed to explore the prevalence of vitamin D deficiency and its association with comorbidities (Type 2 Diabetes, Thyroid Disease, Hypertension, Asthma, Heart Disease, Gastritis and Osteoporosis) among the subjects visiting outpatient departments (OPDs) of public hospitals in Lahore. **Methods:** Adult (≥ 18 years) male and female subjects ($n = 200$) participated in present cross-sectional study. Participating individuals were required to fill questionnaires which recorded their medical history and BMI. Blood samples were taken for laboratory evaluation of vitamin D₃ deficiency. Data was analyzed for evaluation of diverse risk factors. Serum level of vitamin D₃ (25-hydroxyvitamin D [25(OH)D₃]) were measured using standard procedures of measurement using Human Vitamin D₃ (VD₃) ELISA Kit (MyBioSource, Inc. USA). **Results:** Vitamin D deficiency was more prevalent in females than in males. Of the 200 individuals, the highest number of patients suffered from Diabetes Myelitis Type-II (61%) followed by Thyroid Disease (38%). Whereas, the least number of vitamin D₃ deficient subjects were suffering from Osteoporosis (5%). **Conclusion:** It is evident from present study that there are calculable degrees of Vitamin D insufficiency among all age gatherings, sexes, pay levels and areas in Pakistan.

INTRODUCTION

Vitamin D is recognized for its importance in bone health along with the reduced risk of cardiovascular, autoimmune and chronic diseases including cancers [1]. Bone deformities due to vitamin D deficiency among pediatric and adult populations are commonly referred to as rickets [2]. In early twentieth century rickets were treated with exposure to ultraviolet radiations [3] and sunlight [4]. Phosphorus and calcium absorption is critically dependent upon vitamin D and since its production is inexpensive, vitamin D is widely contained in multivitamin supplements, fortified foods and other pharmaceutical preparations [5]. Natural dietary sources such as fish oils (herring, salmon, cod liver), red meat and eggs are vitamin D rich foods [6]. Vitamin D₁ in humans is synthesized in the epidermis by a non-enzymatic UVB-mediated photolytic reaction which converts the pre-cursor 7-dehydrocholesterol to pre-vitamin D₃ and then conversion to vitamin D₃ by thermal isomerization [7]. Cytochrome P450s converts vitamin D₃

to 25-hydroxyvitamin D₃ (25OHD₃) in the hepatic parenchyma [8]. Serum 25OHD₃ is the best indicator of vitamin D and its qualities are determined by affinity assays. Vitamin D deficiency is associated with seasonal variation [9] and associated comorbidities [10]. Furthermore additional factors such as indoor lifestyles [11], lack of sunlight [12,13], obesity [14], socio-demographic factors [15,16], milk consumption and breast feeding [17,18] also attribute to vitamin D deficiency. Vitamin D deficiency has been associated with risk for hip fractures [19], low bone mineral density [20] and muscular weakness [21,22]. Vitamin D deficiency in the pregnant women was observed as a consequence in the fetal skeleton as early as 19 weeks of gestation [23]. Clinical research attributes comorbidities and metabolic disorders such as obesity [24,25], cardiovascular diseases [26], type-2 diabetes [27], insulin resistance [28,29], asthma [30] and hypertension [31] are with vitamin D deficiency.

Although vitamin D deficiency is less common in developed Asian and European countries, multiple epidemiological studies carried out in Middle East, Asia and India have reported vitamin D inadequacy [32-34].

METHODS

Study Design: Adult (>18 years) male and female subjects (n = 200) participated in present cross-sectional study. Participating individuals were required to fill questionnaires which recorded their medical history and BMI. Blood samples were taken for laboratory evaluation of vitamin D₃ deficiency. Data was analyzed for evaluation of diverse risk factors. Following study was approved by the Board of Studies (BOS) at Lahore College for Women University, Lahore as well as from the ethics committees of all the hospitals from which the samples were obtained. All participants of the study signed written informed consent allowing the use of their data and biological samples for scientific purposes. Individuals visiting public and private hospitals in Lahore were recruited for following study. Total 200 subjects were included. All participants were referred by physicians to pathology laboratories for vitamin D₃ deficiency test. Present study included adult male and female subjects >18 years old who had been referred by the physician to pathology lab for vitamin D₃ deficiency test.

Vitamin D₃ Immunoassay: Total 3 ml blood was extracted in serum clot activator tubes via venipuncture technique by a trained lab technician. The tubes were transported to lab in icebox to prevent hemolysis. The collected blood was centrifuged within one hour of the collection at 3000 rpm for 10 minutes. Clear serum was siphoned off using micropipette then stored at 4°C. All serological testing was completed within 3 days of collection. Serum level of vitamin D₃ (25-hydroxyvitamin D [25(OH)D₃]) were measured using standard procedures of measurement using Human Vitamin D₃(VD₃)ELISA Kit (MyBioSource, Inc. USA). Vitamin D₃ cutoff values were defined as severe deficiency (0-8 ng/mL or ≤20 nmol/L), deficiency (10-15 ng/mL or ≤37.5 nmol/L), insufficiency (15-20 ng/mL or ≤50 nmol/L), optimal (30-100 ng/mL or 75-250 nmol/L) and toxic hypervitaminosis D (>150 ng/mL or ≥375 nmol/L). All the collected data was checked and corroborated with lab results on daily basis.

Serological Tests

Quality control and Quality Assurance: Laboratory tests were performed by strictly following standard operating procedures and protocols. All reagents were prepared at room temperature and stored in refrigerator (4°C). Control samples were run once before and together with patient samples in weller polystyrene plates. Patient samples were given unique code numbers which coincided with their laboratory specimens. Standards and samples were

added into corresponding pre-coated (25-hydroxyvitamin D₃ antigen) wells. About 300µl of releasing reagent was added to all wells after pre-dilution of standards and samples and allowed to diffuse for 5-10 minutes. The plates were covered with foil and incubated for 60 minutes at room temperature. About 150µl of anti 25(OH)-vitamin D antibody was added into each well, covered in foil and incubated for 45 minutes at room temperature. The contents were discarded and plates were washed 5 times with buffer. The conjugate (200µl) antibody was added into each well, covered in foil and incubated for an additional 45 minutes at room temperature. The contents were discarded and then second washing was carried out 5 times with wash buffer. About 200µl substrate was added to the wells and incubated at room temperature in dark for 10-15 minutes. Optical density (OD) was measured with ELISA reader at 450nm. Data was recorded and arranged in MS EXCEL. All measurable variables were analyzed and results were correlated.

RESULTS

Population Characteristics: Population characteristics are provided in table 1. A total of 200 males 94(47%) and female 106(53%) subjects testing positive for vitamin D₃ deficiency were included in present study. The average age of participants was 42.8±11.7 (mean±S.D). The male participants were older (43.6±13.3) than the females (42.1±10.1) as shown in figure 1. The mean BMI was 22.2±3.5 in males and 22.3±3.3 in females (figure 2).

Vitamin D₃: The 25(OH)D₃ was determined to be 24.6±11.4ng/mL for males and 24.1±13.3ng/mL for females (Figure 3). The normal range for 25(OH)D₃ is 25-80ng/mL. The difference between the mean values for male and female subjects was found to be insignificant (p=>0.5).

Vitamin D₃ and Comorbidities: The comorbidities in vitamin D₃ deficient subjects are provided in table 2. Vitamin D deficiency was more prevalent in females than in males. Of the 200 individuals, the highest number of patients suffered from Diabetes Myelitis Type-II (61%) followed by Thyroid Disease (38%). Whereas, the least number of vitamin D₃ deficient subjects were suffering from Osteoporosis(5%).

| Characteristics | Males %(n=94) | Females %(n=109) | Total %(n=200) |
|-----------------|------------------|---------------------|-------------------|
| Age | | | |
| ≥18-20 | 1(1) | 0 | 0.5(1) |
| 21-30 | 13.8(13) | 14.6(16) | 14.5(29) |
| 31-40 | 28.7(27) | 35.7(39) | 33(66) |
| 41-50 | 22.3(21) | 25.6(28) | 24.5(49) |
| 51-60 | 12.7(12) | 17.4(19) | 15.5(31) |
| ≥61 | 11.7(11) | 3.6(4) | 7.5(15) |
| BMI | 22.2±3.5 | 22.3±3.3 | 22.3±3.4 |

Table 1: Population characteristics

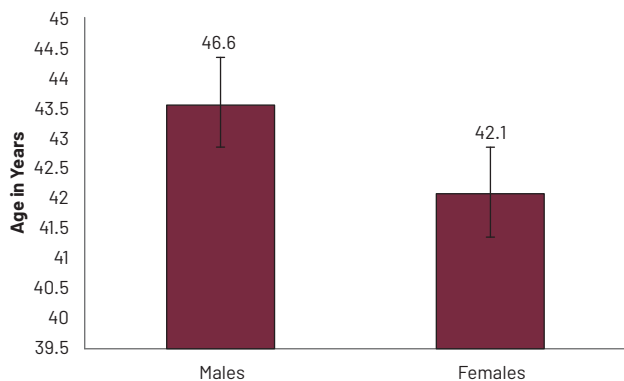


Figure 1: Age of male and female subjects

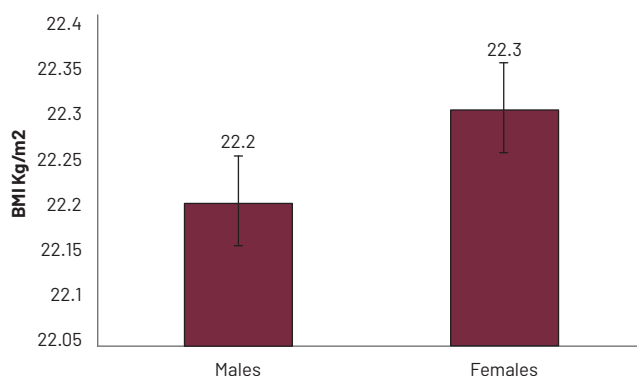


Figure 2: BMI of male and female subjects

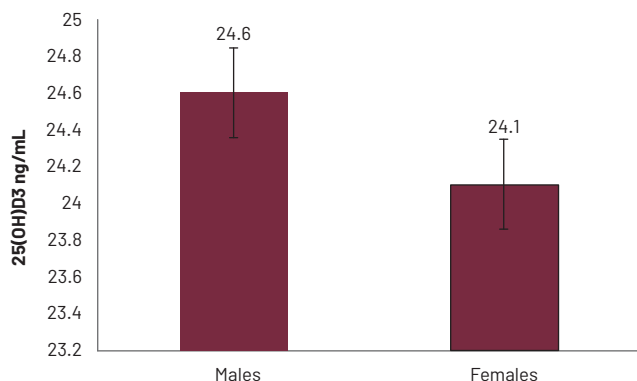


Figure 3: 25(OH)D₃ levels of male and female subjects

| Characteristics | Males %(n=94) | Females %(n=109) | Total %(n=200) |
|-----------------|------------------|---------------------|-------------------|
| Type 2 Diabetes | 29.5(59) | 31.5(63) | 61(122) |
| Thyroid Disease | 17.5(35) | 20.5(41) | 38(76) |
| Hypertension | 16.5(33) | 20.5(41) | 37(74) |
| Asthma | 18.5(37) | 16(32) | 34.5(69) |
| Heart Disease | 8.5(17) | 9.5(19) | 18(36) |
| Gastritis | 7.5(15) | 8.5(17) | 16(32) |
| Osteoporosis | 0(0) | 4.5(5) | 2.5(5) |

Table 2: Comorbidities in vitamin D₃ deficient subjects

DISCUSSION

Vitamin D deficiency (VDD) has been previously reported in

Pakistan [35] in correlation with various comorbidities. VDD has been known to play a major role in a variety of diseases like cardiovascular diseases, neurological disorders, autoimmune disease, depression and cancer. It is deemed as a risk factor for certain birth defects such as rickets in children and osteoporosis, osteomalacia and osteoarthritis in adults. It is also known to cause muscle pain and chronic pain. There are a very few sources of vitamin D in food. Absence of satisfactory daylight exposure and indoor lifestyles are the significant reasons for nutrient D inadequacy [18]. Current study was carried out to evaluate the comorbidities in deficiency of vitamin D. In present study the total of 200 males (47%) and female (53%) subjects testing positive for vitamin D₃ deficiency. The average age of participants was 42.8±11.7 (mean±S.D). These findings are in accordance with another study who has also detailed that a high pervasiveness of nutrient D insufficiency in people in grown-up age. The explanation of high pervasiveness of nutrient D insufficiency related for comorbidities that includes the sun exposure, atmospheric pollution, the degree of physical activity and dietary habits [34]. A study reported that a relation between high BMI measurements and lower vitamin D concentration. Results from current study support a relationship between Vitamin D deficiency and obesity. The 25(OH)D₃ was determined to be 24.6±11.4ng/mL for males and 24.1±13.3ng/mL for females. The normal range for 25(OH)D₃ is 25-80ng/mL. Mean values between the two genders were found to be insignificant (p = 0.5) [36]. Vitamin D inadequacy found in current investigation concurs with previously reported studies in Pakistan and in its adjoining nations. An undeniable degree of Vitamin D inadequacy (40%) was likewise noted among kids in the 2011 National Nutrition Survey conducted by Aga Khan University's Division of Women and Child Health, Pakistan's Ministry of Health and UNICEF with restricted contrasts among metropolitan and rustic regions[37].

CONCLUSION

Present study revealed that 65% men and 91.7% of women with vitamin D deficiency had BMI was 22.2±3.5 in males and 22.3±3.3 in females.

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Original Article

Association of Maternal Age and Presence of Non-communicable Diseases in Consanguineous Marriage with Congenital Abnormalities in Infants

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ABSTRACT

Consanguineous marriages are most common among communities where most families are of traditional and extended types. It has been seen that females who conceive at a very young age or have any non-communicable disease have higher chances to have off springs with congenital abnormalities. **Objective:** To find out the association between maternal age and non-communicable diseases with congenital abnormalities in children. **Methods:** It is a cross-sectional study performed at District Head Quarter Hospital, Okara, including 100 married participants in the age range of 19-55 years after obtaining their informed consent. **Results:** It was observed that 57% of mothers were below 20 at marriage, 42% were above 20 at marriage and 1% of mothers were above 30 at marriage, were having children with congenital abnormalities. 22 out of 100 mothers had obesity, 15 had diabetes and 21 had CVD when pregnant. The infants born to these mothers suffered from brain anomalies, cleft lip and cleft palate, CVD and diabetes. The results were obtained with p less than 0.05. **Conclusion:** Congenital abnormalities were more common among children with mothers aged below 20 or 25 and suffering from any metabolic or genetic disorder.

INTRODUCTION

Consanguineous marriage is defined as a marriage between people who belong to same families having same forefathers and ancestors [1]. They might be close blood relatives or may be a part of an extended family. Cousin marriages are done for supporting relationships, economic ties and for psychological and religious aspects [2]. According to different studies and reports, prevalence of cousin marriages worldwide is estimated to range from 20% to 60% in different regions [3]. Cousin marriages are contracted typically at a young age, when a female might not have developed sufficient nutritional reserves required to bring a healthy infant to life. If the female is already suffering from any non-communicable or communicable disease before conception or during pregnancy, this can also lead to the development of congenital anomalies in the offspring [4,5]. Some of the most common disorders present in females are diabetes mellitus, gestational

diabetes mellitus, hypertension, obesity, arthritis, kidney and liver diseases [6]. Along with these micronutrient deficiencies of Vitamin A, Vitamin D and folic acid are also very common [7]. All these maternal disorders and deficiencies lead to congenital anomalies in infants such as cleft palate and cleft lip, heart problems, spina bifida and other abnormalities of brain [8-11]. Exposure to pre-gestational diabetes mellitus and gestational diabetes mellitus is associated with the occurrence of congenital anomalies of the kidney and urinary tract. Congenital anomalies of the kidney and urinary tract are a diverse group of structural and functional abnormalities of the kidney, collecting system, bladder, and urethra [12]. According to scientific research, diabetes mellitus, metabolic or genetic other disorders and nutritional deficiencies to which a developing fetus, when exposed, can be teratogenic and can induce organ malformation

leading to congenital abnormalities [13, 14]. Obesity during pregnancy has a negative impact on both fetal and neonatal outcomes, including an increased chance of significant congenital abnormalities, which are a leading cause of stillbirth and infant mortality as well as long-term morbidity [15-17]. A wide range of congenital abnormalities, including neural tube defects, cardiovascular anomalies, cleft lip and palate, anorectal atresia, and limb reduction anomalies, are more common in the offspring of obese women [18-20]. Apart from the above-mentioned facts, there is scarcity of data available in this aspect. The current study aims to highlight such congenital abnormalities present at the time of the birth in babies born to parents who are consanguineously married and mothers are young and suffering from any non-communicable disorder. Hence, this study will try to fill the gap in existing knowledge. The purpose of this study is to evaluate the association of maternal young age and presence of non-communicable disorders with congenital abnormalities.

METHODS

It is a cross-sectional study performed at District Head Quarter Hospital, Okara, including 100 married participants in the age range of 19-55 years after obtaining their informed consent. The inclusion criteria were all adult individuals, with and without cousin marriage of both genders were included and the exclusion criteria was non-cooperative individuals. Individuals were assessed through pre-tested questionnaire. Questionnaire was made according to the study objective and was pretested among 10-15 individuals, and was modified accordingly. SPSS version 21.0 was used for data analysis. Frequencies were derived and Chi-square test was applied to find out the association, p value less than 0.05 was considered significant.

RESULTS

The result showed that 57% of mothers were below 20 at marriage, 42% of mothers were above 20 at marriage and 1% of mothers were above 30 at marriage, as shown in Table 1. The results showed that according to BMI scale, 9% of mothers were lying in the normal category, 29% were overweight, 33% of mothers were obese, 27% of mothers were lying in the category of obese grade 1, and 2% were in obesity grade 2 category.

| Age of marriage | Frequency |
|-----------------|-----------|
| Below 20 | 57 |
| Above 20 | 42 |
| Above 30 | 1 |
| Total | 100 |

Table 1: Frequency distribution of age of marriage

| Mother BMI | Frequency |
|------------|-----------|
| 18.4-24.9 | 9 |
| 25.0-29.9 | 29 |
| 30.0-34.9 | 33 |
| 35.0-39.9 | 27 |
| >40 | 2 |

Table 2: Frequency distribution of BMI of mothers

| Children with congenital abnormality | Frequency |
|--------------------------------------|-----------|
| Cleft lip and Cleft palate | 13 |
| Heart problems | 20 |
| Abnormalities of brain | 26 |
| No | 411 |
| Total | 00 |

Table 3: Frequency distribution of children with congenital abnormality

The result showed that 13% of mothers had children with cleft lip and cleft palate, 20% had children with heart problems, 26% had children with abnormalities of brain and 41% of mothers had children with no congenital abnormality (Table 3). The result showed that 22% of mothers had obesity as genetic disorder, 15% of mothers had diabetes as genetic disorder, 21% of mothers had CVD as genetic disorder, 9% of mothers had arthritis as genetic disorder, 18% of mothers had hypertension as genetic disorder and 15% of mothers had no genetic disorder (Table 4).

| Genetic disorder | Frequency |
|------------------|-----------|
| Obesity | 22 |
| Diabetes | 15 |
| CVD | 21 |
| Arthritis | 9 |
| Hypertension | 18 |
| No | 15 |
| Total | 100 |

Table 4: Frequency distribution of genetic disorder in mothers

DISCUSSION

According to the current study, 57% mothers were below age 20 when they delivered their child. Low maternal age at the time of infant birth has been associated with congenital anomalies according to different researches. One such study carried out by Shrim A, Ates S et al in 2011 also supported the significant association of young maternal age with different types of congenital anomalies in infants born to mothers who were below 20 years [21]. Present study indicates that mothers with children having congenital anomalies, suffered from different grades of obesity. 33% mothers were overweight while 27% were suffering from obesity grade 1. This indicated a significant relationship between maternal obesity and infant congenital abnormalities. Same relationship has been indicated by multiple research studies one of them was

carried out in 2018 by Kong L, Norstedt G et al [22]. According to a study conducted in Canada by researchers in 2014, presence of different genetic disorders in mothers impacted the normal cognitive development and health of infants. Present study also strongly indicated that mothers having different disorders such as hypertension, diabetes, obesity and other disorders gave birth to infants' having genetic disorders such as cleft palate, cleft lip, heart diseases and anomalies of brain [23].

CONCLUSIONS

Young maternal age has been associated with increased risk of congenital anomalies in infants. Age lower than 20 years poses a high risk for the development of congenital anomalies in infants due to multiple factors. Along with young age, presence of various genetic disorders in mothers is directly linked to the malformation of different organs, development of physical abnormalities and congenital anomalies in infants.

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Original Article

Frequency and Gram Category-wise Distribution of Clinical Isolates from Patient Samples in Tertiary Care Hospital of Lahore

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ABSTRACT

Antibiotics form an important tool in the treatment of various bacterial infections but their overuse and misuse are resulting in antibacterial resistance among bacteria. **Objective:** to outline the frequency and gram category-wise distribution of clinical isolates from patient samples. **Methods:** In this study, 1000 samples were collected from the patients attending the pathology department of Fatima memorial hospital, Lahore, Pakistan. Identification of bacteria was done through conventional culture and biochemical tests. **Results:** one thousand clinical isolates were identified. **Conclusions:** Gram negative bacterial isolates were prevalent, with 55% frequency distribution and *Staphylococcus aureus* was frequent as it was identified in 410 different samples of patients

INTRODUCTION

Antibiotic resistance is the capacity of certain strains of bacteria to develop a tolerance to specific antibiotics to which they were once sensitive [1]. Increased antibiotic resistance is a great health issue which is directly associated with high incidence rate of infectious diseases. Bacterial resistance also make treatment complicated and expensive [2]. Extensive use of antibiotics in health care sectors is the major contributor of bacterial resistance. This extensive use of antibodies could develop resistance in bacteria [3,4]. Mortality rate due to antibiotic resistance is about 7million per year and may double in upcoming years. Globally the ratio of infectious diseases due to antibiotic resistance is greater than cancers [5]. Now a days, antibiotic resistance has become an emerging issue due to the occurrence of novel bacterial strains. With the increasing demand of antibiotics, antibiotic resistance

creates a huge hindrance in the development and discovery of new antibiotic drugs and treatments [6]. In Pakistan the use of antibiotic without any cause is high and due to overuse of the antibiotics bacteria are getting resistance against these antibiotics. Very few reports were revealed about the occurrence of antibiotic resistance in bacteria linked to different infections in Pakistan. Some examples of antibiotic resistance occurring in Pakistan at different places had been documented. Acinetobacter species had shown resistance to numerous kinds of antibiotics at high level, especially showed resistance to Carbapenem. The resistance to Ceftriaxone and Quinolone in Salmonella species was also increasing in Pakistan [7]. Worldwide bacterial infections diseases are major causes of mortality and morbidity rate. Resistant microorganisms are playing a crucial role in the prevalence of these diseases. Faster

resistance of bacteria towards different drugs further leads toward major health complications such as organ failure is common. With variety of new bacterial variants treatment options are also reduced [8].

METHODS

Total one-thousand samples (blood, swabs, urine, sputum, pus) were collected. Each sample was collected in a sterile container. After sample collection, samples were cultured on selective media plates (Mannitol Salt agar, TCBS Agar, Eosin thiazine Agar, MSA agar, MacConkey Agar, enteric bacteria enteric bacteria Agar) from the sample container. After that plates were incubated for 24 hours at the temperature of 37°C. Then separated colonies were carefully observed. cfu/ml was calculated for a few of the plates and a few showed large growth. Then the colonies were streaked on agar plates to induce pure cultures for storage. Isolates Colonial morphology was determined by their growth on Cystine-Lactose-Electrolyte-Deficient (CLED) agar, Blood agar and MacConkey agar base. Then colonies characteristics were also observed. Further organisms were identified by susceptibility and standard identification techniques. On MacConkey agar dry and small pink color colonies were observed in the case of *E. coli* but yellow dry and smooth colonies were observed on Cystine-Lactose-Electrolyte-Deficient (CLED) agar. While mucoid haemolytic colonies were identified on blood agar that showed the presence of *E. coli*. In the case of *Pseudomonas aeruginosa* smooth, large hemolytic colonies on blood agar were observed. Yellow green non-lactose fermenting colonies on MacConkey agar were observed.

RESULTS

Out of 1000 samples, frequency and percentage of clinical isolates are shown in table 1. According to gram category clinical isolates distribution had been shown in Figure 1. Out of thousand clinical isolates, based on gram staining and microscopy 450 were Gram Positive isolates and 550 were Gram Negative isolates. Total 553 (55.3%) were male patients and 447 (44.7%) were female patients. Frequency of clinical isolates from samples is shown in table 2.

| | |
|--------------|--------------|
| Blood | 9.3%(n=92) |
| Breast fluid | 1.4%(n=14) |
| CSF | 1.7% (n=17) |
| Ear swabs | 2.0%(n=20) |
| Fluids | 4.1% (n=41) |
| Foiet tips | 2.2%(n=22) |
| HVS | 3.7%(n=37) |
| Pleural tips | 2.0%(n=20) |
| Pus | 42.1%(n=421) |
| Semen | 3.8%(n=38) |
| Sputum | 4.5%(n=45) |

| | |
|-------------|--------------|
| Swab | 3.7%(n=37) |
| Throat swab | 1.2%(n=12) |
| Urine swab | 13.9%(n=139) |
| Wound swab | 4.4%(n=44) |

Table 1: Frequency of clinical isolates

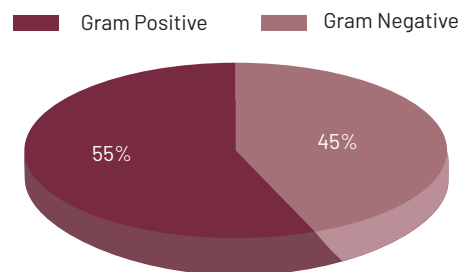


Figure 1: Represents Gram Staining Characteristics of Clinical Isolates

| Micro-organism | Frequency (%) |
|---|---------------|
| <i>Actinobacter</i> | 6 (0.6%) |
| <i>Citrobacter</i> | 2 (0.2%) |
| <i>E. coli</i> | 131 (13.1%) |
| <i>E. coli, Enterobacter</i> | 9 (0.9%) |
| <i>E. coli, Klebsiella</i> | 1 (0.1%) |
| <i>E. coli, Pseudomonas</i> | 13 (1.3%) |
| <i>E. coli, Pseudomonas, Klebsiella</i> | 1 (0.1%) |
| <i>Enterobacter</i> | 64 (6.4%) |
| <i>Enterobacter, Proteus</i> | 27 (2.7%) |
| <i>Enterobacter, Pseudomonas</i> | 75 (7.5%) |
| Hemolytic <i>Streptococci</i> | 6 (0.6%) |
| <i>Klebsiella Species</i> | 31 (3.1%) |
| <i>Klebsiella, Hemolytic Streptococci</i> | 1 (0.1%) |
| <i>Klebsiella, Proteus Species</i> | 2 (0.2%) |
| <i>Klebsiella, Pseudomonas</i> | 2 (0.2%) |
| <i>Klebsiella, Pseudomonas, Staphylococcus aureus</i> | 1 (0.1%) |
| <i>Proteus Species</i> | 9 (0.9%) |
| <i>Proteus Species, E. coli</i> | 3 (0.3%) |
| <i>Proteus Species, Klebsiella</i> | 1 (0.1%) |
| <i>Proteus species, Pseudomonas</i> | 7 (0.7%) |
| <i>Proteus Species, Staphylococcus aureus</i> | 3 (0.3%) |
| <i>Pseudomonas</i> | 144 (14.4%) |
| <i>Pseudomonas, Klebsiella</i> | 2 (0.2%) |
| <i>Pseudomonas, Proteus</i> | 1 (0.1%) |
| <i>Staphylococcus aureus</i> | 410 (41.0%) |
| <i>Staphylococcus aureus, E. coli</i> | 3 (0.3%) |
| <i>Staphylococcus aureus, E. coli, Pseudomonas</i> | 1 (0.1%) |
| <i>Staphylococcus aureus, Enterobacter</i> | 6 (0.6%) |
| <i>Staphylococcus aureus, Klebsiella</i> | 2 (0.2%) |
| <i>Staphylococcus. aureus, Proteus Species, Pseudomonas</i> | 1 (0.1%) |
| <i>Staphylococcus aureus, Pseudomonas</i> | 35 (3.5%) |
| Total | 1000 (100.0%) |

Table 2: Frequency and Percentage of Clinical Isolates

DISCUSSION

Antibiotic resistance is not a latest phenomenon, since the introduction of antibiotic (penicillin), bacteria are noted to possess some resistance. The potential of bacteria to defend against the effects of an antibiotic is called antibiotic resistance. Antibiotic resistance occurs when bacteria or other microbes become resistant to the special effects of an antibiotic after being exposed to them. Antibiotic resistance results in bacteria due to change by some approach that eliminates or reduces the effectiveness of antibacterial agents intended for treatment of infections. The bacteria cause more damage to human body by survival and continuous multiplication [9]. The frequencies that are found in clinical isolates in Lahore are lesser than the past studies from Pakistan (60.4%)[10], 33.5% Russia, 32% in Kuwait, 69.1% in western Nepal 71.4% in Sudan [11], 42% in Iran and 13% in Sri Lanka [12]. Gram positive bacterial infections [13,14]. Gram positive bacteria, *Streptococcus pneumoniae* and *Staphylococcus Aureus* were linked with large ratio of bacterial infections at various sites such as bone, respiratory track, joints, CNS, skin and, bloodstream [15, 16]. In a study on prevalence of clinical isolates e.g. *Staphylococcus Aureus*, causing nosocomial infections, were more in men than in women [17]. The maximum isolates were obtained from pus samples 42.1% followed by urine samples 13.9% and 9.3% in blood samples. The other clinical isolates were 4.5% for sputum samples, 4.4% for wound swabs samples, 4.1% for fluids. These results were in line with the previous studies [18]. A change in the frequency distribution of clinical isolates among Pakistani population, about 2% to 61% [19] and throughout the world [20]. Regarding effectiveness of antibiotics for gram positive isolates, Vancomycin, Klaricid, Fusidic acid, Vibramycin, Erythromycin were much effective. This sensitivity of gram-positive bacteria to Vancomycin was observed similarly in previous study [21]. Gram positive isolates showed resistance with various degrees to Amoxicillin (46.2%), Cefotaxime (12.7%), Ciprofloxacin (4.0%) and Ampicillin (3.1%). Resistance to Amoxicillin was also noted by Shrestha et al [22]. Few of gram positive isolates showed a low resistance to Ampicillin similar to Matute et al [23].

CONCLUSIONS

Non-empirical and inappropriate use of antibiotic has increased the emergence of antibiotic resistance within bacteria both Gram negative and Gram positive. In Pakistan, most of the antibiotics are prescribed inappropriately to patients in hospital.

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Original Article

Comparison of GeneXpert Assay and Fluorescent Microscopy for the Diagnosis of Pulmonary Tuberculosis in Narowal Region

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ABSTRACT

GeneXpert is attributed as one of the latest technical means for diagnosing Tuberculosis in very short period of time. **Objectives:** To evaluate the efficiency of GeneXpert and fluorescent microscopy in the detection of pulmonary tuberculosis (TB); To compare the sensitivity and specificity of GeneXpert and fluorescent microscope in the diagnosis of *Mycobacterium tuberculosis*. **Methods:** In the present investigation, the diagnostic performance of GeneXpert MTB / RIF for tuberculosis was compared with the performance of light-emitting diode fluorescence microscope in TB samples from Narowal, Pakistan. For this purpose, a total of 299 TB positive specimens were obtained. Among these 54% (n = 160) were categorized to be obtained from male and 46% (n = 139) from female population. Data collected was distributed in 4 age groups; 0-20, 21-40, 41-60, and 61-80, in which the percentage and number of samples were found as 2% (n = 6), 60% (n = 179), 27% (n = 80) and 11% (n = 34), respectively. **Results:** The parameters including sensitivity and specificity calculated for GeneXpert were 73% and 100%, correspondingly, while the sensitivity and specificity calculated for LED-FM microscope were 43% and 100%, respectively. **Conclusions:** This indicates that the GeneXpert is more sensitive in detecting MTB in comparison to LED-FM technique. The GeneXpert assay was also found to detect small number of bacillus from samples in comparison to LED-FM method.

INTRODUCTION

Mycobacterium Tuberculosis (MTB) is a causal cause of tuberculosis [1]. The lung is the main organ affected by MTB, but other organs of body may also be severely affected by it [2]. In most cases, the ailment can reduce symptoms, in which case it is called drowsiness or inactive tuberculosis [3,4]. About 10% of lethargic or inactive disease develops into dynamic disease, and when untreated, the slayer will infect more people [5]. Model indicators of dynamic tuberculosis are blood stasis, weight loss, night sweats and fever. Weight loss is what has long been called "consumption." The broad combination of symptoms is caused by contamination of different organs [6]. "When people with dynamic tuberculosis in the lungs cough, spit, talk or sneeze, tuberculosis spreads through airborne droplets. People with inactive tuberculosis do not

spread the disease. HIV/AIDS patients and smokers are prone to active infection [7]. The diagnosis of active TB depends on X-rays of the chest, along with culture and the microscopy of various body fluids. The identification of indolent tuberculosis depends on the special skin test called tuberculin skin test (TST) or by way of blood test [8-10]. Avoidance of tuberculosis includes vaccination against BCG, increased screening hazard, timely identification and correct management of the cases [11]. High-risk group include family members, work environment, and social interactions with active tuberculosis patients. Treatment requires long-term use of various antibiotic agents. The main problem now devouring society is antibiotic resistance, incidence of multidrug resistant tuberculosis and extensive drug resistance tuberculosis. 33% of the

total population worldwide carry the mycobacterium tuberculosis in dormant condition but only 1% of new infections occur in the general population. In 2016, there were more than 10 million cases of dynamic tuberculosis, resulting in 1.3 million deaths. Hence making it an important cause of death due to infection. In most of the developing countries, more than 95% of the deaths occurred more than half of these occurred in Indonesia, Pakistan, the Philippines, India and China. Since 2000, the number of fresh cases has decreased every year. Approximately 80% of the people in different African and Asian countries are tested, whereas in the United States, 5-10% of individual lumps are found to be positive by way of the tuberculin test [12]. Xpert MTB / RIF provides patients with distinct advantages such as early diagnosis and early start of appropriate treatment resulting in improved general health i.e. reducing opportunities for tuberculosis transmission, especially in developing countries [13]. The GeneXpert MTB/RIF technique is used for the diagnosis of TB and rifampicin (RIF) resistance [14]. This study was designed for the association of GeneXpert MTB/RIF assays and fluorescence microscope for rapid detection of TB versus culture.'

METHODS

It is a cross-sectional study. The study will be conducted in the tuberculosis department at DHQ Hospital in Narowal. Sample size was 299 and sampling technique was Judgment/purposive sampling. Inclusion criteria: Patients of all ages, Patients belonging to male and female gender, Patients with active tuberculosis infection and Patients who can actively cough up sputum.

Laboratory Processing: The patient's detailed clinical parameters were recorded and the patient was guided to collect the sputum sample in a defined container. These samples were then assigned a specific laboratory number and processed further.

Preparation of Sample: Smears were prepared from samples after concentration and re-suspension of the pallet. A drop of specimen was placed on a microscope slide. It was spread and allowed to dry. Smear was heated and then kept for fixation at 65-75°C for 2-3 hours.

Staining Procedure: Smear was covered with stain. After 15 minutes of washing, the stains were rinsed and the slides were immersed for 2 minutes in 0.5% decolorizing agent. Slides were washed again and covered with potassium permanganate solution. Slides were rinsed after 2 minutes, air dried and examined under UV light.

Microscopy: After staining, the slides were examined by the Microscopists and me. An eyepiece with a10x amplification and an objective lens with 40x amplification was used. Quantification of acid-fast bacilli was carried out

as meeting the guidelines and criteria of the Centre for Disease Control(CDC).'

| 200X | Report |
|-------------------------|-----------------------|
| No AFB in one length | Negative |
| 1-4 AFB in one length | Confirmation required |
| 5-49 AFB in one length | Exact number (scanty) |
| 3-24 AFB in one field | 1+ |
| 25-250 AFB in one field | 2+ |
| >250 AFB in one field | 3+ |

Table 1: Grading of cells under FM Microscope(WHO,1998)

GeneXpert: Requirements are GeneXpert system (GeneXpert + Computer + Barcode scanner), Sample reagent, Cartridge, Personal protective equipment (N95 mask, gloves, apron, closed shoe, face shield), Vortex, Timer

Procedure: The sample reagent and the sputum collection container lids were opened.02 volumes of sample reagent was added to 01 volume of sputum and lid was replaced. The mixture was thoroughly mixed over a vortex for at least 10 seconds. Then it was incubated for 10 minutes at room temperature and then mixed again. It was incubated for another 05 minutes. The sample was processed till it was perfectly liquid, if it was still viscous, a waiting time of 05-10 minutes was given. The side of the cartridge was labelled with the sample id before its lid was opened. Sample (2ml) was slowly transferred to the sample chamber of the cartridge taking care that care that bubbles don't form. The lid was firmly closed and the test was run on GeneXpert instrument.

Culture

Culture Media: Lowenstein Jensen media was employed to detect the bacilli from samples.

Media Composition: It includes: malachite green, asparagine, potato starch, coagulated egg, mineral salt solution (potassium dihydrogen phosphate, magnesium sulfate, sodium citrate), low levels of penicillin and nalidixic acid are also present in LJ medium to inhibit growth of Gram-positive and Gram-negative bacteria and to limit growth to Mycobacterium species only. Presence of malachite green in the medium inhibits most other bacteria. It is disinfected and solidified by a process of inspissation. Presence of glycerol enhances the growth of *M. tuberculosis*. For cultivation of *M. bovis*, glycerol is omitted and sodium pyruvate is added.

Culture Inoculation: Positive and negative results of samples as found by microscopy and GeneXpert were cultured on Lowenstein Jensen media. After inoculation, the plates were incubated for at least 6 weeks at 37°C. Any visible growth was observed and recorded as MTB and MOTT.'

Statistical Analysis: Using the 2x2 table in the SPSS-20 software and considering the sputum culture as gold

standard. The sensitivity, specificity, PPV and NPV for each assay were calculated to diagnose TB in patients. The kappa(k) test was used to assess the consistency between the tests. The receiver operating characteristic (ROC) curves were performed using SPSS-20 software.

Using the formula, the sensitivity was found as follows:
 Sensitivity % = true positive (TP) / (true positive (TP) + false negative (FN)) X 100.

Specificity was calculated using the formula given below:
 Specificity % = true negative (TN) / (true negative (TN) + false positive (FP)) X 100.

RESULTS

The current study was conducted (in Narowal, Pakistan) to compare the diagnosis of tuberculosis with GeneXpert and fluorescence microscopy. Total processed samples were 299 of which 54% (n = 160) were obtained from male and 46% (n = 139) from female population. Data obtained was divided into 4 groups according to age as; 0-20, 21-40, 41-60, and 61-80.

| Age groups | | | | |
|---------------------|------|-------|-------|-------|
| No. of patients (n) | 0-20 | 21-40 | 41-60 | 61-80 |
| | 6 | 179 | 80 | 34 |
| Percentage (%) | 2 | 60 | 27 | 11 |

Table 2: Categorization of individuals across age groups'

This distribution showed that 2% (n = 6), 60% (n = 179) and 27% (n=80), 11% (n=34) were found in the age range of 0-20, 21-40, 41-60, and 61-80 respectively (Table 2).

Selection of Samples: Samples that fulfilled the inclusion and exclusion criteria were collected and processed.'

Physical Examination of Sputum Sample: Physical examination revealed that out of 299 samples 5% (n=15) were salivary, 49% (n=149) were mucoid, 36% (n=109) were purulent and 10% (n=31) were blood tinted (Table 3).

| Variables | Saliva | Mucoid | Purulent | Blood tinted | Total |
|-------------------|--------|--------|----------|--------------|-------|
| Number of samples | 15 | 149 | 104 | 31 | 299 |
| (n)Percentage (%) | 5 | 49 | 36 | 10 | 100 |

Table 3: Physical examination of sputum sample'

Grading of Samples upon Fluorescent Microscopy: Out of 299 processed samples grading of microscopy revealed that 86% (n=256) samples were negative, 1% (n=4) were scanty, 2% (n=6) were 1+, 6% (n=18) were 2+, 5% (n=15) were 3+ (Table 4).

| Grading | Scanty | 1+ | 2+ | 3+ | Total |
|---------|--------|----|----|----|-------|
| | 4 | 6 | 18 | 15 | 299 |
| | 1 | 2 | 6 | 5 | 100 |

Table 4: Grading of processed samples analyzed on FM

Detection of Tuberculosis with FM: Fluorescence microscopy declared 18% (n=55) as positive (Table 5). The sensitivity and specificity recorded for FM were 43% and 100%, respectively. Furthermore positive predictive value

(PPV) and negative predictive value (NPV) estimated for FM technique were found to be 71% and 100%, respectively (Table 6).'

Detection of Tuberculosis by GeneXpert: Total positive samples as observed through GeneXpert were 31% (n=93) (Table 5). The sensitivity and specificity recorded for GeneXpert were 73% and 100% respectively. Furthermore, PPV and NPV values estimated for GeneXpert assay were found to be 83% and 100%, respectively (Table 6).

| | Methods | |
|--------------------|---------|-----------|
| | FM | GeneXpert |
| Total positive (n) | 55 | 93 |
| Percentage (%) | 18 | 31 |

Table 5: Comparison of diagnostic techniques in detecting tuberculosis

Comparison of FM and GeneXpert: FM had sensitivity of 43% while GeneXpert showed 73% sensitivity. It was also revealed that GeneXpert was more sensitive as compared to Fluorescence Microscopy (Table 6). The area under ROC curves demonstrated that it was greater for GeneXpert (.859) as compared to FM (.703) (Table 8, figure 1), this shows that GeneXpert is more efficient than Microscopy. The concordance value showed the moderate trend as it was noted to be .642 for GeneXpert and FM (Table 7).

| | FM | GeneXpert |
|-----------------|-----|-----------|
| Sensitivity (%) | 43 | 73 |
| Specificity (%) | 100 | 100 |
| PPV | 71 | 83 |
| NPV | 100 | 100 |

Table 6: Comparison of sensitivity and specificity of FM, GeneXpert and Culture

From the above table, it is clear that GeneXpert is more sensitive than FM considering culture as a gold standard. The specificity of GeneXpert and FM is same.

| Diagnosing techniques | k value |
|-----------------------|---------|
| FM x Culture | .434 |
| GeneXpert x Culture | .741 |
| FM x GeneXpert | .642 |

Table 7: Concordance for different methods by kappa test

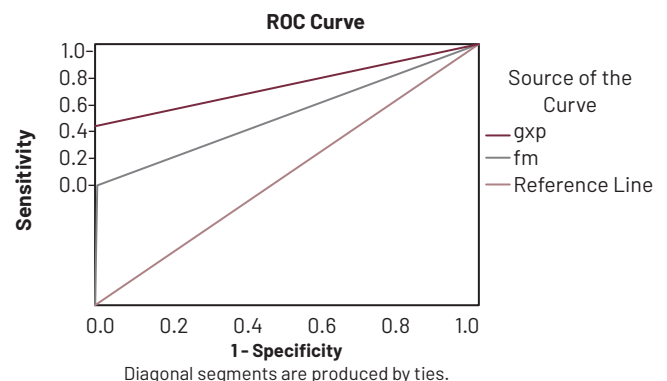


Figure 1: ROC curve

| Test | Area | Significance |
|-----------|------|--------------|
| FM | .703 | .000 |
| GeneXpert | .859 | .000 |

Table 8: ROC area of different methods

DISCUSSION

The purpose of investigation was to investigate diagnostic ability of three different techniques for tuberculosis cases. The findings were paralleled to standard culture techniques. Total 299 sputum samples were examined, with an FM detection rate of 18% (n = 55), GeneXpert of 31% (n = 93) and a standard culture technique of 43% (n = 128). The specificity and sensitivity of the GeneXpert assay were known to be 100% and 73%, respectively, in addition the sensitivity and specificity of the FM microscope were 43% and 100%, respectively. The findings showed culture as better than the two techniques used. This is in contrast with another study comparing the GeneXpert findings and stated GeneXpert to be better [15]. Sajjad Ahmed and his colleagues studied the GeneXpert MTB / RIF assay for the detection of TB on sputum specimens. After meeting the inclusion criteria, a total of 268 participants were included in the study. Their sputum samples were collected and processed by the N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) method and the GeneXpert MTB / RIF assay. The study determined the overall sensitivity and specificity of the MTB / RIF assays, 92.4% (86/93) and 97.1% (138/142), respectively. The sensitivity was 98.4% (60/61) in the culture proven smear positive samples, while the culture confirmed that the smear negative sample had a sensitivity of 93.7% (30/32) using the culture as a reference standard [16]. Few other studies have reported similar findings [17,18]. The result of this study are similar to our study which aimed to evaluate GeneXpert for culture and fluorescence microscopy, and GeneXpert analysis showed sensitivity and specificity of 73% and 100%, correspondingly. In a survey conducted by Elisabetta Walters and her colleagues, 14 samples were analyzed by culture and GeneXpert techniques. Of the 9/14 (64%) cases confirmed by culture, 7 (78%) were positive for broncho alveolar lavage (BAL) samples; in addition, GeneXpert also confirmed two cases with an earlier negative diagnosis (14%). Two drug-resistant cases were identified: one from BAL Xpert and the other from genotyping tests for gastric inhalation culture. All children started receiving anti-tuberculosis treatment and responded well to the treatment [19]. In our study we worked on sputum samples with a sensitivity of 18%, 31%, and 43% for FM microscopy, GeneXpert assay and culture respectively. This study is contradictory to our study which maintains that culture is a gold standard technique, and it's better than GeneXpert and fluorescent microscopy. Lidya Chaidir and her

colleagues studied the application of FM technique for the purpose of diagnosing lung cancer and also HIV related tuberculosis in Indonesian hospital settings. They assessed that fluorescent microscope was more sensitive, but not as specific as ZN. The sensitivity and specificity of FM increase when sputum is concentrated before smear preparation. In people living with HIV, FM exhibit to some extent higher specificity and sensitivity than traditional ZN microscopes [20]. In our study, we did not compare FM microscopy with conventional ZN staining. Though FM microscopy showed 43% sensitivity and 100% specificity. On the contrary, FM takes half the time of the ZN microscopy and has a similar operating cost. Method for diagnosing urinary tract tuberculosis in urine samples by genotoxicity Mtb/rif assay was studied by Yu Pang. Total 167 patients participated in the study. Out of these, 4 (2.4%) patients were omitted from the study. Therefore, 163 patients were analyzed finally, of which 44 (27%) were diagnosed with urinary tuberculosis (UTB) cases based on clinical symptoms and anti-TB treatment and 37 (22.7%) were cultured positive UTB cases. The sensitivity of acid bacillus microscopy and GeneXpert is 40.5% and 94.6% as compared to LJ culture, respectively. After using clinical diagnosis as a reference standard, the specificity and the sensitivity of AFB smears were 98.8% and 18.5% respectively. LJ culture cases are twice times higher than AFB smear cases, with sensitivity and specificity of 45.7% and 100%. In addition, from the clinically diagnosed 81 urinary tract tuberculosis cases, 51 were processed by the Xpert technique, showing the sensitivity of 63% that is considerably higher than AFB smear microscopy and LJ culture method. GeneXpert was only detected in 5 patients with RIF resistance, and all patients had a phenotypic sensitivity test with a sensitivity of 100% [21]. This study is quite similar to present study in which GeneXpert is more sensitive and specific as compared to FM.

CONCLUSIONS

MTB/RIF examinations should create faster resistance testing and, in selected groups, strengthen tuberculosis case detection. If subsidy for improved MDR TB treatment is accessible, then it will be very cost effective and beneficial for rapid screening of tuberculosis patients but this influence is predictably limited by its cost. While this progress should be celebrated and priority should be given to funding in this area, it must be seen as a shocking situation that nearly two million people die from tuberculosis every year and in fact some people will be protected by any type of diagnostic test. Mostly deaths occur in HIV negative individuals, most of them died from medication sensitive tuberculosis, primarily due to insufficient basic affordable healthcare services for the

treatable infectious disease.

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Original Article

Attitude Regarding Dengue Vector Control Among the Community of Tehsil Sahiwal, Sargodha

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ABSTRACT

Dengue fever is one of the most threatening vector borne diseases, causing severe epidemics and has brought the world to take serious steps for its control and prevention. **Objective:** The objectives of the study are to assess the attitudes regarding dengue vector control among community of Tehsil Sahiwal, District Sargodha. **Methods:** It was a community based and cross-sectional study in which 384 residents of Tehsil Sahiwal of District Sargodha were included. Simple random sampling technique was used. Data was collected through questionnaire, which was entered and statistically analyzed using SPSS 20.0. Frequency tables were generated for all possible variables. For quantitative data like age and income, mean and standard deviation were calculated and for qualitative data like sex, education, occupation percentages were calculated. Written consent was taken from respondents. **Results:** Among 384 respondents, mean age was 37.35 ± 11.67 years. 69.8% respondents were males. 58.1% respondents had overall good attitude and 41.9% had poor attitude. Among 207 respondents who had overall good knowledge, 199 (51.8%) had good attitude and 8 (2.1%) had poor attitude. **Conclusions:** Study concluded that attitude regarding dengue vector control among community was found satisfactory 58.1% had overall good attitude.

INTRODUCTION

Dengue fever (DF) is caused by a mosquito-borne human viral pathogen that belongs to the genus *Flavivirus* of the family *Flaviviridae* (single-strand, non-segmented RNA (ribonucleic acid) viruses). Dengue is transmitted in humans by two species of *Aedes* mosquitoes namely *Aedes aegypti* (principal vector) and *Aedes aldopictus* [1]. Dengue virus (DENV) has four serotypes, DENV-1, DENV-2, DENV-3 and DENV-4 [2]. The global incidence of dengue has grown dramatically in recent decades [3]. An estimated 3900 million people in 128 countries are at risk for dengue infection [4]. One recent estimate indicates 390 million dengue infections per year, of which 96 million manifests clinically (with any severity of disease) [5]. Vector control, the most important strategy to prevent and control dengue, requires the cooperation of entire communities, as it is not solely a government responsibility [6]. According to the World Health Organization (WHO), *Aedes aegypti*'s control is mainly achieved by source reduction of

the vector through the elimination of the mosquito breeding sites. [21] Due to *Aedes aegypti*'s domestic ecological feature, their larvae preferably proliferate in small and artificial water-containers, placed inside or near human houses [7]. Therefore, community contribution is, undoubtedly crucial in dengue prevention and control [8]. Community based eradication campaigns are made with the objective of educating the public about the strategies for the elimination of breeding sites to reduce mosquito production. In this method, the public or community people are divided into different groups because all the people of community are not well educated and their level of understanding is not same [9]. In some countries like Cuba, community involvement has been very useful for the effective eradication of *Aedes aegypti* mosquitoes from the environment [10]. However, to get maximum vector control, community involvement should be used in combination of other methods to limit vector density,

[11,12] such as the combination of community based campaigns and chemical control have shown significant results in controlling *Aedes aegypti* in Cuba state [13].

METHODS

The study was community based and cross sectional. The study setting was Tehsil Sahiwal of District Sargodha. A total of 384 subjects were taken at 5% margin of error, 95% confidence interval and expected percentage of good knowledge as 51.7% [14]. Simple random sampling technique was used. Residents of Tehsil Sahiwal of District Sargodha, Age range between (18-60), Both Genders (Male and Female) were included. Data was entered, cleaned and analyzed using SPSS (Statistical Package for Social Sciences) version 20.0. Frequency tables were generated for all possible variables. For quantitative data like age and income, mean and standard deviation were calculated and for qualitative data like sex, education, occupation percentages were calculated. Formal consent was taken from whom to conduct the study. Written consent was taken from respondents. Privacy and confidentiality of data was maintained at all costs in accordance with principles. A semi-structured questionnaire was prepared by researcher and finalized after pre-testing. The residents of Tehsil Sahiwal, District Sargodha were interviewed by the researcher himself and responses were noted on the questionnaire.

Data Analysis Procedure: The software was used for data analysis namely SPSS, through SPSS software, the data was initially arranged with the help of Likert Scale. Parametric or Non-parametric test as also applied for the purpose to smooth the data or as per needs. Descriptive statistics, correlation, regression.

RESULTS

Among 384 respondents, 204 (53.1%) covered their water jars after using immediately while 180 (46.9%) respondents did not cover water jars, 231 (60.2%) had a cover in their water tanks while 153 (39.8%) had no cover. Among 384 respondents, 223 (58.1%) changed the water of the indoor plants every week while 161 (41.9%) respondents did not change. 234 (60.9%) said they ever drain off the water in the plates of the flower pot while 150 (39.1%) respondent said they did not, 134 (34.9%) examined any discarded thing that can hold water around their house while majority 250 (65.1%) did not examine, 123 (32.0%) discarded soft drink plastic bottles with caps while majority 261 (68.0%) did not discard soft drink plastic bottles with caps, 268 (69.8%) changed their air cooler water while 116 (30.2%) did not, 268 respondents who changed their air cooler water, 94 (35.1%) changed every day and 174 (64.9%) respondents changed water every third day, 232 (60.4%) said that they wear full sleeve clothes early morning and evening in mosquito

season while 152 (39.6%) respondents said they did not, 327 (85.2%) used mosquito net/coils in their house while 57 (14.8%) respondents did not use, 327 respondents who used mosquito net/coils in their house, all of them used mosquito net/coils during night (Table 1).

| Frequency Distribution of Respondents Who Covered Water Jars After Using Immediately | | |
|--|-----------|----------------|
| n-384 | Frequency | Percentage (%) |
| Yes | 204 | 53.1 |
| No | 180 | 46.9 |
| Frequency Distribution of Respondents According To Having A Cover In Their Water Tanks | | |
| Yes | 231 | 60.2 |
| No | 153 | 39.8 |
| Frequency Distribution of Respondents Who Change The Water of The Indoor Plants Every Week | | |
| Yes | 223 | 58.1 |
| No | 161 | 41.9 |
| Frequency Distribution of Respondents According to Ever Drain off The Water In The Plates of The Flower Pot | | |
| Yes | 234 | 60.9 |
| No | 150 | 39.1 |
| Frequency Distribution of Respondents According To Examination Any Discarded Thing That Can Hold Water Around Their House | | |
| Yes | 134 | 34.9 |
| No | 250 | 65.1 |
| Frequency Distribution of Respondents Who Discarded Soft Drink Plastic Bottles With Caps | | |
| Yes | 123 | 32.0 |
| No | 261 | 68.0 |
| Frequency Distribution of Respondents According To Change Of Water In Their Air Cooler | | |
| Yes | 268 | 69.8 |
| No | 116 | 30.2 |
| If yes, when | | |
| Everyday | 94 | 35.1 |
| Every third day | 174 | 64.9 |
| Frequency Distribution of Respondents According To Wearing of Full Sleeve Clothes Early Morning And Evening In Mosquito Season | | |
| Yes | 232 | 60.4 |
| No | 152 | 39.6 |
| Frequency Distribution of Respondents According To Use of Mosquito Net/coils In Their House | | |
| Yes | 327 | 85.2 |
| No | 57 | 14.8 |
| If yes, when | | |
| Night | 327 | 100.0 |
| Day | 0 | 0.0 |

Table 1: Questions asked from respondents regarding dengue vector control

| Socio-demographic characteristics | Overall Attitude | | | P-value |
|-----------------------------------|------------------|-------------|--------------|---------|
| | Good | Poor | Total | |
| Age (years) | | | | |
| <40 | 112 (29.2%) | 100 (26.0%) | 212 (55.2%) | |
| >40 | 111 (28.9%) | 61 (15.9%) | 172 (44.8%) | 0.370 |
| Total | 223 (58.1%) | 161 (41.9%) | 384 (100.0%) | |
| Sex | | | | |
| Male | 155 (40.4%) | 113 (29.4%) | 268 (69.8%) | |
| Female | 68 (17.7%) | 48 (12.5%) | 116 (30.2%) | 0.206 |

| | | | | |
|-----------------------------|-------------|-------------|--------------|-------|
| Total | 223 (58.1%) | 161 (41.9%) | 384 (100.0%) | |
| Education | | | | |
| Illiterate | 20 (5.2%) | 18 (4.7%) | 38 (9.9%) | |
| Literate | 203 (52.9%) | 143 (37.2%) | 346 (90.1%) | 0.163 |
| Total | 223 (58.1%) | 161 (41.9%) | 384 (100.0%) | |
| Occupation | | | | |
| Employed | 134 (35.0%) | 97 (25.2%) | 231 (60.2%) | |
| Unemployed | 89 (23.1%) | 64 (16.7%) | 153 (39.8%) | 0.014 |
| Total | 223 (58.1%) | 161 (41.9%) | 384 (100.0%) | |
| Family monthly income (Rs.) | | | | |
| <20,000 | 144 (37.5%) | 95 (24.7%) | 239 (62.2%) | |
| >20,000 | 79 (20.6%) | 66 (17.2%) | 145 (37.8%) | 0.377 |
| Total | 223 (58.1%) | 161 (41.9%) | 384 (100.0%) | |

Table 2: Correlation between socio-demographic characteristics and attitude regarding dengue vector control

Table 2 demonstrates that among 212 respondents who were up to 40 years old, 112 (29.2%) had overall good attitude and 100 (26.0%) had poor attitude. Among 172 respondents who were above 40 years old, 111 (28.9%) had overall good attitude and 61 (15.9%) had poor attitude. The result was found statistically insignificant ($P=0.370$). Among 268 respondents who were males, 155 (40.4%) had overall good attitude and 113 (29.4%) had poor attitude. Among 116 respondents who were females, 68 (17.7%) had overall good attitude and 48 (12.5%) had poor attitude. The result was found statistically insignificant ($P=0.206$). Among 38 respondents who were illiterate, 20 (5.2%) had overall good attitude and 18 (4.7%) had poor attitude. Among 346 respondents who were literate, 203 (52.9%) had overall good attitude and 143 (37.2%) had poor attitude. The result was found statistically insignificant ($P=0.163$). Among 231 respondents who were employed, 134 (35.0%) had overall good attitude and 97 (25.2%) had poor attitude. Among 153 respondents who were unemployed, 89 (23.1%) had overall good attitude and 64 (16.7%) had poor attitude. The result was found statistically significant ($P=0.014$). Among 239 respondents who had family monthly income <20,000 rupees, 144 (37.5%) had overall good attitude and 95 (24.7%) had poor attitude. Among 145 respondents who had family monthly income >20,000 rupees, 79 (20.6%) had overall good attitude and 66 (17.2%) had poor attitude. The result was found statistically insignificant ($P=0.377$).

| Overall knowledge | Overall Attitude | | | P-value |
|-------------------|------------------|-------------|--------------|---------|
| | Good | Poor | Total | |
| Good | 199 (51.8%) | 8 (2.1%) | 207 (53.9) | |
| Poor | 24 (6.3%) | 153 (39.8%) | 177 (46.1%) | 0.000 |
| Total | 223 (58.1%) | 161 (41.9%) | 384 (100.0%) | |

Table 3: Overall attitude of respondents regarding dengue vector control

Above table exhibits that among 207 respondents who had overall good knowledge, 199 (51.8%) had good attitude and 8 (2.1%) had poor attitude. Among 177 respondents who had

overall poor knowledge, 24 (6.3%) had good attitude and 153 (39.8%) had poor attitude. The result was found statistically significant ($P=0.000$) (Table 3).

| Overall Attitude | Frequency (%) |
|------------------|---------------|
| Good | 223 (58.1%) |
| Poor | 161 (41.9%) |
| Total | 384 (100.0%) |

Table 4: Frequency distribution of respondents according to overall attitude

Table 4 describes that among 384 respondents, 223 (58.1%) had overall good attitude and 161 (41.9%) had poor attitude.

DISCUSSION

Age is believed a major factor to create better awareness among community for the prevention of dengue. It is significant to mention that more than half of the respondents (55.2%) were up to 40 years old and 44.8% were more than 40 years old while mean age of the respondents was $37.35 + 11.67$ years. The findings of our study are comparable with a study undertaken by Rehman and coworkers (2015) who also reported that more than half (62.7%) respondents were up to 40 years old while remaining proportion (37.3%) was more than 40 years old [15]. A study carried out by Chinnakali and associates (2012) highlighted that mean age of the respondents was $33.8+12.8$ years. The results of another study performed by Nagoor and collaborators (2017) showed that participants mean age was 34.5 years. It was found during study that most of the respondents (69.8%) were males and 30.2% were females. A similar study carried out by Chinnakali and associates (2012) also confirmed that majority of respondents (78.3%) who participated in the study were males and 21.7% were females [16]. But the findings of a study conducted by Koenraad and partners (2006) indicated that mainstream of respondents (82.0) were females and only 18.0% were males [17]. Study showed very encouraging results that more than half (52.9%) of respondents believed that the mosquito that transmits dengue infection does not lay its eggs in dirty sewage water. But the findings of our study did not show better results than the study carried out by Diaz-Quijano and comrades (2018) who asserted that 81.6% respondents were aware that mosquito that transmits dengue infection does not lay its eggs in dirty sewage water [18]. In our study 54.9% respondents had knowledge that empty stagnant water from old tires, trash cans, refrigerator tray, air-conditioner water collection pot, open water storage tank, old plastic shoes, birds water pots and flower pots can be breeding places for mosquitoes while the results of the study undertaken NurAin and fellows (2017) elucidated that 98% respondents were aware that empty stagnant water from trash cans, flower pots and old tires etc. can be the

mosquitoes breeding places [19]. At present, no proper treatment is available for dengue infection so effective vector control of *Aedes aegypti* can prevent from infection. It was found during study that 59.9% respondents had knowledge that only method of controlling dengue infection is to combat the vector mosquitoes. The results of our study are comparable but showed better situation than the study carried out by Sayavong et al. (2015) who stated that 56.6% respondents had knowledge that the best way to control dengue infection is to combat vector mosquitoes [20]. It was found during study that majority of respondents were aware that stored water containers/tanks for drinking water without being covered should be cleaned after every four days. Study further highlighted that these respondents acquired knowledge about dengue from different sources. Among respondents, main source of infection was health personnel counseling (35.1%), followed by television (25.0%), banners in area (20.1%) and mosque announcement (19.8%). But the study performed by Chinnakali and associates (2012) confirmed that majority (54.9%) of respondents got information from television, followed by newspaper/magazine (51.7%), health personnel (26.9%), radio (7.7%) and past illness with dengue (8.8%) [16]. Another study carried out by Rehman and coworkers (2015) indicated that 74.22% respondents source of information regarding dengue was television, followed by newspaper (12.67%), teachers/professors (7.78%) and other sources (health care providers, radio, friends and internet etc.) (5.33%) [15]. Study also assessed the attitude of respondents regarding dengue vector control and found that most of the respondents covered water jars after using immediately for the prevention of dengue. Likewise, majority (60.2%) had cover in their water tanks but Shuaib and teammates (2010) reported in their study that significant majority (80.4%) had cover in their water tanks [1]. Similarly, majority of respondents changed water of their indoor plants weekly, drain off the water in the plates of the flower pot. There were 34.9% respondents who examined any discarded thing that can hold water around their house, for dengue vector control in their area. But the study done by Shuaib and teammates (2010) confirmed that more than half (56.4%) of respondents examined discarded things to eliminate stagnant water around their house to reduce the mosquitoes [1]. When the overall attitude regarding dengue vector control was assessed among respondents, study highlighted that 58.1% respondents had overall good attitude while 41.9% had poor attitude. The results of a similar study performed by Rehman and coworkers (2015) indicated that 46.0% respondents had good and 54.0% had poor attitude [15]. But the results of another study performed by Said and colleagues (2018) showed that significant majority (99.2%)

of respondents had good while only 0.8% respondent had poor attitude [21].

CONCLUSION

Dengue fever is believed a leading public health issue these days. It has emerged as an important problem worldwide. Current study assessed the attitude regarding dengue vector control among the community of Tehsil Sahiwal, Sargodha. Study concluded that attitude regarding dengue vector control among community was found satisfactory as 58.1% respondents had overall good attitude.

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