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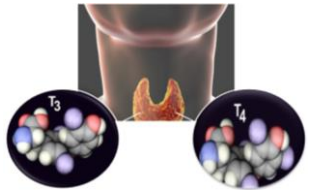

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Deviations in Thyroid and its Regulatory Hormone Profile in Workers Exposed to Welding Fumes

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ABSTRACT: *This study evaluates health risks manifested by the exposure of welding fumes to the labourers working in welding environments. Welding fumes, are the complexes of fluorides, silicates and metal oxides, cause burns, eye damage, hormonal imbalance, organ damage and cataracts. In order to check the changes caused by fumes on thyroid gland, a study was planned to evaluate the variations in thyroid regulatory hormone levels in workers occupationally exposed to welding fumes. For this purpose, blood sampling of the welders (n=24) having exposure to welding fumes was done from different sites in city Lahore, whereas, blood samples of healthy controls (n=24) were collected from University of the Punjab, Lahore. Thyroid (T3 and T4) and its regulatory hormone (TSH) assessment was executed by ELISA. Independent student "t" test at confidence interval of 95% and $P < 0.05$ was applied. A non-significant decrease of both hormones i.e. T3 and T4 was found in the subjects under study as compared to controls. Levels of TSH in blood serum of workers showed a non-significant increase than healthy controls. Elevated TSH and reduced T3 and T4, although, statistically non-significant, predict chances of hypothyroidism due to chronic exposure to welding fumes. Welders are, therefore, recommended to adopt prophylactic measures and safer techniques in order to avoid direct hazardous exposure to welding fumes.*

Keyword: *TSH, T3, T4, ELISA, Welding Fumes, Thyroid*

INTRODUCTION

The environment at the occupational site is of prime importance. This environment serves for the health and working capabilities of workers (Kumara, 2001). Each occupation possesses some degree of risk to the workers and expresses some sort of disease. The risk may be due to physical, or chemical exposure (Gulani, 2008).

The thyroid gland, a vital endocrine gland responsible for producing thyroid hormones (T_3

and T_4) which regulates growth, development and basal metabolic rate (Sarkar, 2015). Thyroid is a butterfly shaped gland, has two lobes; one left and the other right separated by isthmus (Marshall and Bangert, 2008). The gland has thousands of follicles made of thyrocytes with colloid lumen on adjacent sites. This acts as precursor site for thyroglobulin storage. The recommended dose for iodine is 150 µg a day, 1mg per week or 50 mg per year (Triggiani, 2009).

The World Health Organization (WHO) reports an estimated 250 million injuries posed yearly due to work hazards. Out of these the development countries are faced with welding related risks (Sabitu et al., 2009).

In welding, metal objects are joined by means of another metal that acts as filler. The filler is an electrode wire utilized in joining. High level of temperature serves for welding purpose that resultantly produce fumes by welding along with sheer noise and radiation (Antonini, 2003).

Evidences pertaining to toxic fine and ultrafine particles in welding fumes has been reported (McNeilly et al., 2004; Dybdahl et al., 2004; Hirano et al., 2003). Welding, a common indispensable procedure in industry has wide ranging health hazards that include ultraviolet (UV), infrared radiation (IR) exposure. It also includes electrocution, thermal burns, electromagnetic exposure and heat stress (Chauhan et al., 2014).

Retinal damage due to excessive exposure to arc welding can result (Park et al., 2021). Metal fume fever (MFF) can result due to excessive fumigation of various metal atoms involved. This results in influenza like conditions and another respiratory dysfunctions (Ashby, 2002).

MFF largely due to excessive exposure to fumes induces cancer of lungs and larynx to welders (Taylor et al., 2003; ATSDR, 2008). However, the duration of exposure, welding type, the environment and protective equipment can determine the health risk of welders (Palmer et al., 2009).

Thyroid gland plays a vital role in controlling the development and metabolism. Thyroid function is affected by metals due to occupational exposure (Benvenga et al., 2020). Environmental chemicals play vital role in this

regard (Pearce and Braverman, 2009; Boas et al., 2009; Blount et al., 2006; Zoeller, 2005). Polychlorinated biphenyls (PCBs), phthalates, per fluorinated compounds along with metals have relations to thyroid functioning (Pearce and Braverman, 2009; Kashiwagi et al., 2009)

A number of processes generate welding fumes resulting oxidative stress. In welding above 90% of particulates are by electrode filling metal vaporization, core and coating like in arc welding and least to laser beam welding (Shoham et al., 2008). Transformed nanoparticles of metal oxides are formed (about 1%) by condensation of metallic vapors and these nanoparticles after association form particle agglomerates (Sengul et al., 2020).

In children and adults, thyroid hormones have a crucial role in functioning of cardiovascular, nervous and reproductive system (Williams 2008; Danzi and Klein, 2012; Yazbeck and Sullivan, 2012). The main hormones are thyroid stimulating hormone (TSH), tri-iodothyronine (T_3) and thyroxine (T_4). A very small portion of free form of T_3andT_4 enter the cells (<1%). There is negative feedback mechanism between these hormone (Holt and Hanley, 2021). T_3 and T_4 synthesis is by stimulation of TSH. For homeostasis purpose in body, high T_3 levels suppress TSH production while low T_3 andT_4 stimulate the production. Anomaly in thyroid function is usual. A study in US proposed 4.6% of the population faced with hypothyroidism while 1.3% suffers from hyperthyroidism (Hollowell et al., 2002).

Several anomalies are there because of exposure of workers to welding fumes. The current study is aimed to access thyroid and its regulatory hormone levels (T_3 and T_4 and TSH, respectively) among the workers occupationally exposed to welding fumes.

MATERIALS AND METHODS

Experimental Design

Institutional ethical review committee, Institute of Zoology, University of the Punjab Lahore, endorsed the study plan. Welders (n=24) from welding sites of Lahore and healthy controls (n=24) from University of The Punjab were engaged for current case-control investigation.

A comprehensive, self-explanatory proforma was prepared. Information from every participant of both groups was filled in this predesigned proforma. This proforma included information like sample id, name, gender, body mass index (kg/m^2), incidence of any disease, medication, drug addiction and smoking.

Exclusion and inclusion

Individuals not having any history of ailment were included in this study. However, any individual having hepatic, diabetic, renal or any other viral infection were excluded from the investigation. As diseased condition can have impact on outcomes of the study.

Blood Sampling and Processing

Blood samples from all the subjects of both groups were collected after 12 hours overnight fast. Since human were the participants of the study, hence, all precautionary measures were taken. A registered technician was engaged for the purpose of phlebotomy.

Becton and Dickinson (B.D) syringes having sound reliability, originality, in terms of proper sterilization were employed. Also, an expert technician was hired to take the blood from peripheral vein of subjects. During sampling, the part of skin facing peripheral vein was disinfected by rectified spirit. B.D syringe was injected in persons' vein at approximate 45° angle prudently and 5ml of blood was withdrawn, keeping the person sitting and then drained in vaccutainer tubes. After 30-40 minutes, subject's samples were centrifuged (3,000 rpm) for about 10 minutes and serum (supernatant) was separated. The Eppendorf tubes that contained serum was labelled and stored at -80°C. Serum samples were thawed appropriately at room temperature of lab before biochemical analyses.

Hormonal Analyses

The concentration of the tri-iodothyronine (T_3), thyroxine (T_4) and thyroid stimulating hormone (TSH) in controls and welders blood serum were estimated by commercially available ELISA (Enzyme-Linked Immunosorbent Assay) kits in Physiology/Endocrinology laboratory, University of the Punjab, Lahore.

Statistical Analysis

Statistical analysis was done using latest version of Graph Pad Prism version 6.00 software. Results were demonstrated as Mean ± SEM. Un-paired student T test with 95% confidence intervals and $P< 0.05$ was applied to determine variations among the studied groups.

RESULTS

The levels of TSH in controls and welders compared and a non-significant increase of 47.48% was recorded in the blood samples of workers as compared to healthy controls (Fig. 1). An average value of TSH in controls and welders was found to 0.94 ± 0.18 and 1.33 ±0.20 µIU/mL, respectively (Table 1).

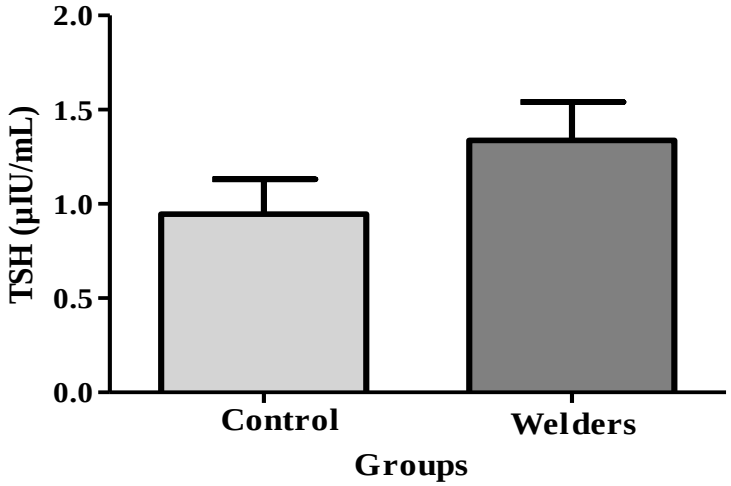

Fig. 1. Levels of serum TSH (µIU/mL) in control and welders

In controls, the approximate mean value of T_3 was 1.47 ± 0.052 (ng/mL) and among the workers it was found to be 1.41 ± 0.057 (ng/mL). The decrease in serum T_3 concentration for workers was a non-significant (Confidence level; 95%, *P* =0.79) (Table 1).

Table 1: An overall comparison of serum thyroid and its regulatory hormone in controls and welders (Mean ± SEM)

Parameters	Control	Welders	t value	P-value	Difference
TSH (µIU/mL)	0.94 ± 0.18	1.33 ± 0.20	t=1.41	0.16	47.48↑%
T_3 (ng/mL)	1.47 ± 0.05	1.41 ± 0.05	0.25	0.79	4.08↓%
T_4 (µg/mL)	41.87 ± 2.51	39.74 ± 2.46	1.10	0.54	5.08↓%

TSH: Thyroid Stimulating Hormone, **T_3:** Tri-iodothyronine, **T_4:** Tetra-iodothyronine, **↓:** decrease, **↑:** increase, **µIU/mL:** micro international unit per milliliter, **ng/mL:** nanogram per milliliter, **µg/mL:** microgram per milliliter

It was noticed the levels of T_3 hormone higher in controls as compared to workers (Fig. 2).

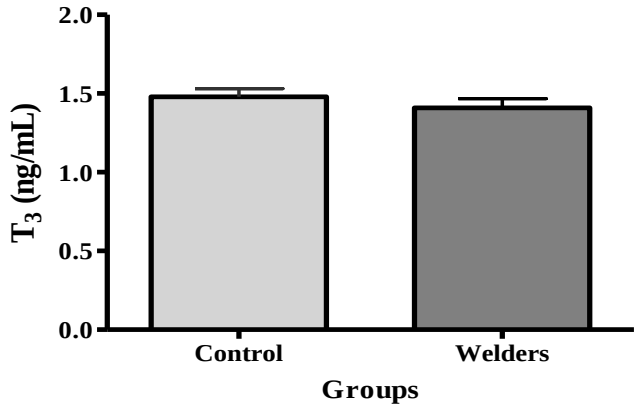

Fig. 2. Levels of serum of Tri-iodothyronine (ng/mL) in control and welders

In controls, the approximate mean value of T_4 was found to be 41.87 ± 2.51 (ng/mL). Among the workers it was calculated as 39.74 ± 2.46 (ng/mL). The decrease in serum levels for T_4 in workers was found non-significant (Confidence level; 95%, *P* value=0.27) (Table 1). The levels of T_4 was noticed higher in controls as compared to workers (Fig. 3).

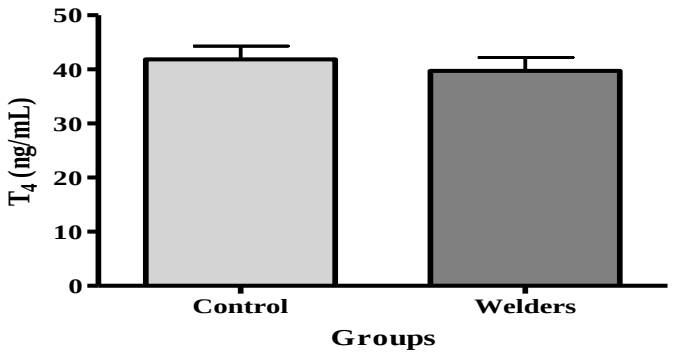

Fig. 3. Levels of serum of Thyroxin (ng/mL) in control and welders

DISCUSSION

Thirteen types of metals are associated with welding fumes that include vanadium (V), manganese (Mn), lead (Pb), chromium (Cr), molybdenum (Mo), zinc (Zn),cobalt (Co), beryllium (Be), mercury (Hg), antimony (Sb), nickel (Ni), cadmium (Cd), copper (Cu) and iron (Fe). Due to chronic Mn exposure particularly to welders cause health risks. Exposure to Mn can also promote the spread of Parkinson's disease that may be a risk factor among welders. While, neurogenic disorders may be caused due to change in the homeostasis of Mn and Fe (Li et al., 2004).

Main constituent of welding fumes includes Mn, Fe and Zn. Whereas, Pb, Cr, and Ni serve as trace elements and can cause change in functioning of thyroid gland. High temperature and exposure to radiations also have an impact on functioning of thyroid (Shakeel et al., 2022).

Welding fumes are formed by complex binding of metal oxides with gases. Metal oxides are the result of oxidation of metal alongside vaporization during welding (McNeilly et al., 2004). Nano particles found in fumes are produced by welding. These fumes pass into blood stream from respiratory tract and in meantime result

in toxic outcomes if they are accumulated in extra pulmonary organs (Roth, 2006). These are accompanied with occupational disorders in welders. Change in functioning of kidney and liver are a result of their inhalation (Antonini, 2003). Inspiration of these particles and gases bring alternations in the kidney and liver physiology (Dumkova et al., 2016).

The disorder of thyroid gland is quite common in Pakistan and hence public's major health burden. Causes are still not understood but considerable findings accomplish chemical exposure in the environment (Iqbal et al., 2016).

The hormones of thyroid are pivotal to regulation of metabolism, differentiation and development. Any amelioration associated with the thyroid biology disrupts whole metabolic machinery in human (Heussen et al., 1993).

Thyroid functioning is properly managed by the levels of T_3, T_4 and TSH that indicate normality in of the gland. Any anomaly in functioning can affect metabolism of thyroid. Calibrating thyroid hormones level in blood can account for proper thyroid working (Yousif and Ahmed, 2009).

The current study highlights evaluates alterations in the levels of TSH, T_3 and T_4 due to welding fume exposure. The blood serum levels for TSH, T_3 and T_4 were analyzed and compared to normal healthy persons.

On the basis of this investigation, abnormal levels of TSH alongside lesser deviations of T_3 and T_4 levels were found. Thyroid dysfunction resulting from compensatory increase in TSH levels have been observed previously by Mortavazi et al. (2009). Consequently, hypothyroidism accompanied with increased risk for coronary artery disease can be anticipated in these workers. Hypothyroidism whether primary or secondary is associated with hyperlipidemia. Thus, decreasing the levels of circulating lipids is the treatment to reduce cardiovascular risks (O'brien et al., 1993).

Thyroid hormones play a major role in spermatogenesis (contrary to previous findings). Evidences of thyroid receptors on nurturing cells for sperms in testis and sertoli cells has accounted research for hormones of thyroid in male reproduction. As sertoli cells back spermatogenesis therefore significant regularity role is played on part of thyroid gland in sperm production. Hence, male fertility alongside spermatogenesis is affected by thyroid malfunctioning (Singh et al., 2011).

In females no significant influence on ovulation rate resulted due to hypothyroidism rather lesser number per litter of implantation sites were reported (Hapon et al., 2010).

Acquired and congenital hypothyroidism can also result in loss of hearing, evidenced both in clinical and laboratory studies (morphological, biochemical, and electrophysiological findings) testify the hypothesis that cochlea is harmed owing to sensory neural loss of hearing in hypothyroidism. Hence, change in middle ear is responsible for conductive component (Villaume et al., 1978).

Clinical hypothyroidism present increased cardiovascular risk and also the patients with sub clinical hypothyroidism exhibit several potential cardiovascular risk factors. Recently, more data has become available indicating mild hypothyroidism affecting cardiovascular system. In patients with mild deficiency of thyroid hormone caused by impairment in function of left ventricular diastole, consistency in cardiac abnormality has been observed (Biondi, 2008).

Involvement in production and regulation of thyroid hormones by contaminants of environment are immense (Zoeller, 2005; Boas et al., 2006; Pearce and Braverman, 2009). Like in T_4 to T_3 conversion, selenium ion accepts the iodine atom released by deiodinase enzyme (Holt and Hanley, 2021). Both selenium and methyl-mercury hold high affinity for each other. Hence, increase in concentration of methylmercury holds selenium, making the thyroid conversions impossible (Soldin et al., 2008; Ursinyova et al., 2012). Substances having percholate and nitrate cause decrease in thyroid's iodine concentration through competitively inhibiting symporter for sodium/iodine leading decreased T_3 and T_4 (Leung et al., 2010; Pearce and Braverman, 2009). Polychlorinated biphenyls and polybrominated diphenyl ethers, that serve as environmental chemicals become structurally similar to thyroid hormones and directly bind to their receptor sites or transporters (Pearce and Braverman, 2009). After binding they can act as agonists or like antagonists.

Certain exposures cause a dysfunction in the thyroid gland secretion that may include hypothyroidism and hyperthyroidism accompanied by abnormality in other organs and systems.

CONCLUSION

The present investigation concluded that welders have chances of manifesting hypothyroid conditions due to the hazardous fumes associated with the welding procedure. Hence,

hypothyroid conditions in these laborers can prognosticate cardiovascular risks, fertility problems in males and associated ailments. Moreover, appropriate personal protective equipment's and prophylactic measures at site of work must be addressed in this sect of workers. There is also need for ample research by quantifying the effects and duration of exposure on health of these welders. As such exposure may have chronic effects on human beings.

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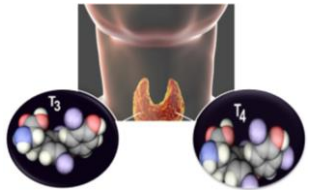

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Assessment of Visual Perception in Children with Autism Spectrum Disorder

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ABSTRACT: *Autism spectrum disease (ASD) is a nervous disorder. In this disease children have sensory processing dysfunction and they unable to communicate, interact, and showed repetition in behaviours. The present study was designed to determine visual impairment frequency in children with autism spectrum disorder (ASD). A descriptive study was performed in Paediatrics Department of Children's Hospital and The Institute of Child Health, Lahore. Data was collected in six months and 52 patients of both gender were considered. A total of 40 boys and 12 girls of 3-8 years old children were included. A structured Questionnaire based on visual perception was designed to determine the visual sensory processing issues in children with an autism spectrum disorder. The findings showed a strong difficulties in the visual sense particularly in differentiation among the colours and eye tracking in autism children. It was concluded that sense of sight highly affected in the patients of autism spectrum disorder.*

Keyword: *Autism Spectrum disorder, Nervous system, Sensory processing issue*

INTRODUCTION

Autism is child's disability in communication and connection with others (Baker et al., 2008; Baranek et al., 2006; Baranek et al., 1997). It is clinically defined by traits such as restricted interests, occupations, repeated habits and behavioural deficits as a diverse neurodevelopmental disease (Web, 2015; O'Neill and Jones, 1997). In some cases cardio-respiratory and touch are also related with ASD (Ming et al., 2016; Miguel et al. 2017). The prevalence of ASD among all children

in the United States is one in 68 now (Centres for Disease Control and Prevention, 2014). There are 4:1 more boy than girls with an autism spectrum disorder (Loomes et al., 2017). The parents reports these children have high pressure regarding care of these children (Larson, 2006). According to the American Psychiatric Association, 2013, ASD is a neurodevelopmental disorder that encompasses a variety of complex developmental disabilities. These include repetitive behaviours, limited interests, ritualized behaviours, behavioural inflexibility, impaired sensory processing, and communication deficits in establishing and maintaining relationships (American Psychiatric Association, 2013).

It is reported in literature that only 6–15% of ASD cases have genetic defects due to Rett's and Fragile X syndromes and other genetic abnormalities (Schaefer, 2008; Helsmoortel et al., 2014). According to one study ASD and ID usually co-occurred with each other and with the other conditions such as seizure disorders, motor problems, and numerous other psychiatric diagnoses (Silverman et al., 2022). Autism is a problem of extreme sensory modulation that can noticed in early age (Ben-Sasson et al., 2007). In this disease the children have high sensory disorders that usually starts at very early age and persist throughout life (Cermak and Ben-Sasson, 1997). However, various sets of causative exhibit some behavioural symptom to assess this issue (Brown et al., 2001; Autism Society, 2015). A case study was also conducted on autism child in early 2 years of age and it was found that no impairment was detected in various domains of the toddler early age (Dawson et al., 2000).

When sensory impulses are unable to be properly organized into appropriate responses, it results in sensory processing disorder (SPD), a neurological illness. Processing sensory data from their environment, such as sound, touch, and movement, is challenging for those with SPD. They may experience sensory input more strongly or less strongly than other people, according to this. Therefore, SPD may affect a person's capacity for social interaction in various settings and for carrying out routine tasks. The present study was aimed to see prevalence of Autism in the existing society.

MATERIALS AND METHODS

Study Period

The present study was designed for six months from 20th August 2017- 20th January 2018.

Study Site

This descriptive study was performed in Pediatrics Department of Children's Hospital and The Institute of Child Health, Lahore.

Inclusion and Exclusion Criteria

A 52 children of both gender were included in the study with 40 boys and 12 girls of 3-8 years While, all other children above this age were not considered.

Questionnaire

A structured questionnaire regarding visual perception was created with the help of expert advisor and a literature review served as the data collection tool for this descriptive study.

Ethical Approval

A written consent of the guardians of the patient was taken before the questionnaire was filled.

Statistical Analysis

Statistical analysis was carried out using SPSS version 21.0 to find the mean and prevalence.

RESULTS

A data of 52 patients were examined in this descriptive study at the Department of Paediatrics, Children Hospital (CH) and The Institute of Child Health (ICH) Lahore. Each patient had to fill questionnaire prepared for them based on their medical history and clinical observations. SPSS version 21.0 was used to examine the entire set of data. An average age of 4.35± 1.26 years was considered during this study (Table 1).

Table 1: Frequency distribution of age

Variable	Value
Mean	4.35
SD	1.26
Minimum	3 years
Maximum	8 years

Abbreviation: SD; Standard Deviation

A total of 52 patients were treated, of which 40 (76.9%) were boys and 12 (23.1%) were girls (Table 2).

Table 2: Participants demographic characteristics

Demographics		N=Frequency	%Frequency
Gender	Boys	40	76.9%
	Girls	12	23.1%

Frequency of sense of vision variables includes diagnosed visual defect observed in 11.5% of patients, difficulty in tracking the eyes was found among 30.7% of patients, while not recorded in all other autism patient. An avoiding brightly lit rooms was recorded in 26.9% of patients, discriminating colours and shapes was found in 80.7% of patients, and frequency of standing in front of a mirror was recorded in 75% of patients (Table 3).

Table 3: A structured questionnaire used for determination of visual defects

Variable	Yes%	No%
Have a diagnosed visual defect	6(11.6)	46(88.4)
Have difficulty eye-tracking	16(30.7)	36(69.3)
Avoid being in a room with bright light	14(26.9)	38(73.1)
Have difficulty discriminating colours and shapes	42(80.7)	10(19.3)
Stands in front of a mirror or reflective surfaces for longer periods	39(75)	13(25)

DISCUSSION

Autism is a disorder of stereotyped behaviour. The stereotypies are usually repetition in behaviour and perceived as age in-appropriation, attention, context, duration or intensity (Kargas et al., 2015; Gal et al., 2002; Wiggins et al., 2009). The goal of the current study was to gain a general understanding of the sight processing difficulties experienced by children with an autism spectrum disorder. The current descriptive study was carried out Children's Hospital Lahore's

Developmental Paediatrics Department and The Institute of Child Health, Lahore. The appearance of symptoms in this disease start to develop before the age of 3 years and similar were recorded in this study (CDCP, 2007). The disease later on exist for an individual's life. This disease affects all races, nations and socioeconomic groups. Its frequency is four times higher in boys than girls and similar results were noticed in the current study with higher percentage that found in boys ass compared to girls. According to the findings of the current study, eye tracking is less common than the trouble differentiating in colours and shapes Children with autism are also more likely to stand in front of a mirror and these findings were in line with those of Dunn, whose study on sensory processing patterns resulted in a model of that pattern (Dun et al., 2002).

It is also reported by Coulter, (2009) that integration of sensory information is a frequently recorded problem that found in individuals with Autism Spectrum Disorders (ASD). In this problem mot probably affected area is vision and the visual symptoms are pervasive and severe. The similar sort of issue in vision also recorded in the present study. These visual symptoms are due to an individual's unique sensory-processing abilities and are biologically based in origin. It was noticed in the present study that autism patients were unable to differentiate colour and similar was reported in a study that children with ASD are unable to discrimination in colours (Franklin et al., 2008). Ludlow et al. (2006) also reported persons suffered in autism only prefer to eat colourless foods and not like to play with certain colourful toys.

Poor eye tracking and fixation skills were noticed in the present study an also reported by Brenner et al. (2007) that highly contribute in gaze version. While, Takarae et al. (2007) reported that difficulty in eye tracking and lack of control in eye movements in the individuals of autism might be due to problem in fronto-striatal and cerebellar circuitry. Similarly, problems to stand in front of mirror and avoiding bright light are also recorded in the patients.

CONCLUSION

It was concluded that the visual problems particularly in differentiation in eye colours, followed by difficulty in standing in front of mirror and eye tracking were high in the patients of autism spectrum disorder. Particularly, the area of standing in front of mirrors was high followed by discriminating colours.

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ETHICAL APPROVAL

The study was approved by the institutional ethical review committee.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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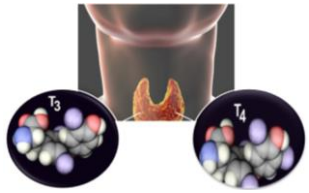

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Impact of Aflatoxins Exposure on Human Health and its Management Strategies

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ABSTRACT: *Aflatoxins are common contaminants in human food supplies that affects the world's developing economies. These are cosmopolitan in distribution and found everywhere in nature and can grow under drought, warm, and humid conditions. The aflatoxins are secondary metabolites and mostly found in feed and food products. These are oncogenic, mutagenic, and immunosuppressive in nature. On the basis of the aflatoxin producing fungi, the aflatoxins are roughly split into two distinct groups: those that penetrate in pre-harvest circumstances and those that are generated in post-harvest conditions. Aflatoxins exposure has great public health impact in economically developing nations and lot of research is taking place to reduce its harmful impacts; as a result, we need to establish preventive strategies that are feasible for these high-risk populations. This review provides in-depth information regarding the presence of aflatoxins, their analysis, and potentially harmful consequences on human health, as well as various detoxification approaches.*

Keyword: *Aflatoxin, toxic, ELISA, detection, prevention, detoxification*

INTRODUCTION

Mycotoxins are secondary metabolites produced by organisms of kingdom *Fungi* and are present in food and feed. Mycotoxins have the ability to cause different diseases and even death in animals and humans. Due to their high toxicity, they are one of the main causes of public health issues. Mycotoxins have been the subject of research around the globe to determine their severity and prevalence in various dietary entities (Berthiller et al., 2018). Mycotoxins influence and jeopardize the economy, global trade, and also cause irreversible health impacts in living things. Contamination by mycotoxins is unavoidable and occasionally unpredictable, making it a unique problem for food safety (Stadler et al., 2018).

Poor hygienic conditions during transportation and storage, moisture, torrential rain, and high temperatures all contribute to the production of mycotoxins including aflatoxins, ochratoxins, patulin, deoxynivalenol, citrinin and zearalenone (Elkenany et al., 2021). Aflatoxin growth is mainly found in cereals, ground nuts, oil seeds, grains and different spices such as wheat, maize, red chilli, turmeric, black pepper, almond, cotton, peanut etc. Aflatoxin contamination can also be found in vegetables and fruits, as well as meat, animal tissues, and animal products. Aflatoxins in feed and food have different permissible limits in different countries (Makau et al., 2020).

Poor storage practices and lack of technical capability have resulted in 50–60 % losses in cereal grains around the world (Akhtar et al., 2018). The loss of stored grains varies from region to region. In temperate climates, the loss is nearly 10 % while in humid tropical climates the loss is about 50 % (Zampieri et al., 2020). Every year, Pakistan loses 76 to

90 million dollars due to the presence of aflatoxins and about $80 million to $1.68 billion per year loss to crops in US. It is anticipated that Indonesia, and Thailand collectively lost $1 billion USD each year due to the contamination of food (Tabak et al., 2019). According to the estimation of Food and Agriculture Organization, aflatoxins affect 25 percent of world food crops each year (FAOSTAT, 2019). It is unprofitable to increase output without lowering postharvest losses because one-third of total output is lost at this stage (Bradford et al., 2020).

Aflatoxins

Aflatoxins belong to the family of harmful metabolites produced by fungi and are found in nature under drought, warm, and humid conditions. The ideal temperature for the production of aflatoxins is 33ºC. The species that produce aflatoxins are mainly *Aspergillus flavus* and Aspergillus parasiticus (Mwakinyali et al., 2019). Aflatoxin-producing fungi are particularly prevalent in warm and humid climates (Agriopoulou et al., 2020). Temperature, soil, humidity, and storage conditions all affect the rate and degree of aflatoxins accumulation (Roila et al., 2021). They were first discovered in the United Kingdom in 1960, when more than one million poultry birds in Turkey died of eating meal contaminated with *A. flavus* (Tahir et al., 2018).

Aflatoxins have catastrophic effects on humans, as well as are potential cause of liver and other organ cancer. Food ingestion, as well as other methods such as dermal contact and inhalation, is common sources to aflatoxin exposure (Garduño-García et al., 2017). There are main types of aflatoxins i.e Aflatoxin B1, Aflatoxin B2, Aflatoxin G1 and Aflatoxin G2, whereas AFM1 is a form of AFB1, mainly found in

milk and other dairy products (Moral et al., 2018). The alphabets “B” and “G” denote the blue and green fluorescence characteristics of aflatoxin when we visualize it under ultra-violet light respectively, while the number 1 and 2 denote the aflatoxins' relative position on thin layer chromatography. The toxicity potential of aflatoxins is B1 > B2 > G1 > G2.

The structures of aflatoxins G1 and B1 were proposed in 1963 and structures of aflatoxins G2 and B2 were proposed shortly thereafter (Fig. 1).

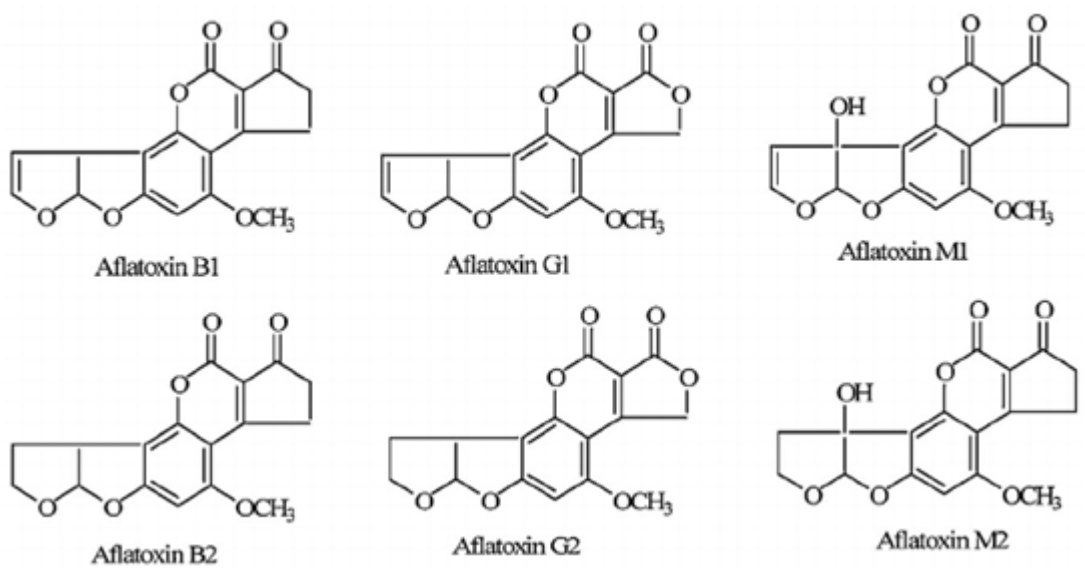

Fig. 1. Chemical Structures of Aflatoxins (https://www.researchgate.net/publication/262878341_Military_potential_of_biological_toxins)

Physicochemical Properties

Aflatoxin B1 undergoes complete catalytic hydrogenation, which culminates in the intake of 3 moles of hydrogen with the creation of the tetrahydrodeoxy derivative (Peng et al., 2020). Aflatoxin B2 is produced in a quantifiable yield when the hydrogenation process is interrupted after taking 1 mole of hydrogen. Additionally, it has been noted that, when a powerful acid acts as a catalyst, aflatoxin B1 reacts additively with a hydroxyl group (Javanmardi et al., 2020).

Although there have been few comprehensive investigations on the stability of aflatoxins, general experience would seem to suggest that some degradation occurs under a variety of circumstances. For instance, the compounds seem to partially disintegrate when left standing in methanolic solution. Beyond work related to structure elucidation, there has been comparably little systematic investigation of the chemical interaction and conduct of the aflatoxins (Yunus et al., 2019). The physical features of aflatoxins are given in Table 1.

Table 1. A **s**summarized data on physical features of aflatoxins

Aflatoxins	**Structural Formula**	**Molecular Mass**	**Liquefaction Point (˚C)**
G_1	$C_{17}H_{12}O_7$	328	244-246 ˚C
B_1	$C_{17}H_{12}O_6$	312	268-270 ˚C
B_2	$C_{17}H_{14}O_6$	314	286-289 ˚C
G_2	$C_{17}H_{14}O_7$	330	237-240 ˚C

Hazardous Health Effects of Aflatoxins

A large exposure to aflatoxin in the case of acute aflatoxicosis can result in about 25% of deaths. Aflatoxin exposure is common in countries that are still developing, resulting in major disorders and death (Puzanov et al., 2017). The incidence of acute aflatoxicosis, on the other hand, is relatively low because human beings are mostly immune to these toxins, and people generally keep away from taking food that is tainted with aflatoxins. However, this problem is frequent in penurious areas where people have no other option but to eat the poisonous food (Tahir et al., 2018, de Sousa et al., 2022).

Dullness, hydrops, liver necrosis, liver cirrhosis, jaundice, enlarged liver are all symptoms of severe aflatoxicosis. The immune system of children is less strong as compared to adults, so aflatoxins contamination is more vulnerable to children (Kumar and Kalita, 2017). Chronic aflatoxins exposure has a significant impact on an animal's nutritional status. In such cases, aflatoxins bind to DNA covalently shortly after exposure and protein synthesis start decreasing as a result (Asghar et al., 2018). There are several allowable limits for aflatoxins in food and animal feed components that have been adopted by different authorities.

The permitted levels of aflatoxin in multiple food items in different nations are listed in Table 2.

Table 2. Acceptable levels for aflatoxins (Yadav et al., 2021)

Aflatoxins	Maximum Permitted Value µg/kg	Products	Country
Aflatoxins (B2, G2, B1, G1)	10	Nuts	Australia, Taiwan, Indonesia, Malaysia
(AFB1, AFB2, AFG1, AFG2)	10	Groundnuts	Japan, Thailand, Egypt, Turkey
Aflatoxins (G1, B1,G2,B2)	4	Groundnuts	European Union
Total aflatoxins	10	Food entities	Vietnam
Total aflatoxins	20	All foods except milk	USA
Aflatoxins (B1, B2, G1,G2)	5	Groundnuts	Singapore
Total aflatoxins	30	Food stuffs	India
Total aflatoxins	20	Nuts	Philippines
Total aflatoxins	15	Nuts and their derivatives	Canada
Total aflatoxins	20	Groundnuts	Kenya

Techniques for Detection and Quantification of Aflatoxins

Various analytical techniques are present for aflatoxins detection and quantification. Some of the techniques are given as follow:

1. Thin Layer Chromatography

One of the most common separation techniques used for the detection of aflatoxins is thin-layer chromatography (TLC). It comprises a stationary phase that is made up of silica or alumina that is immobilized on an inert material made up of plastic or glass known as matrix (Folarin-Ottun 2018). The mobile phase includes water, methanol and acetonitrile. The sample is carried through the solid stationary phase by the mobile phase. The difference in solvability of the analytes in the two phases determines the distribution of aflatoxins between the mobile and

stationary phases in TLC (Jallow et al., 2021). The TLC method can be used to detect several types of mycotoxins in a single run. Although, TLC has excellent sensitivity, it also needs an expert to run it, sample pre- treatment, and this equipment is expensive. The development of High-Pressure Thin Layer Chromatography (HPTLC) has overcome the problems related to TLC (Sipos et al., 2021).

2. High Pressure Liquid Chromatography

The most widely used chromatographic technique for separating and determining organic compounds is High Pressure Liquid Chromatography (HPLC). HPLC is used to determine about 80% of all organic compounds on the planet. HPLC technique is also comprised of stationary and mobile phases. The sample which is to be analyzed is coated on top of the column, it flows through both the mobile and stationary phases and distributes evenly. HPLC also necessitates time-consuming pre- and post-column derivatization processes to refine aflatoxins detection limits. A change in the HPLC method, in which the HPLC is incorporated with mass spectroscopy, was developed to tackle the challenges associated to aflatoxins analysis (Malviya et al., 2010). High Pressure Liquid Chromatography-Mass Spectrometry (HPLC-MS), on the other hand, is a large and expensive piece of equipment that can only be handled by an expert and professional personnel (Kumar et al., 2021).

3. Gas Chromatography

In GC, the mobile phase is a carrier of gas and stationary phase is a liquid that is placed on inert solid particles. The sample which is to be analyzed is evaporated into gas phase and is detected using either Lame Ionization Detector (FID), Electron Capture

Detector (ECD) or Mass Spectrometry (MS). Gas chromatography is not commercially used as it is an expensive technique (Kaminiaris et al., 2020).

4. Enzyme Linked Immuno-Sorbent Assay

ELISA is a safe and suitable technique for the determination of aflatoxins. In ELISA, the antigens or antibodies are not labelled with isotopes but enzymes. ELISA is sensitive and simple to use. It also gives accurate measurement of anti-body or antigen concentration (Pereira et al., 2020).

Agricultural products are being detected for aflatoxins using ELISA method and different ELISA kits are available in market these days. The ELISA test can be performed on a 96-well assay platform, allowing for the simultaneous analysis of a large number of samples. ELISA kits are inexpensive, easy to use and there are no such health risks involved. However, ELISA method involves washing process multiple times which is laborious and time consuming (Omar et al., 2020). The strengths and weaknesses of aflatoxins detection tools are given in Table 3.

Table 3. Strengths and weaknesses of aflatoxin detection tools

Approach	Strengths	Weaknesses	References
HPLC	• Precise and efficient quantitative approach	• Quite pricey to test a significant number of samples • Samples destruction during preparation	(Abbas et al., 2004)
ELISA	• Fast, precise and quiet easy to use • Simultaneous examination of several samples • Partial use of organic solvents	• Chances of false negative/ positive results • Confined detection limit	(Do and Choi, 2007)
GC/MS	• Concurrent analysis of aflatoxins • Sensitive • Provides confirmation (MS detector)	• High variability in regard to repetition and replication	(Kasoju et al., 2020)
TLC	• Involves no complex equipment • Easy and reliable semi-quantitative way	• Outdated method • Samples destruction during preparation • Need HPLC analysis for quantification of results	(Zahra et al., 2019)
Strategies for Preventing and Reducing Aflatoxins	Due to frequent presence of aflatoxins in food and feed, a variety of strategies		

have been developed to remove contamination and to restore food quality and edibility. Pre-harvest and post-harvest are the two types of control strategies. Pre-harvest strategies include use of genetically modified crops that show resistance against Aspergillus infection, use of pesticides and planting time of crops (Rushing and Selim, 2019). Post-harvest strategies include physical, chemical and biological methods. Physical methods include heat, washing, proper drying, storage techniques, use of pesticides etc. Chemical methods include treatment with HCl, citric acid, lactic acid etc. Biological methods include treatment with plants extracts such as Allium sativum, Nigella sativa.

Detoxification of Aflatoxins

The reduction of mycotoxin in food requires optimal storage conditions. Mycotoxin growth may be influenced by elements such as temperature, water activity, gas composition and microbiological interactions of the preserved items (Kumar et al., 2022). These factors could be effectively controlled if they were all under an integrated control. For the removal of mycotoxins, numerous methods are present. Maintaining food's savoriness and nutritional value is important. Many strategies and techniques have been examined to reduce mycotoxin infection rate in both food and feed products (Tiwari et al., 2022). These techniques fall into three categories: chemical, biological and physical.

Physical Methods

Physical techniques like color-based sorting, mechanical separation, fines removal and density segregation can lower the amount of mycotoxin contamination that is present in food. Sodium carbonate or distilled water is used to wash the grains of different food and feed to lower the levels of aflatoxins (Shen et al., 2021).

Chemical Methods

Because aflatoxin infestation is unpredictable and unavoidable, it poses a unique threat to the safety of both feed and foods because it can cause both animals and people discomfort. Despite the availability of other chemicals, it has been discovered

that 0.5% HCL is the most effective one for removing aflatoxin B1 from fish feedstuffs and fowl samples (Awan et al., 2022). Ammoniation is indeed an effective technique that has been around for a while and is mostly used to reduce the amount of aflatoxins in feed, but it has an impact on food quality. Similar to this, aflatoxin levels in food items may be decreased by alkalization, heat treatment and acidification. Deamination also reduces the toxicity of AFB1 (Yang et al., 2022).

Biological Methods

Mycotoxins can be reduced through biotransformation by adsorption, binding, or detoxification. In order to diminish mycotoxins as biocontrol agent, probiotic microorganisms (Saccharomyces cerevisiae and Lactobacillus delbrueckii) were studied. This research revealed the use of probiotics as an alternate option to stop the synthesis of aflatoxin in food items (Nazir et al., 2021). Plant extracts of Nigella sativa and Allium sativum are very effective for the detoxification of aflatoxins (Zahra et al., 2019).

CONCLUSION

Aflatoxicosis may result in serious, fatal diseases. The presence of various aflatoxins in feed and food products could be harmful to both animal and human health and can also badly affect the nation's economy. To prevent severe and difficult situations, there must be strict regulation of food standards in both developed and developing countries alike. Aflatoxin exposure can sometimes be unavoidable and result in significant financial damage. Regarding the quality of food stuffs, precautionary measures are important. To ensure food safety, there must be strict enforcement of laws and regulations in every state that allows the presence of these dangerous aflatoxins within permissible ranges. Adequate aflatoxin control can be achieved by using approaches like HACCP (Hazard analysis and critical control points).

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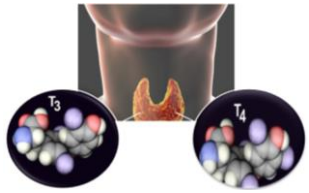

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Prevalence and Frequency Distribution of HLA-A, HLA-B and HLA-C Alleles in the Punjab, Pakistan

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ABSTRACT: *The Human Leukocyte Antigen (HLA) system, a set of highly polymorphic genes, has been found to play an effective role in the disease resistance and disease susceptibility. In this study, prevalence of class-I alleles of HLA was characterized at HLA-A,-B and -C loci in the 216 individuals randomly selected population at different regions of Punjab province of Pakistan. The study revealed that at HLA-A locus the allele 11 was most prevalent with 16.7 % frequency. Similarly, at HLA-B locus the allele 51 was found abundant with 15% frequency and at HLA-C locus the allele 7 was prevalent with 24% frequency. Among the two-locus HLA class I haplotypes, B*08/C*07 was found to be the most prevalent followed by B*35/C*04. Surprisingly, HLA-B*29 and HLA-B*36 alleles were found in the Punjabi population which is contrary to the previous reports.*

Keyword: *Allele frequency, HLA, MHC-I, Pakistan*

INTRODUCTION

Major histocompatibility complex (MHC), human leukocyte antigen (HLA), consists of genes which are highly polymorphic and play significant role in immune response, especially in adaptive immune system. These genes encode proteins that are involved in foreign organ rejection (Stephen and John, 2000). The region of HLA spans 3600 kbp on the chromosome 6 (Terasaki, 1990). The HLA is further divided into two classes: HLA-A, -B and –C have been placed under class I, while HLA-DR,-DQ and –DP have been placed under class II.

On cell surface, MHC proteins are expressed endogenously and exogenously to discriminate between self and nonself-antigens through adaptive immune response (Harjanto et al., 2014) . Molecules of HLA class-I present the cytosolic intracellular peptides to the CD8+ cytotoxic T cell receptors that kill the infected cells directly (Ghodke et al., 2005; Suheir and Amos, 2014) (Carapito et al., 2016). HLA class-I molecules help in recognition and binding of intracellular peptides and present these intracellular peptides to the cytotoxic T-cells while extracellular peptides are recognized by HLA class II molecules which present them to the helper T-cells. Earlier researches on transplant failure have revealed the importance of HLA variability complex to induce adaptive immunity (Billerbeck et al., 2013; King et al., 2002; Reiher et al., 2017)

HLA individual alleles are responsible for susceptibility and prevention from autoimmune and infectious diseases. Previous studies reveal that these alleles are associated with different infectious and autoimmune diseases so the prevalence of an allele can help to predict the susceptibility of a disease in the studied population (Ghodke et al., 2005; Howell, 2014). Due to its extreme polymorphic nature, the frequency of HLA alleles can vary among different populations and hence this can provide important information about susceptible of a given population towards diseases. HLA genetic variability occurs due to alterations in

the alleles of HLA genes. HLA antigens are the proteins recognized by the immune system of host during organ transplantation, blood transfusion and pregnancy (Reiher et al., 2017)

Pakistan possesses great diversity among its civilization and ethnicity (Blood, 1996). Major ethnic groups include Punjabi, Sindhi, Saraikis, Pashtuns, Baloch, Muhajir, Pathan, Hindkowans, Rajputs and Chitralis (Mushtaq, 2009). Punjab is the most populous province of Pakistan with an estimated population of about 30.4 million belonging to various ethnic groups (Ahmed, 1990).

The study of HLA has been conducted in various countries of the world in the recent past that has ultimately helped in prevention of several diseases (Abedini et al., 2021). In this study, the distribution of HLA alleles was determined in 216 unrelated individuals from different divisions of Punjab based upon geographical distribution. The purpose of this study was to explore the diversity of HLA class I alleles among the Punjabi population for the establishment of a reference data set for further studies such as HLA and its association with chronic diseases.

MATERIALS AND METHODS

For HLA genotyping, blood sample of 216 healthy individuals was taken which were randomly selected from different regions of Punjab. DNA extraction was done from whole blood sample using salting out method. Briefly, proteinase K and lysis buffer containing SDS were used for lysing cells. Afterwards, supernatant was treated with Phenol-chloroform-

isoamyl alcohol. The process was repeated to completely remove the RBCs. At the end the, salting out method was applied for DNA isolation .PCR sequence specific priming (SSP) was used for typing HLA-A, -B and –C alleles according to manufacturer's instructions (One Lambda, Inc., USA). For calculating allele frequencies, we used direct counting method (No of copies of alleles /2n in the sample population) (González-Galarza et al., 2015). Gene frequencies were calculated using the formula;$(GF = 1 - \sqrt{1 - AF})$ (Kaštelan, 1991). Hardy Weinberg law was applied for the calculation of haplotype frequencies (Serre, 1997)

RESULTS

It was observed that our studied population contained 54 HLA class I alleles 27 HLA-A, 14 HLA-B and 13 HLA-C alleles. Allele frequencies obtained from HLA class I alleles has been described in Table 1.

It was noted that for HLA class-I A locus, allele A*11, A*02 and A*01 were the most common; at locus B allele B*51, B*01 and B*40 were the most common, whereas, at locus C allele C*07, C*06 and C* were the most prevalent.

Table 1: Allele frequency of HLA Class 1

Alleles n=216	AF (%)	GF (%)	Alleles n=216	AF (%)	GF (%)	Alleles n=216	AF (%)	GF (%)
A*01	12.3	6.351722	B*07	3.9	1.969393	C*01	3.2	1.613009
A*02	15.3	7.967397	B*08	14.6	7.58788	C*02	0.9	0.451017
A*03	9.8	5.026319	B*13	3.2	1.613009	C*03	6.9	3.511659
A*11	16.7	8.731166	B*14	0.7	0.350615	C*04	10.8	5.554248
A*23	1.4	0.702467	B*15	5.6	2.840338	C*05	1.4	0.702467
A*24	7.6	3.875081	B*18	1.9	0.954556	C*06	13.6	7.0484
A*26	9.5	4.868512	B*27	1.6	0.803226	C*07	24	12.82202
A*29	1.4	0.702467	B*29	0.2	0.10005	C*08	3.2	1.613009
A*30	0.9	0.451017	B*35	10.2	5.237138	C*12	10.8	5.554248
A*31	4.4	2.224748	B*36	0.2	0.10005	C*14	6.2	3.1496
A*32	5.6	2.840338	B*37	2.3	1.15669	C*15	12.9	6.672619
A*33	7.4	3.771106	B*38	0.5	0.250313	C*16	4.2	2.122526
A*68	6.9	3.511659	B*39	1.4	0.702467	C*17	1.1	0.551521
A*74	0.2	0.10005	B*40	10.9	5.607204			
			B*41	0.9	0.451017			
			B*44	4.9	2.480771			
			B*45	0.9	0.451017			
			B*48	0.9	0.451017			
			B*49	0.7	0.350615			
			B*50	4.6	2.327076			
			B*51	15	7.804555			
			B*52	5	2.532057			
			B*53	0.4	0.2002			
			B*55	1.9	0.954556			
			B*56	0.2	0.10005			
			B*57	4.6	2.327076			
			B*58	2.5	1.257912			

Different haplotypes of HLA class I (Haplotype A/B, A/C and B/C) have been shown in Table 2.

Table 2: Most prevalent HLA class I haplotypes (A/B, A/C and B/C) in Punjab population of Pakistan

A*02/B*40	3.937	A*11/C*07	4.839	B*08/C*07	8.469
A*26/B*08	3.937	A*26/C*07	4.839	B*35/C*04	5.22
A*11/B*07	3.6	A*02/C*15	3.687	B*40/C*15	4.292
A*11/B*51	2.925	A*01/C*07	3.226	B*51/C*14	3.596
A*03/B*35	1.8	A*33/C*07	2.88	B*51/C*07	3.364
A*31/B*51	1.8	A*02/C*07	2.65	B*51/C*15	3.248
A*01/B*51	1.687	A*11/C*06	2.535	B*50/C*06	3.016
A*11/B*08	1.687	A*01/C*06	2.419	B*57/C*06	2.9
A*11/B*35	1.687	A*02/C*06	2.304	B*52/C*12	2.32
A*11/B*40	1.687	A*11/C*12	2.189	B*44/C*07	2.204
A*11/B*44	1.687	A*11/C*15	1.959	B*40/C*07	1.972
A*02/B*51	1.575	A*03/C*06	1.843	B*15/C*06	1.74
A*26/B*51	1.575	A*01/C*15	1.728	B*07/C*07	1.508
A*68/B*51	1.575	A*03/C*12	1.728	B*40/C*12	1.508
A*01/B*08	1.462	A*11/C*04	1.728	B*08/C*12	1.392
A*02/B*08	1.462	A*02/C*03	1.613	B*37/C*06	1.392
A*01/B*57	1.35	A*01/C*12	1.498	B*07/C*15	1.276
A*03/B*50	1.237	A*03/C*04	1.382	B*40/C*03	1.276
A*03/B*51	1.237	A*03/C*07	1.382	B*58/C*03	1.276
A*11/B*57	1.237	A*11/C*03	1.382	B*08/C*15	1.16
A*33/B*08	1.237	A*24/C*07	1.382	B*13/C*06	1.16
A*01/B*40	1.125	A*31/C*14	1.382	B*15/C*03	1.16
A*24/B*40	1.125	A*24/C*15	1.267	B*52/C*07	1.16
A*02/B*15	1.012	A*33/C*04	1.267	B*35/C*07	1.044
A*03/B*08	1.012	A*02/C*04	1.152	B*39/C*12	1.044
A*68/B*08	1.012	A*24/C*04	1.152	B*51/C*16	1.044
A*01/B*07	0.9	A*32/C*04	1.152	B*13/C*04	0.928
A*01/B*15	0.9	A*68/C*15	1.152	B*15/C*07	0.928
A*01/B*35	0.9	Av02/C*12	1.037	B*35/C*06	0.928
A*01/B*52	0.9	A*32/C*07	1.037	B*08/C*04	0.812
A*02/B*50	0.9	A*68/C*07	1.037	B*35/C*12	0.812
A*02/B*57	0.9	A*24/C*12	0.922	B*44/C*05	0.812
A*11/B*52	0.9	A*33/C*03	0.922	B*51/C*06	0.812

A*24/B*35	0.9	A*68/C*04	0.922	B*51/C*12	0.812
A*24/B*51	0.9	A*01/C*04	0.806	B*55/C*01	0.812
A*32/B*35	0.9	A*02/C*14	0.806	B08/C14	0.696
A*33/B*35	0.9	A*26/C*04	0.806	B*08/C*16	0.696
A*33/B*44	0.9	A*26/C*12	0.806	B*18/C*07	0.696
A*68/B*35	0.9	A*31/C*07	0.806	B*41/C*17	0.696

The prevalence of HLA-A, -B and –C frequencies in Punjab population in comparison to human populations belonging to Italy, Jordan, Brazil, China, Syria, France, Sudan and Lebanon has been described in Table 3 and Table 4.

Table 3: HLA-A Allele frequencies in Punjab population of Pakistan compared to other countries.

Allele	Pakistan (n=216)	Italy (n=500)	Jordan (n=15141)	Brazil (n=1559)	China (n=26266)	Syria (n=105)	France (n=130)	Sudan (n=250)	Lebanon (n=1994)
A*01	0.123	0.121	0.150	0.103	0.024	0.129	0.277	0.168	0.230
A*02	0.153	0.254	0.123	0.249	0.313	0.190	0.423	0.484	0.405
A*03	0.098	0.114	0.088	0.082	0.019	0.129	0.246	0.176	0.250
A*11	0.167	0.060	0.041	0.050	0.226	0.030	0.085	0.072	0.105
A*23	0.014	0.025	0.039	0.053	0.002	0.030	0.023	0.076	0.065
A*24	0.076	0.122	0.104	0.095	0.173	0.131	0.200	0.172	0.205
A*26	0.095	0.050	0.044	0.036	0.026	0.036	0.085	0.040	0.070
A*29	0.014	0.036	0.030	0.049	0.007	0.030	0.108	0.024	0.065
A*30	0.009	0.050	0.087	0.064	0.060	0.090	0.077	0.220	0.080
A*31	0.044	0.025	0.017	0.042	0.036	0.052	0.092	0.048	0.015
A*32	0.056	0.051	0.035	0.038	0.009	0.010	0.138	0.064	0.055
A*33	0.074	0.022	0.035	0.027	0.089	0.052	0.046	0.032	0.040
A*68	0.069	0.000	0.048	0.062	0.005	0.071	0.108	0.120	0.000
A*74	0.002	0.000	0.008	0.012	0.000	0.000	0.000	0.024	0.000

Table 4: HLA-B Allele frequencies in Punjab population of Pakistan compared to other countries.

Allele	Pakistan (n=216)	Italy (n=500)	Jordan (n=15141)	Brazil (n=1559)	China (n=26266)	Syria (n=106)	France (n=130)	Sudan (n=250)	Lebanon (n=1309)
B*07	0.039	0.056	0.032	0.0631	0.018	0.043	0.146	0.084	0.085
B*08	0.146	0.059	0.023	0.0496	0.007	0.048	0.169	0.064	0.045
B*13	0.032	0.034	0.028	0.0144	0.105	0.033	0.038	0.048	0.065
B*14	0.007	0.036	0.041	0.0589	0.0009	0.062	0.092	0	0.043
B*15	0.056	0	0.049	0.0871	0.15	0.024	0.154	0	0.029
B*18	0.019	0.048	0.046	0.0564	0.003	0.048	0.092	0.048	0.075
B*27	0.016	0.019	0.016	0.0176	0.031	0.024	0.062	0.028	0.03
B*29	0.002	0	0	0	0	0	0	0	0
B*35	0.102	0.159	0.141	0.1208	0.043	0.186	0.154	0.112	0.355
B*36	0.002	0	0	0	0	0	0	0	0
B*37	0.023	0.014	0.009	0.0105	0.01	0.01	0.062	0.032	0.01
B*38	0.005	0.035	0.042	0.0269	0.027	0.057	0.038	0.068	0.07
B*39	0.014	0.027	0.014	0.0275	0.028	0.01	0.046	0.132	0.005
B*40	0.109	0.026	0.022	0.0423	0.159	0.024	0.115	0	0.035
B*41	0.009	0.013	0.057	0.0141	0.0008	0.01	0.031	0.156	0.105
B*44	0.049	0.092	0.061	0.1057	0.025	0.076	0.262	0.028	0.135
B*45	0.009	0.004	0.021	0.0134	0.002	0.019	0.031	0.032	0.035
B*48	0.009	0.000	0	0.0051	0.0191	0	0	0.012	0
B*49	0.007	0.035	0.067	0.0285	0.0008	0.057	0.054	0.08	0.085
B*50	0.046	0.018	0.066	0.0214	0.004	0.019	0.023	0.104	0.04
B*51	0.15	0.104	0.099	0.0863	0.068	0.081	0.115	0.176	0.155
B*52	0.05	0.014	0.046	0.0176	0.027	0.071	0.023	0.1	0.07
B*53	0.004	0.008	0.025	0.0266	0.0004	0.03	0.031	0.06	0
B*55	0.019	0	0.019	0.0096	0.03	0.024	0.062	0.008	0.045
B*56	0.002	0	0.002	0.0041	0.005	0	0	0.004	0
B*57	0.046	0	0.029	0.0387	0.01	0.005	0.069	0.116	0.015
B*58	0.025	0	0.025	0.025	0.068	0.014	0.031	0.028	0.025

DISCUSSION

Our results suggested high diversity of HLA class I alleles in Punjab population of Pakistan. Previous studies suggest that allele HLA-A*02 is higher in prevalence at HLA–A

locus in the human population throughout the world (Clayton and Lonjou, 1997), however in our studies we found that allele HLA-A*11 with 16.7% was the most prevalent in the studies population. Moreover, prevalence of HLA-A*11 was intermediate when compared with other human populations. The human populations of Italy, Brazil, China, Syria, France and Sudan indicated that the most prevalent allele is HLA-A*03, whereas in our studies population allele HLA-A*03 was second in prevalence (Ayo et al., 2015; Dafalla et al., 2014; Du et al., 2007; Dubois and Gebuhrer, 2004; Ikhtiar et al., 2018; Rendine et al., 1998). In addition allele HLA-A*01 is third in prevalence in population which is consistent with human populations of Italy and France (Du et al., 2007; Dubois and Gebuhrer, 2004). It was interesting to notice that allele HLA-A*74 which was least common in our population was also found least common in human populations of Jordon, Brazil and Japan (Ayo et al., 2015; Du et al., 2007). However, some other studies showed that it was not observed in human populations of Italy, China, Syria, France and Lebanon (Dafalla et al., 2014; Du et al., 2007; Dubois and Gebuhrer, 2004; Elbjeirami et al., 2013; Ikhtiar et al., 2018).

For B locus of HLA, it was found that the most prevalent alleles in our cohort study (prevalence >10 %) were HLA-B*51, HLA-B*08, HLA-B*40 and HLA-B*35. HLA-B*51 was the most prevalent in our sample population while the most prevalent allele in human population of Italy, Jordan, Brazil and Syria was HLA-B*35 (Ayo et al., 2015; Elbjeirami et al., 2013; Ikhtiar et al., 2018; Rendine et al., 1998). However, HLA-B*51 was second in prevalence in human population of Italy, Jordan and Syria. Interestingly, it was noted that the second most prevalent allele in our sample population HLA-B*08 was lower in prevalence in our studied cohort, except in France (Ayo, C.M. et al., 2015; Dafalla et al., 2014; Du et

al., 2007; Dubois & Gebuhrer, 2004; Elbjeirami et al., 2013; Ikhtiar et al., 2018; Rendine et al., 1998; Salti and Shaya, 1997a). Moreover, our study also indicated that the allele HLA-B*29 and HLA-B*36 found in our studied population were not present in our compared cohort population.

For HLA-C locus, it was found that HLA-C*07 was the most abundant allele in our sample population with 12.8 % prevalence, while HLA-C*02 was the least prevalent allele with 0.9 % prevalence in our sample population. When two-locus haplotype frequency was determined in the sample population, it was found that B*08/C*07 was the most prevalent, which is also the most prevalent haplotype around the world, whereas, B*35/C*04 was found to be second in prevalence which is consistent with the Syrian population studies. Moreover these frequent haplotypes have also been found predominant in Mediterranean populations such as: Europeans (Nunes et al., 2014), Iranians (Abroun and Farzanehkhah, 2010) and Lebanese (Salti and Shaya, 1997b).

CONCLUSION

Allelic prevalence and frequency determination at different HLA loci help finding compatible HLA match individuals. Knowledge of association of various alleles with different diseases can also help avoiding the onset of certain diseases. The study indicated that allele HLA-A*11 with 16.7% was the most prevalent in the studied population. At HLA locus B, HLA-B*51 was the most prevalent in our sample population, For HLA-C locus, it was found that HLA-C*07 was the most abundant allele in our sample population.

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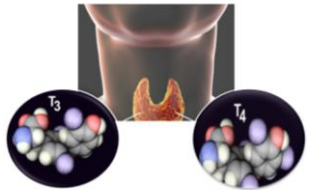

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Prevalence and Management of Endoparasitic Worm load in Ostriches of Different Captive Conditions Housed in Punjab, Pakistan

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ABSTRACT: *Ostrich farming has attained a status of a fast-growing agribusiness in the livestock industry due to the wide range of associated benefits attached to it. This study was designed to assess the prevalence of endoparasites in this economically important bird as well as their treatment to provide better guidelines for successful ostrich farming. The study involved 385 feacal samples of ostriches taken from 55 commercial farms and captive sites in Punjab during the period of January 2020 to December 2020. The relevant data and samples were collected from 15 districts of Punjab. Feacal samples were tested against gastrointestinal worm load by using floatation and sedimentation techniques. Ostriches of 11 commercial farms and Lahore Zoo, Jallo Wildlife Park Lahore, UVAS Ostrich farm Pattoki and Bahawalnagar Wildlife Park were found to harbor protozoan parasites such as Eimeria spp. Balantidium coli, and Amoeboid cyst. It was noteworthy that the use of herbal dewormers showed much better results than chemical anthelmintics. Similarly, those wildlife parks where ostriches were given a natural environment showed negative results for endoparasites. At some farms combination of chemical and herbal anthelmintics was also used. The absence of a reliable nutritional management system caused the mortality of ostriches at 40 farms due to gastric problems and choking. The mortality rate at the age of 2-4 months was 73% while mortality of adult birds from 1-7 years was 27%. Among selected farms, 50% of ostriches were facing lameness, leg deformities, and retarded growth due to improper space, a congested environment, and poor feeding systems. Lastly, more research is needed to make this agribusiness flourished.*

Keyword: *Endoparasites, Ostrich, Prevalence, Anthelmintics, Managemen*

INTRODUCTION

Ostrich (*Struthio camelus*) is the world's largest flightless carinate bird which is the only living member of the genus *Struthio*, family ratite, and has the fastest speed among all the birds on land due to its long strong legs. Almost 150 years ago commercial ostrich farming started only for feathers and somewhere for leather (Huchzermeyer, 2002). Nowadays Ostrich farming is emerging in the world as a new horizon in the livestock industry and it is producing meat, skin, oil, and feathers as major products (Abbas et al, 2018). The brain, eyes, and tendons of ostrich leg are also used in the treatment of human diseases (Shanawany, 1995).

In Pakistan, ostrich farming was gaining popularity because of its valuable products. Especially the climatic conditions of Punjab are very favourable for ostrich farming, keeping this in view Livestock and Dairy Development Department (L&DD) was playing a significant role in the promotion of the ostrich industry in Punjab. According to collected data the Government of Punjab registered almost 10,000 ostriches and gave a subsidy of Rs10,000 to farmers for each bird for promotion of the ostrich farms in Punjab (Abbas et al., 2018).

As in all production system parasites are a problem, the results obtained from a study showed the presence of various protozoa (Martínez-Díaz et al., 2000) flagellates, ciliates and coccidian (Martínez-Díaz et al, 2000). In Africa problems found in ostrich framing were tapeworms, nematodes, anthrax, ticks, lice, and ophthalmia (Barton and Seward, 1993; Davis, 1998). In ostriches, no specific infectious or contagious diseases are seen except *Libiyostrongylus* (wire-worm), which is the only true specific infectious pathogen of ostrich, found in the stomach (Huchzermeyer, 2002). Ostrich shows varying degrees of immunity regarding parasites and the females that select their mate on the bases of ornamentation could acquire males with better resistance to parasites (Bonato et al., 2013). According to previous research reported by Lozano et al., 2021 in ostriches from South America (Mario-González et al., 2017), Asia (Eslami, 2007), Polynesia (More, 1996), Africa (Mukaratirwa, 2004), and Europe, *L. douglassii* was found infecting both ostriches and emus (Jansson and Christensson, 2000). In another study of ostrich endoparasites, Forty-three stool samples out of a total of eighty from ostriches (N=80) tested positive for the presence of eggs per gram (epg) from the parasites Cappilaria, Ascaridia, and Eimeria spp. Out of 43

birds, 19 had mature parasites like Cappilaria, Ascaridia, and Eimeria. (Ambreen et al., 2021). But still, these findings are not enough therefore superficial knowledge is not sufficient therefore benefits from the ostrich industry can only be derived after gaining thorough knowledge of ostrich behavior and health-related issues (Mshelia et al., 2010). Ostrich farming is still at its dawn and a lot of research and growth is a requisite to reach the level of success for which the poultry industry is known (Nemejc and Lukesova, 2012).

This study was designed to find the prevalence of endoparasites in ostriches raised in commercial farms and wildlife parks of Punjab at a large scale to provide better insight into health issues and management of these important big birds with big health benefits.

MATERIALS AND METHODS

STUDY AREA AND SAMPLE SIZE

A total of 385 feacal samples were collected over a period of year (January 2020 to December 2020) from Lahore Zoo, Zoo Safari Lahore, Jallo Wildlife Park Lahore, UVAS Ostrich Farm Pattoki, Bahawalnagar Wildlife Park, and 50 commercial farms located at 15 different districts of Punjab and ostriches presented by L&DD at different exhibits. Ostriches of all age groups and sex were included in the sampling. For ≤50 birds 4 samples were taken and for ≥51 birds 7 samples were taken.

SAMPLE COLLECTION

Samples were collected from the ground by a gloved hands and transferred into coprological sample pots which were placed in a cooler bag with an icepack. Each sample pot was labelled with the date, the owner's name, the address of the sampling area, species of bird, and its sex. Once transported to the laboratory, they were stored for a maximum of five days at 4 °C at the Conservation Biology Lab Institute of Zoology, University of Punjab, Lahore. In three holdings, repeated sampling was planned at different times of the year, to establish whether seasonal conditions influenced the parasite found. In order to view parasitic eggs/oocysts of nematodes, cestodes, and protozoa, both qualitative (flotation, sedimentation, and micrometry) and McMaster quantitative techniques were applied (Papini et al., 2012)

QUALITATIVE TECHNIQUES

i. Flotation Technique

A total of 1 gram feacal samples were taken in a beaker and 15 ml flotation solution added to it and stirred well. The solution was strained by using a tea strainer and poured into a 15ml tube. Tube was placed in a test tube

rack, leaving a convex meniscus and gently a cover slip was placed on the top of the test tube. Let the test tube stand for 15 to 20 minutes. Then the coverslip was placed on a clean glass slide and observed under a microscope (4X, 10X, 40X) (Deplazes et al., 2013).

ii. Sedimentation Technique

It is a method for detecting trematodes eggs in which heavier nematode eggs were settled in sediments. Approximately 3 grams of feaces were added in 40-50 ml of tap water taken in a beaker and mixed it well with a stirrer and the solution was strained by using a tea strainer. The solution was poured into a test tube and let stand for 5 minutes. Decant the supernatant carefully and the solution was re-suspended with 5ml water. This step was repeated 3 to 5 times until the supernatant became clear. Then a drop of sediment was poured on a clean glass slide. A cover slip was placed on a glass slide and observed under a microscope (Deplazes et al., 2013).

MICROMETRY OF SAMPLES

Microslides were made from floatation and sedimentation samples and examined under the microscope to spot endoparasites.

Mcmaster Quantitative Technique

Each feaces sample weighed 2 grams, and 28 milliliters of a saturated sugar solution (specific gravity 1.2) was added to the mixture before being filtered and placed on a McMaster slide. Under a light microscope (100X), parasites were identified and enumerated down to a detection limit of 50 OPG and 50 EPG (Zajac and Conboy, 2012).

STATISTICAL ANALYSIS

Using SPSS version 20, we performed statistical analyses on the data collected during the study trials, including the T-test and repeated measures multiple ANOVA (Galen et al., 2022)

RESULTS

A total of 385 feacal samples from 55 locations were examined in this study for the presence of endoparasites and ostriches at 15 locations were found to be positive for endoparasites. Among positive samples *Eimeria spp*. was 66%, *Amoeboid* cyst was 26% and *Balantidium* was 6.6%. Identification of helminths ova and oocyst was done according to the shape and dimensions of eggs, by using standard parasitological techniques. It was seen that younger ostriches less than 1 years of age are more likely to have the infection as compared to those adult ostriches. The effect of sex on the prevalence of endoparasites in both groups (ostriches

in farms and wildlife parks) did not show any significant effect.

During study commercial farms and wildlife parks were visited during this study and findings from questionnaires revealed that extensive deworming was observed for parasites. Both chemical and natural dewormers were used during this period. Parasites found and their ova are presented in the table 1.

Table 1: Endoparasitic worm load of captured farmed ostriches in Punjab

Age Group	**No. of positive samples**	**Species of Endoparasites**									
		***Eimeria tanella* (23×19µm)**		***Eimeria* maxima (30×20µm)**		***Eimeria mitis* (16×15µm)**		***Amoeboid* cyst (30×20µm)**		***Balantidium coli* (50×68µm)**	
		No.	**%age**	**No.**	**%age**	**No.**	**%age**	**No.**	**%age**	**No.**	**%age**
Young Ostrich	65	15	18.5	7	8.6	25	30.8	14	17	4	4.
Adult Ostrich	16	-	-	-	-	8	9.8	8	9.8	-	-

The Table 1 and Fig. 1 represent different species of gastrointestinal parasites in the ostrich of Punjab. Out of 81 positive samples, 65(80.2%) and 16(19.7%) were found in young and adult ostriches respectively. Among *Eimeria* spp, *Eimeria tanella*, *Eimeria maxima*, and *Eimeria mitis* were identified by using micrometry. Clinical signs like weight loss, emaciation, and bloody diarrhea due to *Eimeria tanella* were seen in ostriches of a farm in Gujranwala.

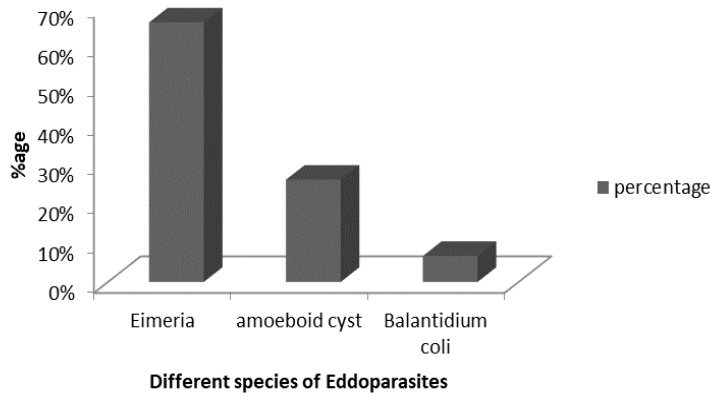

Fig. 1: Seasonal Distribution of different Species of endoparasites found in Ostrich

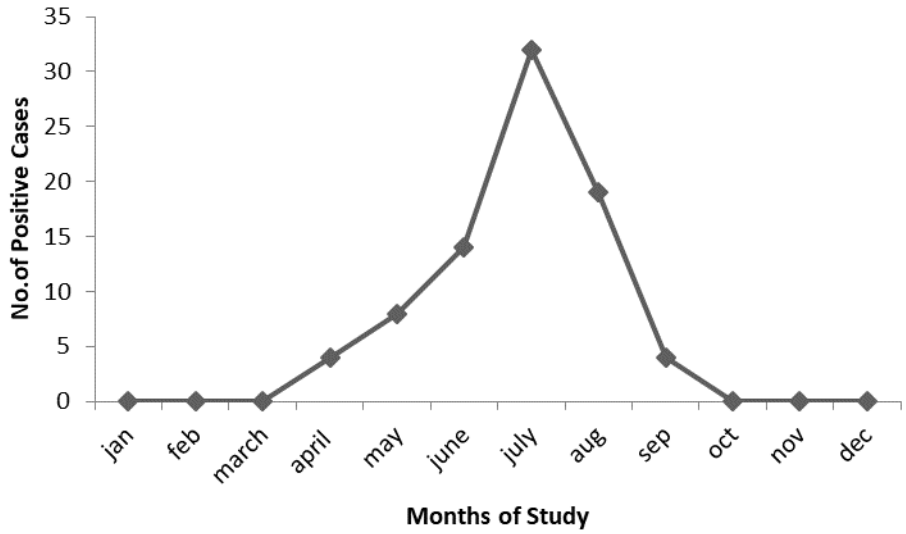

Fig. 2: Seasonal prevalence of endoparasites

It is t very clear from Fig-2 that the maximum number of positive samples in the month of July is the rainy season in Pakistan and facilitates the parasitic population.

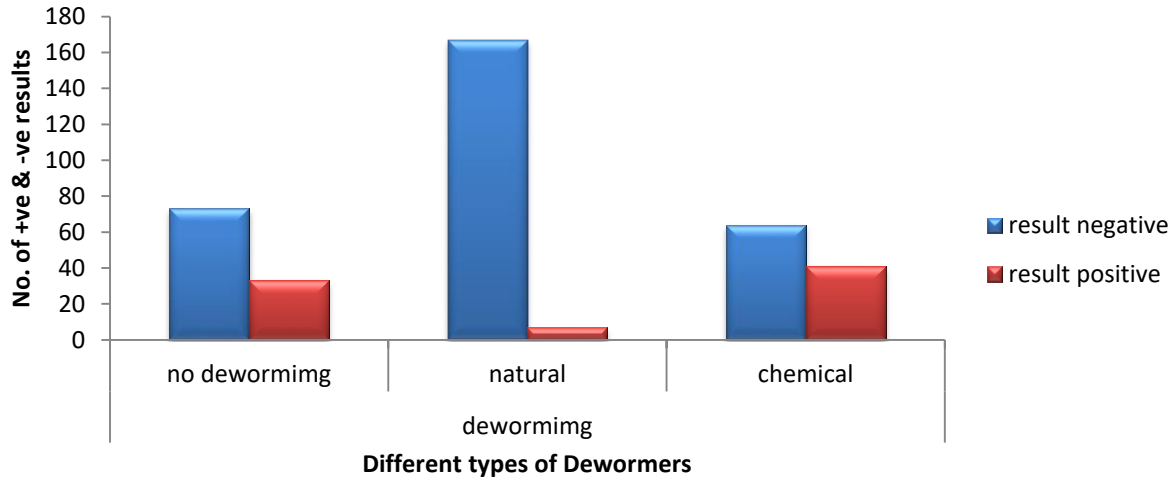

Fig. 3: Comparison of Chemical, Herbal, and No Deworming at Farmed and Captive Sites of Punjab for Anthelmintic Properties

Chemical deworming showed more positive results than herbal deworming as shown in Fig. 3. The herbal medicine, PAK-1(1Kg- *Mentha arvensis*, 1Kg- *Cichorium intybus*, 1Kg- *Silybum marianum*, 1Kg-*Trachyspermum ammi*, 1Kg- *Azadirachta indica*, 500 grams -*Pimpinella anisum*, 500gms-*Curcuma longa*, and 500 grams-*Cassia ngustifolia*) showed good results for ostriches at commercial farms. The farms that were not using any kind of anthelmintic had negative and positive results similar to farms using chemical anthelmintic for their birds. The risk factor analysis of Age and Season with parasitic worm load using the chi-square test gives p-value<0.00, hence the association between age and season with parasitic load is significant.

DISCUSSION

Due to the worldwide importance of ostrich farming, control of parasites in ostrich is now becoming an emerging issue. Unfortunately, despite its great economic potential, the ostrich received little attention from scientists and there are only a few studies focused on its endoparasites. The present study shows very little endoparasitic infection in both, the farmed ostriches and that of wildlife parks. A sampling at 55 farms & captive sites was performed from January 2020 to December 2020.

In the winter season, all samples were found negative, this can be attributed to climatic conditions like low temperature, low humidity and low rainfalls which inhibit the growth and propagation of parasites. A study on the prevalence of gastrointestinal

parasites in desi fowl in and around Gannavaram, Andhra Pradesh, India also supports this fact. The results showed a significant relationship between seasonality. The data obtained from results indicated a high prevalence in the rainy season (43.41%) followed by the summer (39.91%) and winter (17.68%) seasons (Sreedevi et al., 2016). On the contrary, a study in Sweden showed that nematode (L3-stage larvae of L. douglassii) can survive in the winter season (Jansson et al., 2002).

In summer a few oocysts of *Eimeria*, not enough for sporulation, were seen in the faecal examination of some ostriches. *Isospora struthionis* is reported on zoo ostriches in Russia(Huchzermeyer, 1998). During the summer and rainy season, From May 2020 to September 2020, 15 farms out of 55 were found to be infested with *Eimeria spp*. *Amoeboid* cyst and *Balantidium coli* similar was reported by Priyanka etal, 2021. A similar study was carried out on 7 ostrich farms in three states of northern Nigeria from May to September.

All farms except one had samples positive for Eimeria (Mshelia et al, 2010). Whereas in research of Gaborone a decline was found during June and July months, and Coccidia oocyst was not found in any of the ostriches (Binta et al., 2003). At a commercial farm in Faisalabad faecal sample was found to have cysts of (50µm×68µm), and according to the dimensions it was identified as *Balantidium coli* which is not reported yet from ostrich. The infection of this parasite could be due to local environment of farm because ruminants were also present on the farm. A very low pathogenicity in ostriches was attributed to these parasites because eggs and oocyst count were generally very low. However, in two farms, one in Gujranwala and the other in Lahore ostriches were found to have enteritis, faeces with blood, diarrhoea and death due to coccidiosis infection.

The prevalence of very low endoparasitic count can be attributed to the extensive use of dewormers and natural herbs used on farms along with feed. A herbal dewormer named PAK-1 (1Kg- *Mentha arvensis*, 1Kg- *Cichorium intybus*, 1Kg- *Silybum marianum*, 1Kg-*Trachyspermum ammi*, 1Kg- Natural dewormers (comprised of 500 grammes each of *Azadirachta indica, Pimpinella anisum, Curcuma longa,* and *Cassia ngustifolia*) have been used with positive outcomes, outperforming chemical dewormers commonly used on farms. The recommended dosage of PAK-1 was 10ml/30lit water, twice weekly. There was no statistically significant variation in the prevalence

of endoparasites between sex groups of ostriches. These results are consistent with those reported by Jamil et al. in 2022. Herbal medicines are an effective source of prime components for drug detection and the formation of phytopharmaceuticals in the control of devastating parasitic infections. There is a prerequisite to applying traditional medicine information in clinical applications via value addition.

The effect of age on the prevalence of infestation, however, showed chicks (less than 12 months) had a higher prevalence as compared to adult ostriches. This could be due to a lack of immunity (Soulsby, 2015). Commercial farms and wildlife parks were visited during this study and findings from questionnaires revealed that extensive deworming was observed for parasites. this extensive use of chemical dewormers has many negative effects on captive birds' health and funds. According to the literature, urban zoos frequently draw wild animals like rats, herons, vultures, and pigeons. This greatly contributes to the spread of disease among confined animals because it allows them to interact with wild animals more easily. The enclosures and treatment areas must be properly disinfected, and intermediate-host populations must be reduced through targeted treatments and effective sanitary administration. (Melo et al., 2022).

This increased both chemical and natural dewormers being used during this period. Even though no helminths parasites were detected during this study, it is highly likely that birds can suffer from helminths infestation and more research should be conducted on this topic.

CONCLUSION

In conclusion, the present study demonstrated for the first time the prevalence of endoparasites in ostriches raised in Punjab. Ostriches showed a very low prevalence of endoparasites which is a good aspect of ostrich farming in Pakistan. Maintenance of good sanitary conditions, feeding management, and avoidance of contact with domestic animals at farms might be found as helpful factors for ostrich farming. However further studies on problems of ostrich production such as feeding management which can negatively affect the production need to be investigated in Pakistan. This study will essentially be helpful to the researchers and local veterinarians to develop strategies for the treatment and control of diseases.

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ETHICAL APPROVAL

The study was approved by the Institutional ethical review committee.

CONFLICT OF INTEREST

No conflicts of interest were reported by the authors.

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Efficacy of *Aloe vera* in human health especially against COVID-19

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ABSTRACT: *The world is full of enchanted natural flora and one of its charmed plant is Aloe vera. It showed the presence of number of novel compounds that engaged in multiple pharmacological activities and are in use to cure diseases. The present review of A. vera is currently about the debacles of COVID-19 i.e., its component involvement as stumbling block for virus proteases. It has also been noted as an effective anti-COVID cloth coating that will bio-actively lower the impact of differential microbe's expansion along with minimizing the spread of COVID-19. It boosts the immune system in variable circumstances potentially being an anti-inflammatory, anti-cancerous, anti-microbial and wound healing agent. A. vera has made drastic contributions regarding differential human ailments such as wound healing, dental issues, digestive complications, and skin diseases. Moreover, commercial applications of A. vera focuses on manufacturing of bioethanol and nutritionally engaging it in food. Furthermore, inquiries are being carried out to unfold the new wonders of this plant specifically against COVID-19.*

Keyword: *Aloe vera, Antimicrobial, covid-19, Anticancer, Pharmacology*

INTRODUCTION

In traditional system, *A. vera* has been utilized therapeutically in Unani, Ayurveda, Siddha medicine and also in homeopathy since ancient times (Pathak and Sharma, 2017; Gupta et al., 2018). Due to therapeutic aims, in literature, *A. vera* has been endorsed with multiple names such as heaven's blessing, pharmacy in a pot, wand of heaven and also as the silent healer (Gupta and Rarawt, 2017; Tiwari and Upadhayay, 2018). *A. vera* is succulent, perennial, xerophytic plant having pointed, serrated, lance shaped, fringed leaves in rosette configuration (Fig. 1A) (Minwuyelet et al., 2017; Gupta et al., 2018). It belongs to arid and hot climatic regions (Rahman et al., 2017; Amin et al., 2018). This plant belongs to family Asphodelaceae, although, traditionally

there have been many contradictory views in literature about its family (Yohannes, 2018). It also expresses some relationship with Liliaceae family members such as garlic, turnip and onion families based on the possession of certain chemical constituents (Tiwari and Upadhayay, 2018).

A.vera leaves are basically categorized into 3 stratums; the outer most is the rind which has protective nature, the middle one is the sap which is yellow in color, bitter, with juice produced from cells present below epidermis of leaves comprises of glycosides and anthraquinone and the inner parenchymatous layer is mostly the crystal clear mucilage gel encompasses customarily polysaccharides (Fig. 1B). (Minwuyelet et al., 2017; Yohannes, 2018). *A. vera* has long stalked flowers that extend beyond leaves, dazzling yellow color, tubular organized in loose spike form.

The plant accomplishes maturity in almost four years and ends its life cycle in 12 years (Abakar et al., 2017; Pathak and Sharma, 2017).

1.1. Potential Pharmacological Virtues of *A. Vera*

A. vera is the superlative naturally occurring medicinal plant in this whole realm being widely used in herbal medication as a vital therapeutic source for numerous ailments since historical times until in present times too.

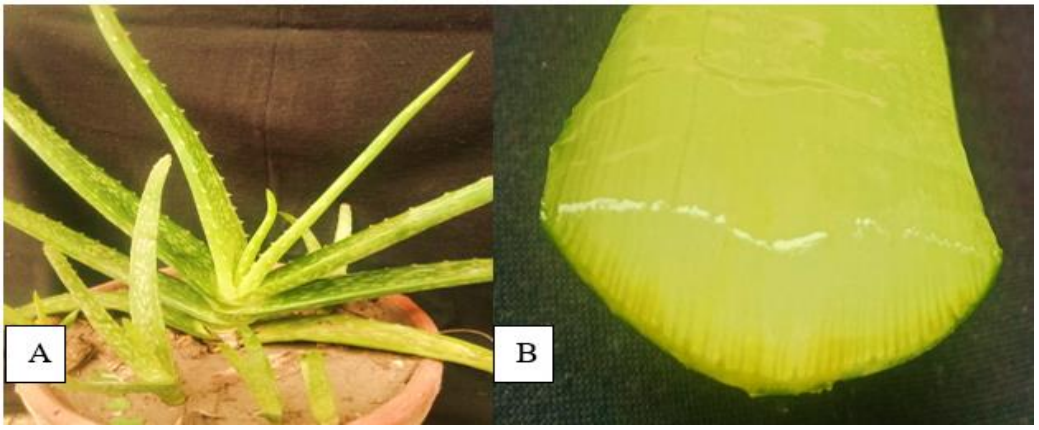

Fig. 1 A). *A. vera* plant B). Leaf of *A. vera*: Inner parenchymatous gel layer

Also its voluminous biological activities have been pharmacologically approved and cited in literature various times for curing infirmities as arthritis, analgesic, anemia, AIDS, anti-allergic, appetite stimulant, anesthetic, blood pressure, anti-biotic, anti-bacterial, cataracts, conjunctivitis, chronic

ulcers, anti-diabetic, diuretic, decoagulant, demulcent, digestive disorders, eczema, emollient, edema, anti-fungal, anti-inflammatory, gastrointestinal ulcers, heat burn, anthelmintic, hypoglycemic, hair stimulatory, insecticides, immune-stimulatory, liver stimulant, anti-leukemia, laxative, lowering blood lipid levels, mouth infections, migraine, anti-oxidant, psoriasis, parasitic killer, anti-pyretic, rejuvenating agent, radiation burns, skin disorders, anti-septic, skin cancer, tuberculosis, anti-tumor, tonic, anti-toxic, vaginitis, wound healing, and still many more. It is also being utilized as a vital ingredient for enhancing beauty in cosmetic merchandise in various ways such as in moisturizers, shampoos, facial products (Tiwari and Upadhayay, 2018).

1.2. *A. vera* as an Anti-Covid-19 Agent

A. vera can exhibit anti-COVID-19 activity as reported in the Democratic Republic of Congo (Mpiana et al., 2020a). Through in-vitro studies, it was observed that the polymerase activity of RNA in arterivirus and coronavirus can be inhibited by Zn2+ and also such viruses' replication in the cell culture can be obstructed by the ionophores of Zinc. Zinc is generally crucial as a co-factor of enzymes and it's intracellularly increase blocks the reproduction of retroviruses involving the SARCoV-1 (Abiri et al., 2020; Mpiana et al., 2020a; Khan and Al-Balushi, 2021). In SARS coronavirus, aloe-emodin impedes the slitting of 3C-like protease and this enzyme is a key part of replication of virus by means of proteolytic mechanism at replicase phase (Mpiana et al., 2020a).

1.2.1. COVID-19 Protease Inhibition

Plants are the natural architect of viral inhibiting proteins through the expression of metabolites and the phenomenon of natural protease inhibitors at present are the best known therapies to combat COVID-19 (Origbemisoye and Bamidele, 2020). The current research trend is to obstruct the key protease of COVID-19 (Mpro), a pharmacological prey by means of numerous secondary phytochemicals extracted from medicinal plant's one of them being *A. vera*. The virtual analysis of *A. vera* compounds as inhibitors for protease 3CLPro was carried out through ADMET and molecular docking process. The outcome of ADME is brought out by Lipinski's ruling of five which revealed that among the other ligand compounds feralolide is the most competitive against the drugs and has also been known for its anti-cancer, anti-fungal and anti-oxidant properties (Mpiana et al., 2020b). Another study has also reported

feralolide from *A. vera* as novel component for Mpro inhibition of SARS-COV-19 and its efficiency can be increased by substituting the hydroxyl group with each hydrogen of feralolide. This study done through molecular dynamic simulation techniques and ab initio fragment molecular orbital (FMO) calculations (Shaji et al., 2022). Also Aloe-resin and 9-dihydroxyl-2-O-(z)-cinnamoyl-7-methoxy-Aloesin are proven to be relatively effective for inhibition plus they are inclusive in the anti-inflammatory process. Such conclusions are founding the pathway for medical research based on herbal medication towards COVID-19 (Mpiana et al., 2020b).

In another study, *A. vera* has also proven to be a switching mode as herbal remedy for COVID-19 because in-silico molecular docking mechanism against viral cycle SARS-COV-2 comprising of Spike glycoproteins, RNA-dependent RNA-polymerase (RdRp) and main protease (Mpro), it has concluded that efficient retarding influence of *A. vera* components on proteases has observed constraining affinity towards the target regions (Khanna et al., 2020). It has proven that *A. vera* constituent's Catechin and Quercetin, uncovered great binding interactions with proteases and RNA-dependent RNA-polymerase, respectively showing potential energies against the antiviral drugs (Pandit and Latha, 2020). The anti-viral metabolites in *A. vera* i.e., anthraquinones working alone or either synergistically with pharmaceutical drugs to attack the SARS-CoV-2 protease (CLPro) (Origbemisoye and Bamidele, 2020). Aloe-emodin has also been studied as a barrier for biosynthesis of nucleic acid eventually blocking the production of proteins (Mpiana et al., 2020a). Emodin in *A. vera* combine with the S proteins of SAR-CoV preventing its entry into the host cells and also interrupting the 3CLpro capability of virus eventually stopping the synthesis of Nsp. The experimental trials have proven that specific concentrations of emodin can block the collaboration of ACE2 and SAR-CoV S-protein thus inhibiting the channels of 3a ion and interfering with the release of fresh coronaviruses (Llivisaca-Contreras et al., 2021). Jain (2020) have recently described that among many herbal plant extracts, *A. vera* extract had showed the utmost inhibiting activity against COVID-19 protease (6LU7).

1.2.2. *A. vera* as an Anti-COVID Cloth Coating

Health care workers are at utmost risk of COVID-19 in aspects of treating patients. They require special protecting coverings to secure themselves but that covers a lot of differential risky drawbacks. To

resolve this issue, currently varieties of green innovations are being utilized to manufacture antimicrobial clothing's specially antiviral to protect oneself from various diseases. Currently plant extracts are being selected for material coatings due to their bioactive, economical and eco-friendly nature by means of their significant anti-bacterial, anti-viral and anti-fungal aspects and one of them i.e., all in one possessing all these abilities is *A. vera*. The significantly abundant polysaccharides and anthraquinones in it are the key factors in anti-viral activities. Mechanically, *A. vera* physically inhibits the binding regions between the cells of host and virus.

The main resolve of *A. vera* coating is basically obstruction of virus on the surface through repelling its aerosol droplets. In its anti-viral mechanism's anthraquinones along with its derivatives actively impede the connotation of aminoacyl-transfer-ribonucleic acid (aa-t-RNA) and the ribosome of viral cell eventually hindering synthesis of proteins. All of this occurs due to the interaction between the negative charge of anthraquinones and its derivatives with the positively charged aa-t-RNA. Polysaccharides have showed lessen adsorption time and replication of virus in the in vitro studies. It has been declared that polysaccharides of acidic nature work by forming the associations through their anti-adhesives and those comprising of high-pitched uronic acid of negative charge form an attraction with the t-RNA obstructing the preparation of proteins. Hence, it has been established that *A. vera* is a capable anti-COVID agent for cloth coating. Besides, *A. vera* dependent coatings will lessen the need of kits of PPE and multi-wrapped clothes eventually reducing the transmission of viral infections but still further investigation is under process for testing of anti-viral coatings to establish finalize conclusive remarks soon in future (Chauhan and Kumar, 2020).

Nanofibers are highly specialized in managing, blocking, deactivating the viral and microbial activities. These are considered to be potentially applicable in developing protective surgical clothing products such as masks, gloves, PPE, surgical and gowns etc. On this basis, several experiments are carried out using variable concentrations of *A. vera* with Polyvinyl Alcohol (AV/PVA) for developing electrospun fibers that possess highly effective anti-microbial properties and had shown strong repulsion and electrostatic force among the molecules that boost up due to the presence of H groups in Aloe gel and PVA ensuing the compactness of fiber molecules. Various experiments were also carried out by using these strategic

components demonstrating the effectiveness of AV/PVA fibers which was further modified and tested by using ZnO NPs. Electrophied nanofibers were prepared with variable concentrations of *A. vera* gel and fixed ZnO NPs, fix Aloe gel concentration and variable quantity of ZnO NPs, all these groups were tested against various microbes and affirmed by techniques such as SEM and FTIR (Khanzada et al., 2020; Montagner et al., 2021; Munir et al., 2022).

1.3. *A. vera* as Immune System Booster

The immuno-modulation mechanism involves stimulation of cytokines by activation of lymphocytes which in turn enhances the action of macrophages (Origbemisoye and Bamidele, 2020). The glycoproteins and macromolecular polysaccharides of *A. vera* has been described as immunomodulatory and immunoregulatory agents. Acemannan play their role in immunoregulatory action through macrophages initiation, production of cytokines TNF-a and IL-6, collectively working with interferon IFN-c thus promoting the liberation of NO, superficial antigens expression and thus eventually induction of morphological deviations of the cells (Gao et al., 2018). Acemannan advances the metabolism through standardizing cellular functions and modifying the nutrients flow and regularizing the wastage movement of cells (Sushen et al., 2017).

1.4. *A. vera* as Anti-inflammatory Agent

The mode of action of *A. vera* as anti-inflammatory agent is that it hinders the pathway of cyclooxygenase, lowers the production of E2 prostaglandin and hinders the linkage of pro-inflammatory cytokines and leukocytes. Anthraquinones of *A. vera* are the structural correspondent of tetracycline and it obstruct the site of ribosomal A in bacteria eventually inhibiting the synthesis of proteins. The presence of pyrocatechol in *A. vera* is proven to be lethal towards microorganisms (Paul et al., 2020).

A. vera possesses the enzyme peptidase bradykinase which collapses the bradykinin i.e., the key inflammatory element involved in causing pain. Bradykinase not only shuts down the bradykinin action but diminishes pain and also hastens the healing mechanism. C-glucosyl-chromone, a new element from *A. vera* extract, possesses sturdy anti-inflammatory action. Anti-prostaglandin components and sterols have been described as anti-inflammatory agents. Sterols have proved to be lessening inflammation effect by 37%. Lupeol is the utmost dynamic anti-inflammatory sterol (Pathak and Sharma, 2017; Maan et al., 2018; Mikołajczak, 2018;

Yohannes, 2018). It stops the relocation PMN neutrophil cells to the inflammatory tissues of vein as to inhibit the process (Suhardono et al., 2020).

1.5. *A. vera* as Anti-cancerous agent

Acemannan, anthraquinone and aloe emodin components of *A. vera* have the capability to conquer the malignant cancerous cells growth, affecting their assault, migration, hindering in cell proliferation, cyclic arrest, cell death initiation and modulating the signaling of the immune system. They seem to have their influence in the anti-neoplastic, pleiotropic and anti-proliferative way (Nazir and Ahsan, 2017; Mikołajczak, 2018). Juice of *A. vera* is also being reported as a contribute in curing cancer, also the mutilation by chemotherapy and radiotherapy since it annihilate the normal cells rudimentary of retrieval (Pathak and Sharma, 2017). Glycoproteins, barbaloin, aloesin and many other polysaccharides are seem to be involved in cytotoxicity activity to counter severe myeloid leukemia and lymphocytes leukemia cells (Minwuyelet et al., 2017; Maan et al., 2018).

1.6. Antimicrobial activity

Anthraquinone, dihydroxyanthra quinones, aloe emodin and saponins have been anticipated as possessing direct antimicrobial actions. A polysaccharide element, acemannan indirectly enhances antimicrobial activity by stimulating phagocytic leukocytes (Dubey et al., 2017). Its acemannan and glucomannan compounds possess anti-bacterial action by soothing the immune system through enhancing macrophages. Anthraquinones express the anti-bacterial effect similar to strong antibacterial drug Tetracycline (Mikołajczak, 2018). Gupta (2017) has also verified that the solid referential existence of bactericidal activity is all because of dynamic combos of various pharmacological components i.e., aloe-emodin, anthraquinones, aloin, aloeride, antranol, anthracine, chryosophanic eroding, barbaloin, resistanol and saponin etc. Aloe-emodin and Aloin polyphenolic structural composition led them to suppress the protein unification of bacteria.

A. vera antiviral potential is basically due to the anthraquinones but several other compounds are also involved such as emodin, acemannan, quercetin, acyclovir, kaempferol, aloin and catechin hydrate which lay their art as an antiviral agent. Various minerals such as Ca, Co, K, Mg, Zn, and Fe found in A. vera also contribute to its anti-viral potential. Several compounds i.e., lectins, chrysophanic acid, acemannan, aloin, and aloe-emodin are present in *A. vera* have been identified

to participate in the anti-viral activity (Mpiana et al., 2020a). They have also been described in literature against different viruses such as Human papillomavirus, Cytomegalovirus, Poliovirus, Herpes Simplex virus type 1 and 2, HIV-1, Varicella-Zoster virus and Hemorrhagic Viral Rhobdovirus Septicemia (Mpiana et al., 2020a; Abiri et al., 2021). Also, polysaccharides in *A. vera* gel are effective emollients against anti-viral infections, specifying *A. vera* as herbal cure (Demeke et al., 2021; Soleymani et al., 2022).

1.7. *A. vera* as Wound healer

A. vera polysaccharides principally acemannan is greatly cooperative in curing wounds by accelerating the synthesis of collagen, stimulating the activation of macrophages to release the fibrotic cytokines, producing hydroxyproline and hyaluronic acid in fibroblasts. It influences the collagen composition enhancing the cross-linking. Also *A. vera* comprises of 96% water that minimizes the drying of lesions plus surges the epithelial cells movement and keratinocytes (Gao et al., 2018; Mikołajczak, 2018). Amino acids and numerous electrolytes such as copper, calcium, chromium, iron, magnesium, potassium and zinc are present in *A. vera* playing a dynamic part in the process of wounds remedy. It excites the production of antibodies and initiates the liberation of growth factors for healing of wounds. It fastens the healing process, averting the formation of scars, helping the creation of cells in the deeper films of skin (Maan et al., 2018).

Mannose, 6-phosphate in *A. vera* helps in curing the 1st and 2nd grade burns and wounds. In contrast to silver sulfadiazine, healing proportion is rapid almost one-half in response of mannose-6-phosphate (Gao et al., 2018). Glucomannan, a calmative polysaccharide having moisturizing features and gibberellin hormone also play their part in wound healing (Dubey et al., 2017). All of these work in a similar way as compared to acemannan polysaccharide interacting together exciting the formation of collagen through in taking *A. vera* orally and topically (Pathak and Sharma, 2017; Upadhyay, 2018). Applying *A. vera* in topical way efficiently upsurge the dermatan sulfate and hyaluronic acid synthesis in the wound granulated tissue endorsing the healing of wound. Also collagen production is being enhanced through gibberellin and glucomannan (Giroh et al., 2019). *A. vera* has also been described as an effective substance to increase the re-epithelialization of cornea reducing the inflammation occur due to alkalotic burns although further studies are required to signify

this mechanism (Moghadam et al., 2020).

1.7. Green impact of *A. vera* for human health improvement

1.7.1. Dental ailments

Since old times, oral cleanliness is a significant part of individual welfare (Isadkar et al., 2018). *A. vera* usage for dental hygiene date back to 1700 and 3700 BC especially in the time of archaic Egyptians highlighting its curative possessions (Gupta, 2017). Anthraquinones were majorly involved in fighting against the mouth bacteria, reducing the inflammatory effects (Sushen et al., 2017). It has been found that *A. vera* was readily influential against different oral microbes such *Candida albicans*, *Lactobacillus acidophilus*, *Pseudomonas aeruginosa*, Peptostreptococcus *anaerobius*, *Prevotella intermedia* and *Enterococcus faecalis*. Also it has been reported that *A. vera* gel has been used to compose toothpastes and mouthwash which greatly exhibit anti-microbial activity (Gupta, 2017).

In dentistry, *A. vera* is being exploited for treating numerous dental problems such as periodontitis, cellular propagation of periodontal ligament, gingivitis, antedating halitosis, stomatitis, aphthous ulcers, oral sores, curing endodontic, specific cheilitis, collagen type I, positive growth factor differentiation, dental grafts, and alkaline phosphate property in humans etc. It has been utilized in multiple forms such as tooth gel, denture cleansers, mouth wash and denture adhesives etc (Gupta, 2017; Isadkar et al., 2018; Salehi et al., 2019). *A. vera* reduces gum infections, edema of tissues and thus, subsequently bleeding of gums (Abdulwahhab and Jassim, 2018). The gel from *A. vera* has been considered as an endodontic being utilized as a biocompatible remedy for pulpal tissues (Maqbool et al., 2020).

Traditionally in old times, *A. vera* gel was mixed with charcoal for oral hygiene (Sushen et al., 2017). The mouthwash of *A. vera* was considerably effective for curing oral lichen planus and also helpful in reducing gingivitis and plague (Deepthi and Kumar, 2018). Gibberellins and glucomannan in *A. vera* advances the potential of reinforcing fibroblast multiplication, quickening patching with epithelial tissues extension counteracting the diseases which delay the wound recovery (Gupta, 2017; Deepthi and Kumar, 2018). The lessening of gingiva through *A. vera* is attributed to the proximity of sterols as vindicating experts and also lupeol as sparkling pain reliever. It has been reported that *A. vera* lessens the chances of halitosis and clinically, local application of it is considerable in adjunctively treatment of scaling and planning of root in case

of lingering periodontitis (Gupta, 2017).

A. vera has also been a great medicament for burning oral syndrome, xerostomia, submucous fibrosis, candida denture stomatitis and radiation mucositis (Giroh et al., 2019). Acemannan the prominent starch, a composite mannose polymer in *A. vera* possesses influential regeneration activity and intuitive steadiness advances the curative action of dental area in humans through extracellular grid prearrangement, dental mash cell development, parting and mineralization making it perfect for denture strengthening purposes. Acemannan in *A. vera* is found to be greatly involved in treating aphthous ulcer through reinforcing the growth of fibroblast cells to boost the recovery of ulcers, also endorses the generation of collagen by the process of mannose-6-PO4 to fibroblast receptors. Barbaloins and alloins components are operative in lubrication case and in sedative covering. Also acemannan hydrogel decreases the prevalence of alveolar osteitis. Saponin specialize frothing is better for purifying purposes in aloe toothpaste and give equivalent results without enhancing the fluoride content (Gupta, 2017).

1.7.2. Anti-diabetic activity

Polysaccharides in *A. vera* are involved in developing insulin level and expressing hypoglycaemic possessions (Pathak and Sharma, 2017). *A. vera* basically lessen the disturbed lipid profiles and chronic hyperglycemia which are the main features of Diabetes mellitus, also influencing cardiovascular mellitus (Yohannes, 2018).

1.7.3. Digestive complications

A. vera is greatly endorsed against multiple digestive issues such as stomach ulcers, indigestion, irritated bowl syndrome, heartburn, yeast formation, reassures digestive bacteria, reducing intestinal toxicity, constipation and many other gastrointestinal problems (Sushen et al., 2017; Mikołajczak, 2018). A. vera plays its part in curing gastric, silver, mouth, leg ulcers and sores where it influence in lessening the size of ulcer, exudation and erythema (Maan et al., 2018). *A. vera* anthraquinones are key ingredient being potential laxative agent through surges of intestinal peristalsis, intestinal water content and stimulating mucus secretion (Yohannes, 2018).

1.7.4. Skin dilemmas

A. vera has sensational and wonderful influence on skin improving its integrity, reducing erythema and lines, healing pimples, enhancing fibroblast stimulation, increasing skin elasticity and softness. Its muco-polysaccharides are greatly involved in moisturizing the skin by increasing the moisture

binding potential of skin. Amino acids are helpful in giving softness while zinc causes the pores tightness (Minwuyelet et al., 2017; Gao et al., 2018). In cosmetics, 95% dermatologist products have essential extracts which involve *A. vera* as a core ingredient in them (Sushen et al., 2017).

1.7.4. Reproductive Improvement

It has been reported that *A. vera* plant that comprises of some phyto-estrongenic components influencing the estrogen levels in a positive way leading the growth of follicles as effective as the

sexual hormones and these compounds reduces the hormone levels of LH and FSH. In male rats' studies, it has been concluded that it causes certain histological differences in testis surging the content of Sertoli cells, spermatids, spermatocytes and spermatogonia (Koshkaki et al., 2020).

1.8. Commercial Applications of *A. vera*

1.8.1. Bioethanol production

The leaf rind of *A. vera* has proven to be a capable source for the production of bioethanol. The biomass of its leaf rind cellulose has also been used to produce cellulosic nanofibers and also acid-hydrolyzed leaf rind biomass utilization has also been reported to analyze its potential for bioethanol manufacturing (Rajeswari and Jacob, 2020).

1.8.2. Usage in beverages

The juice of *A. vera* is useful in making of several beverages such as in papaya beverage formation, it is being used in a different blended ratio for a ready to serve (RTS) beverage and this will have better storage efficiency and quality. In mango nectar, *A. vera* is basically a core component in improving its potential and quality features and can be stored up to 6 months. It is also being used in the preparation of herbal wines and possesses better anti-bacterial activity against the common pathogens of food. *A. vera* products basically involve it in a variety of concentrations and combinations in numerous food applications. The concentrated quantity *A. vera* is present in jams and jellies, cakes, also in mixtures with water and teas. The fillet of gel is available in chewing gums, candies, bars, prompt tea granules and fruit smoothies. Its juice exists in different types of drinks such as sports drink, soft drink, vegetable mixtures juice, alcohol and whiskey, dairy products i.e., probiotics dahi and lassi. Powder of *A. vera* occur in ice-cream, curds, lassi, yoghurt and laddu (Kukreti et al., 2016).

1.8.3. Food supplements

Variable compositions of *A. vera* in different dosages is present in food

supplements available in market. *A. vera* (99%) extract (30ml) can be taken twice per day for multipurpose as a supplement. Its fibrous juice is used as a dietary enhancement purpose (20ml to 30ml diluted with in equal quantity). Simple juice of 30ml utilized only one time is purposed for various digestive disorders. Pure juice of *A. vera* (98%) dosage 30ml, used once a day is meant for immunomodulation, antioxidants and also as a storehouse for nutrients. Purified extract (100%) in capsules, only 5 per day can heal the internal intestinal complications (Mulay and Ogale, 2018).

1.8.4. Food coatings

A. vera can also be demonstrated as efficient source of ecofriendly antimicrobial coatings for biomedical products because it has been studied that *A. vera* pristine Pluronic merged solution possess effective antimicrobial potential enhancing the structural and physical properties of it (Seifunnisha and Shanthi, 2020). *A. vera* gel has been widely used as an edible source of coatings for different fruits being whole, fresh-cut or in raw ones e.g., in grapes, strawberries, plums, apples, litchis, papayas, cherries, mangoes etc. The gel basically reduces the synthesis levels of ethylene, activities of peroxidases, and polyphenol oxidase. It also lessens the fungal effect and browning on fruits. Besides, being an edible source of coating, it comprises of vitamins acting as antioxidants and several amino acids for essential humans (Farina et al., 2020a, b). The fruit coatings inclusive of polysaccharide as key component are greatly operational in inhibiting the transfer of gases, lowering the rate of respirations, having less toxic effects and are being biodegradable. Currently, nano-coatings are being highly considered due to their great adhesive potential (Suriati, 2022). Recently, it has been studied that the combination of A. vera gel with lemongrass essential oil in variable concentrations showed significant results for edible fruit coating in strawberry by reducing acidity, maintaining firmness of fruit, resisting microbial growth and also increasing the fruit shelf life for storing it (Hassan et al., 2022).

CONCLUSION

The outcome of current investigation has revealed that *A. vera* secondary metabolites are a vital ingredient of various activities and have been proved through literature especially in certain actions for boosting the immunity. *A. vera* had been an efficient stone mark of health strength in various cases and it has been utilized through historical times until now. Several recent discoveries in the past few years are also being carried out to prove its impact on human health pharmacologically although

been employing it for extended time and improvising its commercial utilization. Most radically, *A. vera* anti-viral association with COVID-19 which has shaken the world health system to its core, has led to a remarkable twist in the pharmacological way of drug development and also its herbal cure, however in this regard, further significant molecular evidences are required to provide its influencing facts and mechanisms.

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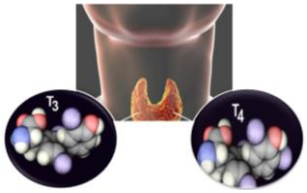

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In Silico Comparative Metagenomic Analysis of Microbial Communities of Chromium Contaminated Sites

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ABSTRACT: Chromium is one of the highly toxic and carcinogenic heavy metals. Due to increased anthropogenic activities, high concentration of chromium is found in many areas. Many microorganisms have the ability to detoxify chromium. Metagenomics allow us to comprehensively study microbial communities present at different sites without culturing them. The objective of this study was to analyze the abundance of microbial groups in different environments contaminated with chromium. For this purpose, chromium contaminated soil, anaerobic sludge and reactor samples were chosen. 16S rRNA data of these samples was retrieved from NCBI SRA database. The sequences were analyzed by Mothur software accessed via Galaxy server, and were classified using SILVA database. Venn diagram, phylogenetic tree, heatmap, relative abundance graphs and Krona pie charts were generated. Statistical analysis was also performed in the form of AMOVA and HOMOVA tests. According to results of our study, *Proteobacteria*, *Leucobacter*, *Actinomycetales*, *Actinobacteria*, *Arthrobacter*, *Rhizobiales*, *Sphingomonas*, *Bradyrizobium* and *Nucardioidaceae* were present in all the samples. *Firmicutes*, *Planctomycetes*, *Verrucomicrobia* and *Bacteroidetes* were more abundant in chromium contaminated samples as compared to control samples. The results were also found to be statistically significant. The above-mentioned bacteria can be targeted and studied to discover their roles in bioremediation of chromium contaminated sites.

Keyword: Chromium; bioremediation; metagenomics; bacteria; microbial community analysis

INTRODUCTION

Heavy metals are of great interest in today's era (Joseph et al., 2019). Despite of their toxic properties, they are still being used widely in different industries, putting the environment at risk (Esmaeili and Beni, 2108). Until now, 53 elements have been identified as heavy metals as they have densities greater than or equitant to 5g cm^{-3} and atomic weight more than 20. Due to increased industrial effluents, highly urbanized societies and over-population, many hazardous materials in the environment are increasing, causing serious health problems (Li et al., 2019). Chromium (Cr) is one of the naturally existing heavy metal having a molecular weight of 51.1 a.m.u and density of 7.19 g cm^{-3}. It is 17^{th} most abundant element on Earth and is highly reactive to oxygen presence in air, and hence various oxidation states have been reported ranging from 0 to +6 (Oliveira, 2012). Among all other, Cr (III) trivalent and Cr(VI) hexavalent are considered as most stable forms of Cr in the environment. Different industrial processes like burning of coal and oil, drilling of oil wells, metal refining, leather industries, stainless steel production, and chemical dye production use Cr (Coetzee et al., 2020). Cr contamination via air emissions and industrial effluents reach the local population and affects them (Welling et al, 2015). Cr is considered as Group I carcinogen by the International Agency for Research on Cancer (Ray, 2016). Exposure to high concentration of Cr can have detrimental health effects on human beings as it may affect the working of different organs such as lungs, kidney, liver and brain, and can results in different types of cancers as well. Studies have also shown that long term exposure to Cr can cause muscular and neurological alteration, Alzheimer's disease, Parkinson's disease, muscular dystrophy and even cancer (Pushkar et al., 2021).

Cr affects the microbes in various ways and can alter the composition of microbial communities (Pei et al., 2018). However, many microbial species are known to exhibit resistance against Cr and can detoxify it. Different microbial remediation techniques (bioremediation) such as biosorption, bioaccumulation, biotransformation, and bioleaching have been employed to remove Cr and other heavy metals from industrial wastewaters (Fernández et al., 2018).

Metagenomics is an approach which allow us to analyze directly the extracted genetic materials from sites and decipher the information of uncultivated microbial communities present in them. It can be a good tool to trace out the microbes and the genes involved in bioremediation (Datta et al., 2020). Next Generation Sequencing technologies (NGS) have made possible the enormous data output from metagenomics samples. This gives a very comprehensive picture of the microbial communities, both culturable and non-culturable. *In silico* metagenomic analysis plays a crucial role in unraveling the intricate complexities of microbial communities and their functions within diverse ecosystems. By harnessing the power of computational tools and algorithms, this approach allows researchers to study the genetic content of entire microbial populations without the need for culturing individual species. Such metagenomics analysis can provide insights into the diversity, composition, and functional potential of microbial communities, shedding light on their roles in nutrient cycling, disease development, and environmental processes (Mitra et al., 2015). Such *in silico* studies can serve as a powerful tool for advancing our knowledge of microbial ecology and can pave the way for the development of targeted interventions and strategies for harnessing the potential of these complex microbial communities (Llorens-Marès et al., 2015).

The objective of the present study was to compare microbial communities of different sites contaminated with Cr in order to find out the bacteria common in all those sites. Such bacteria might have a role to play in Cr detoxification. For this purpose, 16S rRNA gene sequences of metagenomes of Cr affected microbial communities were downloaded from internet databases and were analyzed comparatively.

Methodology

Sample selection

Soil, anaerobic sludge and bioreactor sites contaminated with Cr were selected for this study. Sequence data published on National Center for Biotechnology Information (NCBI) Short Read Archive (SRA) was downloaded with accession numbers. A total of 24 samples were randomly selected. Out of these 24 samples, 9 samples were of soil, 4 were of reactor, 5 samples were of anaerobic sludge and 6 were selected of control soil (Table 1)

Table 1. List of samples retrieved from NCBI SRA

No	Sample	SRA	No. of spots	Published
1	Soil	SRR12524814	89,977	2020-12-01
2	Soil	SRR12524811	90,490	2020-12-01
3	Soil	SRR12524812	85,982	2020-12-01
4	Soil	SRR12524829	72,900	2020-12-01
5	Soil	SRR12524822	92,943	2020-12-01
6	Soil	SRR12524821	90,379	2020-12-01
7	Soil	SRR12524813	87,836	2020-12-01
8	Soil	SRR12524805	97,779	2020-12-01
9	Soil	SRR12524803	83,440	2020-12-01
10	Reactor	DRR218029	21,232	2020-09-03
11	Reactor	DRR218030	33,806	2020-09-03
12	Reactor	DRR218031	25,714	2020-09-03
13	Reactor	DRR218032	27,722	2020-09-03
14	Anaerobic Sludge	SRR10015225	48,497	2019-08-26
15	Anaerobic Sludge	SRR10015226	51,373	2019-08-26
16	Anaerobic Sludge	SRR10015227	48,540	2019-08-26
17	Anaerobic Sludge	SRR10015228	44,026	2019-08-26
18	Anaerobic Sludge	SRR10015229	47,111	2019-08-26
19	Control	SRR16005226	54,660	2021-09-22
20	Control	SRR16005227	41,830	2021-09-22
21	Control	SRR16005228	43,288	2021-09-22
22	Control	SRR16005233	51,433	2021-09-22
23	Control	SRR16005234	47,857	2021-09-22
24	Control	SRR16005235	62,514	2021-09-22

All the samples had been sequenced through Illumina MiSeq paired end sequencing technology. The data selected was comparable to each other according to 16S rRNA gene region sequenced (V3-V4), base numbers, number of spots, etc.

Processing of sequences:

The processing of the sequences and the analysis was performed in Mothur software (Schloss et al., 2009) through Galaxy server (https://usegalaxy.org/). All the collected and downloaded data and the reference files [such as Silva.v4.fasta for alignment, Trainset9_032012.pds.fasta and Trainset9_032012.pds.tax for classification obtained from Mothur Miseq SOP website (https://mothur.org/wiki/miseq_sop/)] were uploaded on the Galaxy server. Contigs were made by aligning both

reverse and forward files of the data. The data was cleaned by eliminating all unnecessary sequences and by reducing the number of ambiguous reads by using commands such as screen.seqs and unique.seqs

Sequence Alignment

Sequences were aligned to the SILVA reference file of 16S rRNA dataset named "Silva.v4.fasta" (Quast et. al., 2012). It improves the clustering of the sequences. Any read which did not overlap the V4 region of 16S rRNA gene was removed. The next step was to merge near-identical sequences together to further reduce the spread of the data. Sequences that differed by 1 in every 100 bases were likely to represent sequencing errors, not true biological variation, and therefore these were combined to further reduce the data.

Taxonomy classification

After the alignment of the sequences, next was to assign taxonomy to the sequences. For this *classify.otu* command was run using RDP reference dataset *Trainset9032012.pds.fasta* (Cole et al., 2014). *Remove.lineage* command was used to remove the undesirables such as the sequences belonging to chloroplast, mitochondria, unknown groups and eukaryota. After this *Summary.seq* was run to summarize the quality of sequences.

OTU clustering

For this, *Cluster.split* command was used which assigned the sequences to OTUs and to split large matrices of the data to reduce further computation. To find out how many sequences are in each OTU from each group, *make.shared* command was used. Then *classify.otu* command was used to learn about the taxonomy for each of OTUs. For this step, level 0.03 was considered which actually means 97% similarity threshold or specie level. *Count.group* was performed to find out the number of sequences in each sample.

Diversity Analysis

Diversity analysis was performed in two ways, alpha and beta diversity analysis. In alpha diversity analysis, *summary. single* command was used to calculate the diversity indices of the Cr-contaminated sites. For beta diversity analysis, heatmap tool was used which is a graphical representation in which shades of red and black are used to depict the extent of similarities and dissimilarities between the samples. *Venn diagram* was used to determine species richness and diversity. Phylogenetic tree was made to find out

how different sites are grouping with each other based on their sequence similarities.

Krona pie chart

It was used to visualize the proportions of all the bacteria present in the samples. For this purpose, *input file* was selected as *"Taxonomy-to-krona"*. In first krona pie chart, all the samples were plotted together to see the diversity of all the bacteria present in the samples. Krona pie chart of each sample was also made to visualize diversity of bacteria in each sample.

Relative Abundance

To further find out the relative abundance of the bacteria present in control and Cr-contaminated sites visually, relative abundance bar graph was generated by using biom (biological observation matrix) format which is used to represent OTUs. The results were visualized and analyzed in Phinch tool https://www.phinch.org/.

Statistical Analysis

To assess whether the diversity of bacteria present in control and different Cr contaminated sites have statistically significance differences, AMOVA test was performed. An alternative way to observe whether the spread of data differs between groups is by comparing the homogeneity of molecular variance (HOMOVA). It is molecular based statistical test. Parsimony analysis was also performed to compare communities with similar structure. All the statistical analysis was performed in Mothur software through Galaxy server, as mentioned above.

RESULTS

Cleaning, alignment and classification of sequences

A total number of sequences in the start were 1355509. The shortest sequence had a chain length of 36 base pairs and contained three polymer repetition. The longest sequence had 601 base pairs. The number of sequences decreased to 821612 after *screen.seqs*. The total number of sequences declined further as we removed unwanted, ambiguous and duplicated sequences. The total number of sequences gradually declined to 356249 in the end, with the longest sequence having a chain length of 636 base pairs. On alignment with the reference database silva.v4.fasta, most of the sequences aligned starting at base pair location 638. The analysis was further conducted by clustering the samples into operational taxonomy units (OTUs) and classifying them.

Diversity Analysis

Alpha diversity analysis

The command *summary.single* showed different diversity metrics that indicated the species diversity and richness in the samples (Table 2).

Table 2: Diversity indices of the control and chromium contaminated samples

Sample	Chao	Simpson	Shannon
ChromiumAS1	3945.778	0.592248	1.841371
ChromiumAS2	2953.97	0.213452	3.036724
ChromiumAS3	961.2419	0.278226	3.126684
ChromiumAS4	839.8261	0.033787	4.356874
ChromiumAS5	7672.648	0.014806	5.572186
ChromiumS1	48263.94	0.00203	7.74438
ChromiumS2	5309.645	0.002695	6.82159
ChromiumS3	42452.19	0.002428	7.539418
ChromiumS4	38661.15	0.005417	7.305137
ChromiumS5	51454.69	0.001781	7.841346
ChromiumS6	43433.49	0.005209	6.962666
ChromiumS7	39666.46	0.005297	7.55263
ChromiumS8	49367.84	0.003491	7.606628
ChromiumS9	49650.59	0.001852	7.822735
Control01	5707.387	0.036701	5.5797
Control02	4952.492	0.059163	5.285571
Control03	5505.077	0.041767	5.465372
Control04	6047.503	0.025453	5.767816
Control05	8176.775	0.022387	6.058845
Control06	10200.18	0.016929	6.131441

Shannon index indicated the richness and evenness and according to results, in anaerobic sludge site highest value was 5.57219 of sample ChromiumAS5 and lowest value was of sample ChromiumAS1. In soil site, highest value was of ChromiumS5 sample and lowest value was of ChromiumS2 sample. Chao indicated the total richness in each sample, the results showed that in anaerobic sludge site the sample ChromiumAS1 had the highest

value and ChromiumAS4 showed the lowest value. While in soil site, ChromiumS5 was the sample having highest value and ChromiumS2 showed the lowest value. Simpson showed the diversity of the bacteria, and in anaerobic sludge the sample ChromiumAS3 had the highest diversity and ChromiumAS5 had the lowest. While in soil, ChromiumS4 sample showed highest diversity and ChromiumS5 showed the lowest value.

Beta diversity analysis

In diversity analysis, different tests were used to find the similarities and differences between the samples. In Heatmap analysis, shades of red and black colors showed that whether the samples were more similar or dissimilar (Fig. 1).

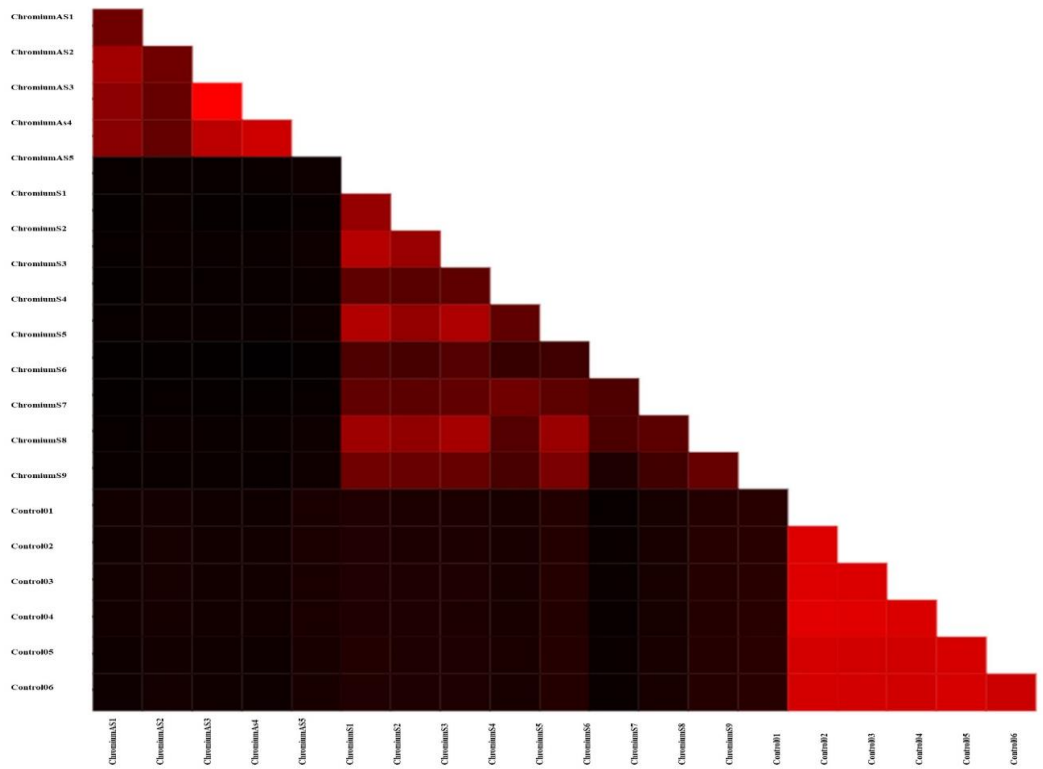

Fig. 1. Heatmap of the control and chromium contaminated samples

Brighter red shades indicated high similarities between the samples whereas darker shades indicated higher differences between the samples.

According to results, samples of anaerobic sludge showed higher dissimilarities as compared to the samples of soil. Where Cr-contaminated soil samples showed dissimilarities when compared to the control samples. Venn diagrams were created based on a four-way analysis of samples to determine the number of sequences common in different samples (Figure 2). The similarity among bacterial diversity was represented by nine different Venn diagrams each comprising of 4 random samples. The overlapping circular areas in each Venn diagram indicated the number of OTUs common in those samples. 129 OTUs were found to be common in ChromiumAS5 and ChromiumAS3 samples, and 48 OTUs were common in ChromiumS6 and ChromiumS9 samples. No common OUT was found among ChromiumS6, ChromiumAS3 and ChromiumAS5 samples (Figure 2). Similarly, 220 OTUs were found common in Control01 and Control04 samples. Overlapping of Control04, Control01 and ChromiumAS3 7 common OTUs. When Control04, ChromiumS6 and ChromiumAS3 samples were compared, no common OTUs were found (Fig. 2).

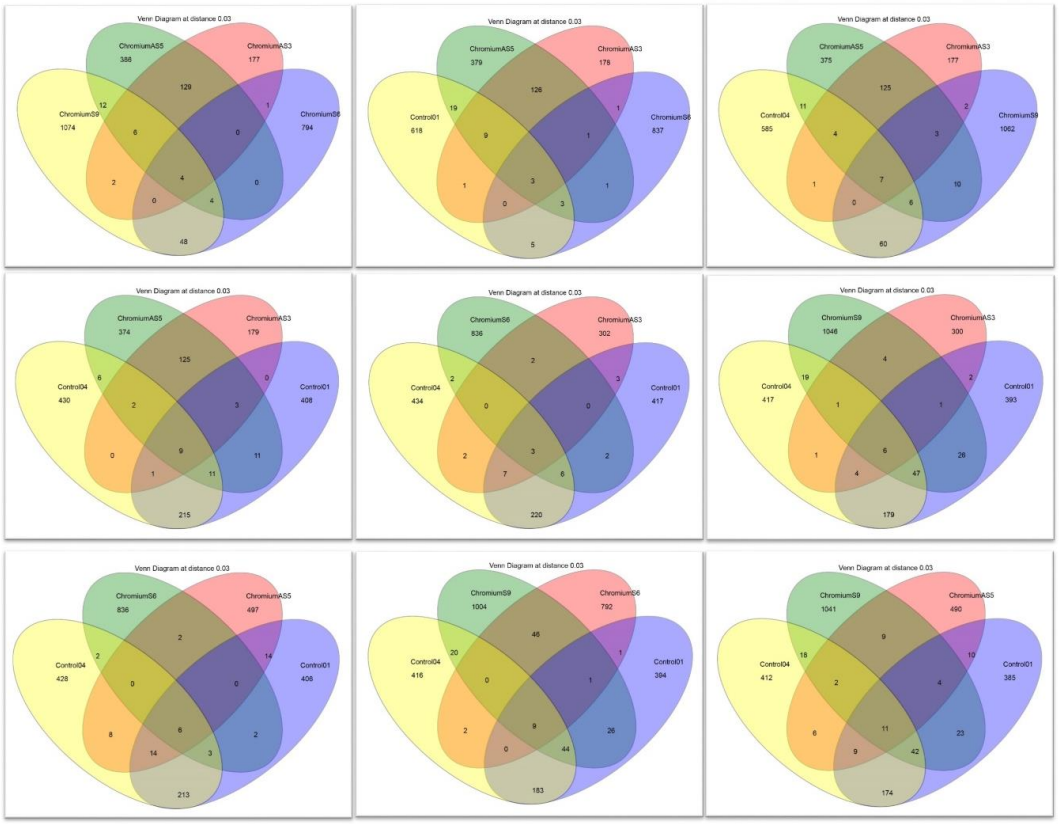

Fig. 2. Selected Venn diagrams showing common and exclusive number of sequences in different samples

A phylogenetic tree was generated to determine how the samples group with each other based on the similarity of the sequences (Fig. 3).

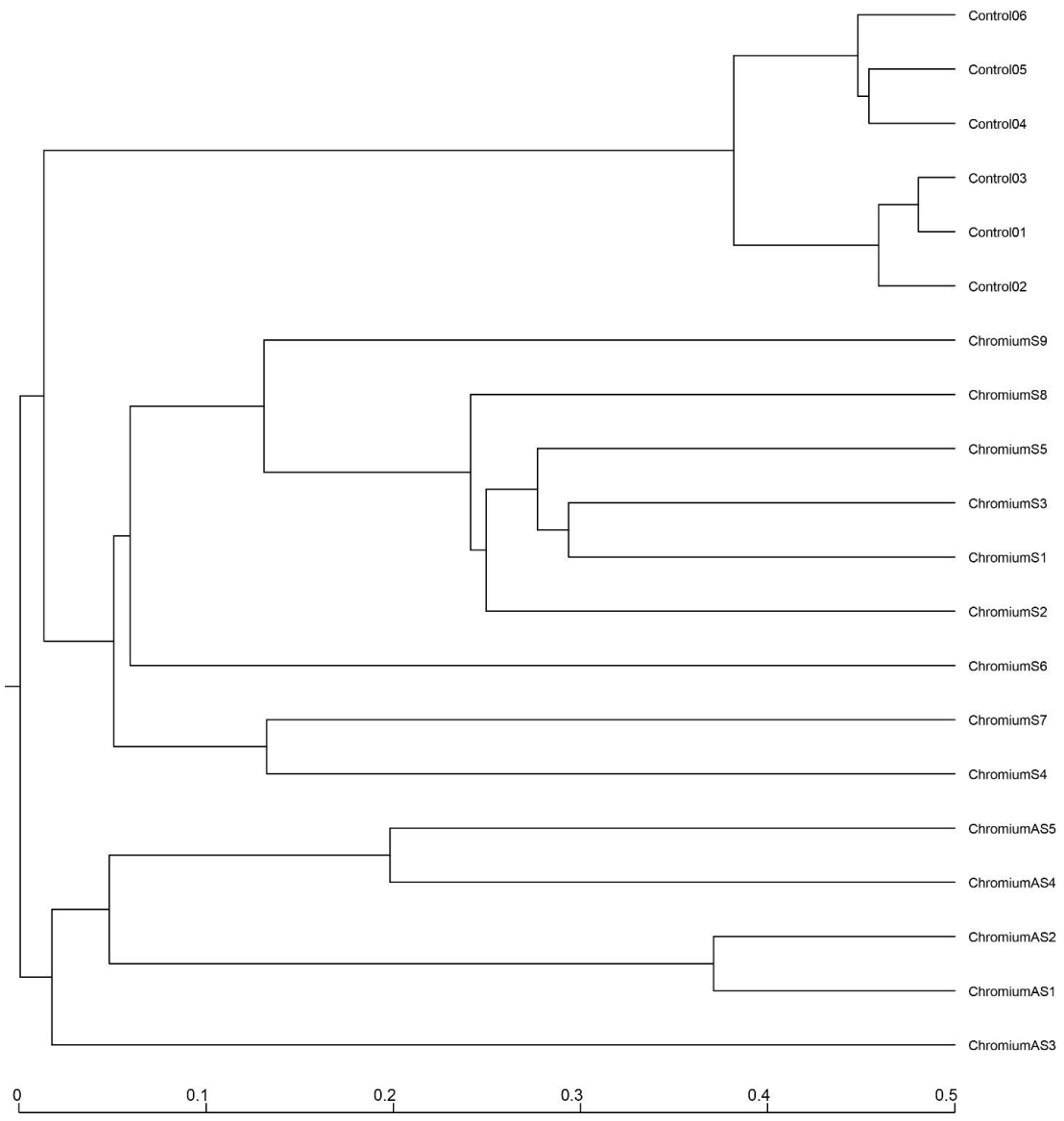

Fig. 3. Neighbour-joining Phylogenetic showed grouping of the samples based on their sequences similarities

The samples ChromiumS1, ChromiumS5, ChromiumS3 and ChromiumS8 showed more similarities with each other as compared to other samples.

Krona pie chart Analysis

Krona pie chart was used to visualize the microbial composition of the samples (Fig. 4).

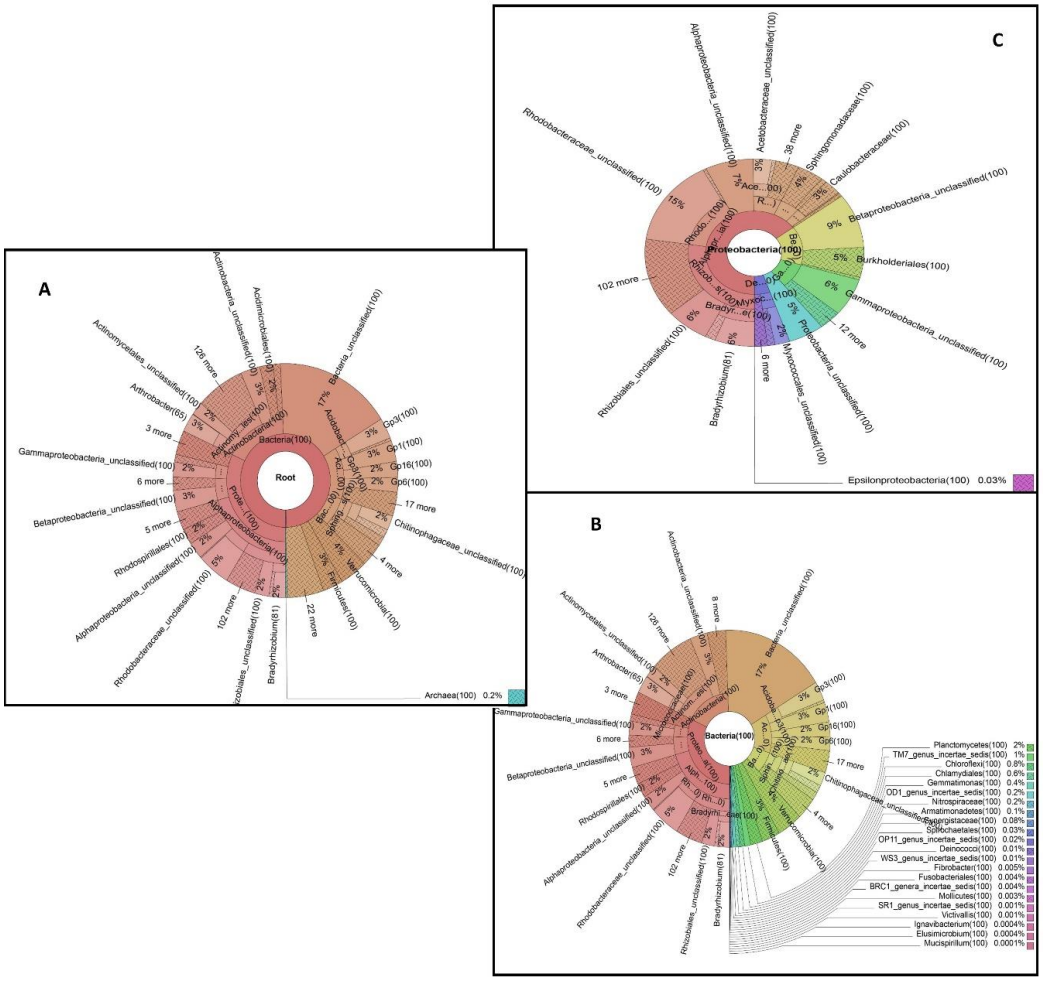

Fig. 4. Krona pie charts showing proportions of different microorganisms in the samples. **A:** the percentages of all the microorganisms, **B:** the percentage of different bacterial groups, **C:** the percentage of different groups of proteobacteria

In krona pie chart, total number of sequences were 703092 out of which 701597 sequences (99.8%) were classified as bacterial sequences, and 1495 sequences (0.2%) were classified as archaeal sequences. In the bacterial domain, 224779 (32%) classified sequences belonged to *Proteobacteria* (7% unclassified sequences of Alphaproteobacteria, 9% unclassified

sequences of Betaproteobacteria, 6% unclassified sequences of Gammaproteobacteria, 1% unclassified sequences of Deltaproteobacteria). Unclassified sequences of *Acidobacteria* were 24%, *Actinobacteria* were 3%, and *Bacteriodetes* were 2%. Acidimicrobiales classified sequences were 2%. *Arthrobacter* classified sequences were of 3%. Unclassified *Rhodobacteraceae* sequences were 5% while classified *Planctomycetaceae* sequences were 2%.

Relative abundance

Relative abundance was determined by using biom file and was visualized in Phinch software (Fig. 5).

Fig. 5. Relative abundance of different bacterial groups in the control and chromium contaminated samples

TableAccording to it, different Cr (anaerobic sludge, control and soil) samples differed by the percentages of bacteria present in them. Figure 5 represents the percentage and relative abundance of major bacterial taxa found in the samples.

The *Actinobacteria* were present in all the samples but were most abundant in ChromiumAS3 and control samples. *Proteobacteria* were present in all samples but were most abundant in ChromiumAS2 sample. *Acidobacteria* were present in all soil and control samples but were not detected in anaerobic sludge samples. *Firmicutes* were also found in all the samples but were most abundant in the sample ChromiumS7. Another bacteria *Verrucomicrobia* was present in samples of anaerobic sludge and soil but was found in very less numbers in control samples. *Planctomycetes* bacteria was abundant in soil samples but it was either absent or in very few numbers in anaerobic sludge and control samples. High abundance of *Planctomycetes* was found in ChromiumS9 samples. *Chloroflexi* was one of the bacteria which were present in all samples, but in some samples it was found in very numbers while in other samples it was found in large proportions. In anaerobic sludge ChromiumAS4 sample, *Chloroflexi* was abundant while the least amount of this bacteria was found in soil samples (ChromiumS3 and ChromiumS6). In some samples the presence of *Bacteriodetes* was abundant while in others these bacteria were found in very low numbers. In soil sample ChromiumS3, *Bacteriodetes* was found in abundance.

Firmicutes, *Planctomycetes*, *Verrucomicrobia* and *Bacteroidetes* were present in very low numbers in control, while were abundant in all Cr-contaminated samples. Relative abundance of *Acidobacteria* and *unclassified Bacteria* in control and Cr contaminated sites were almost similar.

Statistical analysis

Statistic tests AMOVA, HOMOVA and Parsimony were performed. When AMOVA was applied on all the samples, and when comparing samples of any two sites at a time, every time the p-value obtained was <0.001, indicating that the difference between the samples was statistically significant.

In HOMOVA, the results showed that the p-value was less than 0.01 which determined it as significant. It showed

that all the samples were different to each other. Parsimony was a general test for comparing communities with similar structures. It could only specify the probability of groups having the same structure but did not indicate the level of similarity.

DISCUSSION

Exposure to high concentrations of Cr can alter the physiological and biological functioning of living organisms (Urbano et al., 2012). Metagenomics is an advance technique by which we can extract total DNA of microbial communities directly from the sample without culturing the microbes. In order to determine microbial diversity of Cr contaminated sites in this comparative metagenomic study, 16S rRNA sequence data was used and analyzed through Galaxy server (Kozich et al., 2013). 16S rRNA sequence data was chosen as it is both highly conserved and has variable regions as well, and huge reference databases are available. All these samples had been sequenced via Illumina sequencing technology. Next generation sequences (NGS) techniques are very strong and effective tools to find out comprehensive information of complex prokaryotic communities.

In this analysis, Silva reference file was used for alignment of the contigs of 16S rRNA genes, whereas RDP based database file was used for the purpose of classification of OTUs. Diversity analysis was performed in two ways: *alpha diversity* (number of species coexisting within a local site) and *beta diversity* (the magnitude of similarity in species composition among different sites) (Zhou et al., 2020). In heatmap analysis, the brighter shade of red color showed more similarities between samples while the darker shade showed more dissimilarities. According to this graphical representation, number of bacteria which are more dissimilar were abundant among the samples, while a few samples showed similarities of bacteria. Neighbour joining Phylogenetic tree was made to find out similarities and dissimilarities among the samples. According to this analysis, the samples that grouped together were more similar e.g., ChromiumAS3, ChromiumAS1 and ChromiumAS2 were more similar to each other and dissimilar to the other samples and control. Diversity indices such as Chao, Simpson and Shannon were also determined. Chao indicates the total richness of microbial communities,

Shannon indicates the richness and evenness while Simpson shows the diversity. To further analyze the data, relative abundance graph was generated and visualized in Phinch software. It was found that although the relative abundance of bacteria varied from sample to sample, but similarities were also present.

In one of the previous studies about Cr contaminated soil, Actinobacteria and Proteobacteria (Alphaproteobacteria and Gammaproteobacteria) were found to be the important phyla showing resistant to Cr toxicity at different contamination levels. *Lactobacillus*, *Pseudomonas*, *Nitrospira*, *Clostridium*, *Bacillus*, and *Escherichia* were found to be the dominant genera in active mining areas (Pradhan et al., 2020). While according to another study *Ochrobactrum* sp. and *Microbacterium* sp. were rich in abundance (Kao et al., 2021). According to our results of krona pie chart, *Proteobacteria* (Alphaproteobacteria, Betaproteobacteria,

Gammaproteobacteria) were abundant in all samples of Cr contaminated sites and less in control. Most of the studies showed *Pseudomonas* is one of the best potential microbe for the bioremediation of Cr. As Arisah et. al. (2021) stated that *Pseudomonas* showed high efficiency of Cr (VI) removal, up to 85%. Another study by Gong et. al. (2020) investigated Cr removal efficiency of *Pseudomonas* sp. and according to this study the bacteria was able to resist high concentrations of Cr. Under the optimal conditions, the removal rate of Cr was 94.26% in contaminated soil. One of the research by Sousa et. al. (2023) evaluated bioremediation of Cr by *Rhodobacter* sp. According to the results of this study, under optimal conditions this bacteria showed complete removal of hexavalent Cr. Rajyalaxmi et al. (2019) reported the potential of the photosynthetic bacterium *Rhodobacter* to remediate hexavalent Cr. According to this study, *Rhodobacter* sp. showed reduction of Cr(VI) up to 35 µM on 8^{th} day of its incubation under anaerobic light conditions. Some researchers have also reported the presence of Rhizobiales in Cr contaminated soil (Araujo et al., 2023). Relative abundance bar graph in our study showed that samples of Cr contaminated soil had higher abundance of Proteobacteria, Acidobacteria and Bacteroidetes, while Actinobacteria, Verrucomicrobia, Planctomycetes, Chlamydiae, Gemmatimonadetes,

Nitrospira, Firmicutes and Armatimonadetes were found in less abundance.

According to one of the studies about microbes present in heavy metal contaminated anaerobic sludge, Proteobacteria, Firmicutes, Bacteroidetes, and Thermotogae were observed in high percentage as compared to other species of bacteria (Lim et al., 2017). In our study, anaerobic sludge sites showed that Proteobacteria and Actinobacteria sp. were highly abundant. while Bacteroidetes, Firmicutes, Acidobacteria, Verrucomicrobia, Terenicutes and Planctomycetes were present in lower proportions.

CONCLUSION

According to this study, *Chloroflexi*, *Firmicutes, Planctomycetes, Gemmatimonadetes, Verrucomicrobia* and *Bacteroidetes* were present in less numbers in control, while more in all Cr-contaminated samples. *Actinobacteria* were abundant in control while less in all other groups. Relative abundance of *Acidobacteria* and *Proteobacteria* in control and other Cr contaminated sites was almost similar. Bacteria which are abundant in Cr contaminated sites might have some role to play in detoxification of Cr. There is also strong need to focus more on functional genes of these bacteria related to Cr detoxification in order to find out better Cr remediation solutions.

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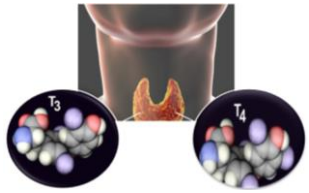

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Diversity of Human Skin Microbiota in Healthcare Workers of South Punjab, Pakistan during COVID-19

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ABSTRACT: *Human skin microflora plays important role in the functioning of skin and is modulated by several intrinsic and extrinsic factors including hygiene practices. During Covid-19 pandemic, focus has been particularly directed towards improving hygiene. We explored the skin microflora of healthcare workers in local population of Multan, Pakistan. A total of 44 samples of skin were collected from healthy workers along with the administration of questionnaire regarding hygiene practices. After isolation, bacteria were characterized by morphology, staining and biochemical tests. Majority of the workers was 20-30 years old females. Most of them reported to wear gloves during practice, wash hands 8-10 times a day, use sanitizer 8-10 times a week and took bath 12-15 times a month. Isolated microflora (n=110) included Staphylococcus aureus (62%), Escherichia coli (16%), Pseudomonas spp. (9%), Proteus spp. (5%), Enterobacter spp. (5%) and Klebsiella spp. (3%). Presence of pathogens, although in small numbers, emphasizes the necessity of disseminating knowledge regarding adoption and maintenance of hygienic practices, specifically among healthcare workers.*

Keyword: Skin microflora; Healthcare workers; Hygiene; Covid-19; Microbiome

INTRODUCTION

Skin is the largest organ of human body and it provides protection against the external environment. Microorganisms inhabiting the skin are considered to be of great interest by the researchers as they play major role in shaping the

host's immunological responses towards environmental factors. There is a wide variety of microorganisms inhabiting our skin which make up natural microbiota. It is also called normal or residential flora of skin. Surprisingly, human skin has been proven to be unsuitable environment for bacterial growth (Bojar and Holland, 2002). Still many bacterial species successfully inhabit skin among which, commensals are the dominant resident species on skin (Barnard, 2017). This human microbiota plays major role in modulating the immune system by acting as a barrier against the foreign pathogens. In humans, immunological responses are frequently associated with the microorganisms living on skin (Skowron et al., 2021). Skin microbiota colonization is influenced by several factors related to an individual's lifestyle choices such as occupation, kind of clothes, use of antibiotics etc (Callewaert et al., 2020). Many opportunistic microorganisms can also live on the skin and they can cause severe infections of skin by evading the skin barrier (Bay et al., 2020). Bacteria capable of residing on skin are classified into four phyla including Actinobacteria, Firmicutes, Bacteroidetes and Proteobacteria. Among these, the most dominant opportunistic microorganisms include *Staphylococcus* spp. (Peng and Biswas, 2020).

Several culture-based techniques and molecular methods are being used for detection of the skin micro flora predominantly including 16s rRNA sequencing which is the most reliable technique used for skin microflora detection. However, it is limited, to some extent, by no possibility of differentiating between the resistant and non-resistant strains of bacteria (Maroniche et al., 2017). By using traditional culturing techniques, *Staphylococcus* spp. and *Corynebacterium* spp. have been revealed as the most abundant bacterial species present on moist parts of skin (Emter and Natsch, 2008). Besides the bacterial species, fungal communities can also survive on skin but their population does not fluctuate with physiological changes in comparison with the bacterial communities (Wu et al., 2020). The antimicrobial resistance (AMR) acquired by the microflora residing at one place may be transferred to other places via human transportation. Many pathogenic microorganisms have been implicated in fatal infections owing to multidrug resistance (MDR)

genes. Those multidrug resistant microorganisms are referred to as superbugs due to their AMR in opposition to multiple drugs which were originally developed to fight them (Davies and Davies, 2010). MDR microorganisms thrive successfully in our surroundings and cause severe health issues to both human beings and animals.

Particularly exposed in this regard are the healthcare workers, who are at great risk of encountering the pathogens in their daily routine (Cho and Blaser, 2012). Those infections which occur during the healthcare are specifically developed either in hospital or another healthcare facility within 48 hours of admission. It is also possible that they may occur at home within 30 days after receiving the healthcare. In all circumstances, these are known as healthcare associated infections (HAIs) (Revelas, 2012). The Agency for Health Care Quality and Research concluded that HAIs are the major and common complications faced in hospital care and are included among the top 10 causes of mortality in USA (Haque et al., 2018). Healthcare sector is, therefore, facing huge threat in the form of hospital acquired infections. During COVID-19 epidemic, because of contact with infected patients and contaminated equipment, the health care workers were on the front line of vulnerability towards getting the infection. MDR pathogens have been widely distributed in various ecological niches in the hospital environment, as reported previously (Cruz-López et al., 2023). Among commonly found skin microbiota of healthcare workers isolated from their cell phones, resistance has been reported against the most commonly used antibiotics to treat skin infections highlighting the prevalence of MDR pathogens among skin microflora of these workers (Banawas et al., 2018).

Some hygiene conditions such as regular hand washing and using the sanitizer haven been recommended and employed for the purpose of prevention (Bowdle and Munoz-Price, 2020). It has been proven by many research groups that improving hand hygiene conditions can lessen the chances of developing HAIs. During the COVID-19 epidemic, hand hygiene conditions have been reported to be associated with decreased rate of HAIs (Roshan et al., 2020). Healthcare workers must consider maintaining the hand hygiene conditions as an important responsibility because they are well-taught about the risks of causing infections if they do not take it

seriously. The present study was conducted to analyze the composition of skin microflora of healthy healthcare workers and to ascertain the hygiene practices adopted by them.

Methodology

Data Collection

This perspective study involved 44 skin samples of healthy healthcare workers belonging to urban as well as rural population of Pakistan employing non-random sampling technique. The samples were taken from the Bakhtawar Amin hospital, Multan and Nishtar Hospital, Multan. They were processed in the Microbiology Laboratory of The Women University, Multan. All healthcare workers were administered a questionnaire to fill and provide consent to participate in this study. Informed written consent was taken from individuals whose sample were taken and they were guided to fill these questionnaires. All the samples were distributed in three different age groups including; group 1 of 20-30 years old, group 2 of 30-40 years, group 3 of 40-50 years old persons. Males and females were equally distributed among these three categories of age. All the processes involving collection and processing of samples were in compliance with the declaration of Helsinki.

Questionnaire Administration

Questionnaire included questions relevant to their age, gender, occupation and the hygiene practices that are being followed during the duty hours. As described above, respondents were randomly selected to view the consent scenarios and were asked “Would you consent to share your samples and information with researchers in such manner”. After viewing the consent scenario, they said they would share samples and data for this study. This suggests that considering some of the potential risks and benefits of participation may inform and influence people’s decision to take part in the study.

Sample Processing

The standard Nutrient agar medium was prepared and poured in the autoclaved petri plates. All skin samples were processed on Nutrient agar plates by swabbing technique and incubated at 37^{o}C for 24 hours. After 24 hours, diversity in the skin microflora was recorded by observing the growth of

different colonies on media plates. Morphological identification of isolated bacterial strains was done by following the colony morphology chart that includes the color, shape, elevation, texture and margin of the colonies. The selected isolates were then purified by quadrant streaking method. Gram staining was subsequently done to differentiate between gram-positive and gram-negative isolates on the basis of their staining characteristics. Gram-positive bacteria retain the primary dye crystal violet that stains their walls purple and gram-negative bacteria retain the secondary dye safranin that stains the bacteria pink. Results of microscopy were recorded subsequently.

Biochemical Characterization

Following the Bergey's Manual of Systematic Bacteriology, identification of isolated bacteria was done with the help of biochemical tests including catalase test, oxidase test, triple sugar iron (TSI) test, citrate test, coagulase test and indole test. In order to perform all of these tests, 18-24 hours fresh culture was used as prescribed. Specifically, for gram negative bacteria, oxidase, glucose fermentation and indole tests were performed. On the other hand, for gram positive bacteria, catalase, TSI and coagulase tests were performed. After performing these tests, different species isolated from skin in this study were identified. The bacterial diversity of skin microflora was expressed as relative abundance (%) among the skin microflora community.

Results

Sample Characteristics

A total of 44 swab samples from skin of paramedical staff working in hospitals and microbiology labs were collected for conducting this study along with administration of a questionnaire which included questions related to age, gender, and occupation as well as about the hygiene conditions and practices during the Covid-19 pandemic. Majority of the samples taken belonged to group 1 which included 20-30 years old individuals followed by group 2 and 3 (Fig. 1). They were mostly lab workers followed by clinical assistants and doctors. A vast majority (73%) of them were females. The least prevalent were the pharmacists and only few were males.

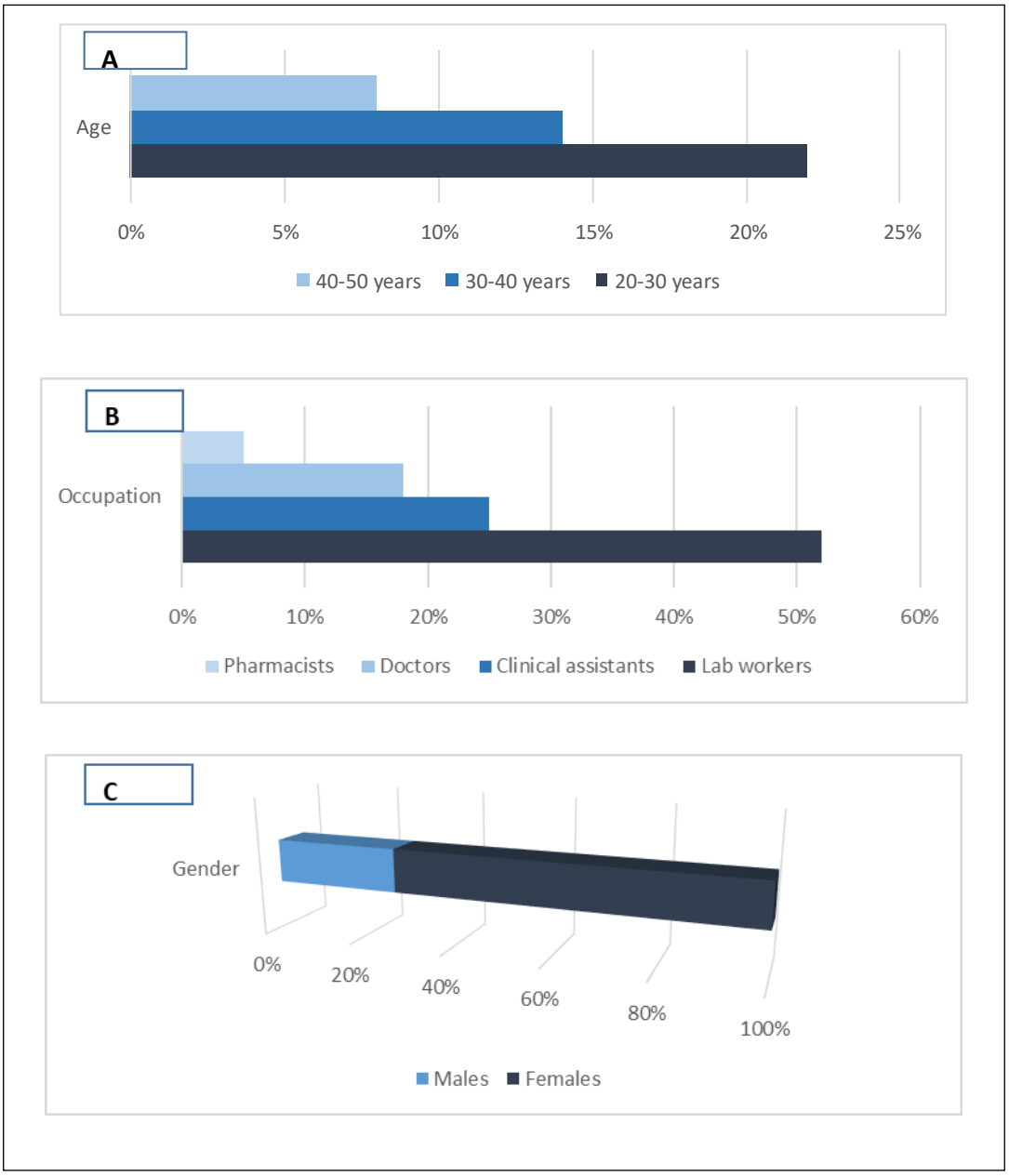

Figure 1: Baseline Characteristics of the Sample Population including A) Age, B) Occupation and C) Gender

Hygiene Practices

In addition to this information, other questions regarding their hygiene practices were asked such as the frequency of washing hands, frequency of bathing, frequency of using sanitizers and whether or not they were wearing gloves during the working hours

(**Figure 2**). They reported a change in hygiene practices during pandemic era with regard to the frequency of all factors. For instance, there was a high frequency of washing hands which was at least 8-10 times per day, exactly. There was high rate of bathing i.e. 12-15 times per month at the minimum. The use of sanitizer per week was not as high as it should have been or as expected. It was 8-10 times per week. In addition, 73% of the healthcare workers used to wear gloves while serving the community whereas, the remaining 27% did not.

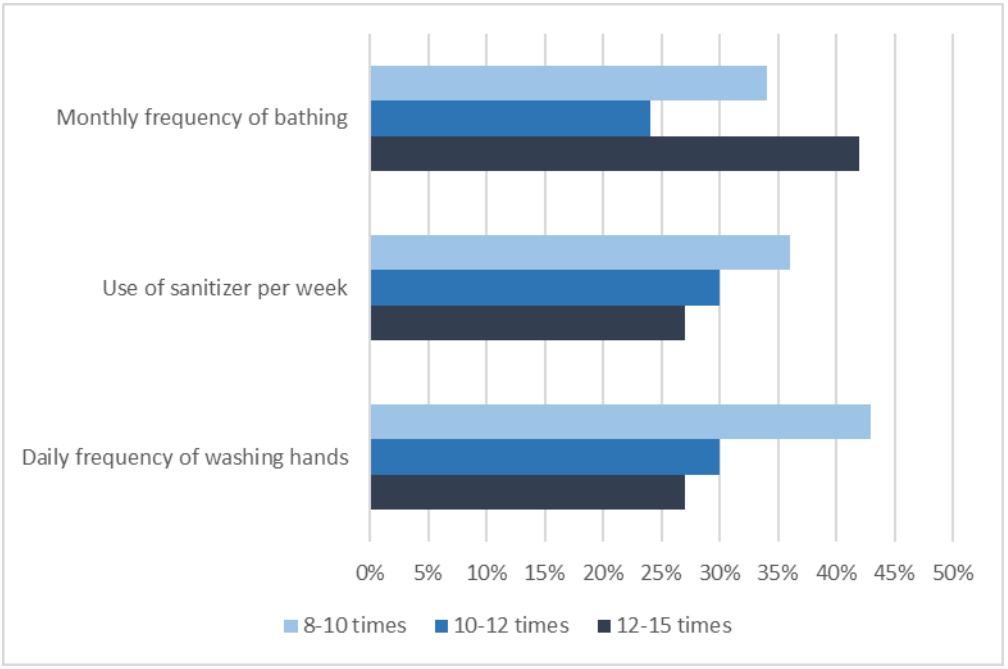

Figure 2: Basic hygiene practices during Covid-19 among healthcare workers

Gram Staining

Individual cells of isolated strains of bacteria were studied by gram staining technique and the data was recorded. Gram staining helped in distinguishing 110 isolated strains into two groups of gram positive and gram negative bacteria. It was revealed that majority of the isolated bacteria were gram-positive. In total, there were 67 gram positive and 43 gram negative isolates. The results are mentioned as percentage in Fig. 3.

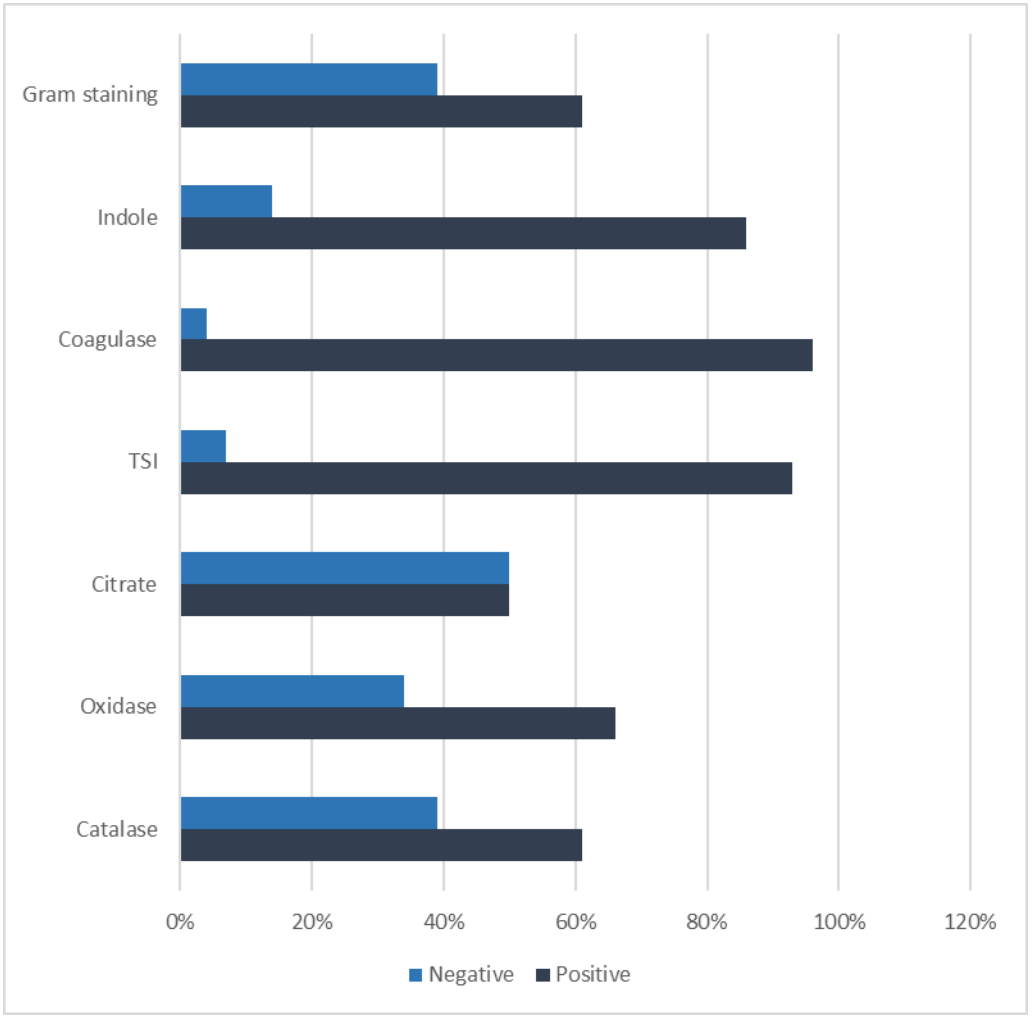

Figure 3: Results of Biochemical tests and Gram Staining for Skin Microflora of Healthcare Workers

Biochemical Characterization

Various biochemical tests were performed in order to identify the bacterial isolates up to species level in accordance with the scheme provided by Bergey's manual of Systematic Bacteriology. Biochemical characteristics of the isolated bacterial strains were observed and the data was recorded. 94 isolates were Indole positive and 16 isolates were Indole negative; 105 isolates were coagulase positive and 5 isolates were coagulase negative; 102 isolates were TSI positive and 8 isolates were TSI negative; 72 isolates were oxidase positive and 38 isolates were oxidase negative; and, 67 isolates were catalase positive and 43

isolates were catalase negative. For citrate utilization test, half (55) of the isolates produced positive and remaining half (55) gave negative test result. The results are mentioned as percentage in **Figure 3**.

Prevalence of Skin Microflora

To understand the diversity of skin microflora that prevails among healthcare workers working in clinical environments, a comparative study was done among healthy healthcare workers serving in the labs and hospitals. Out of a total of 110 bacterial strains which were isolated and identified based on the results obtained from different biochemical tests, 68 species were of *Staphylococcus aureus*, 17 species were of *Escherichia coli*, 9 species were of the genus *Pseudomonas*, 5 species were of the genus *Proteus*, 5 species were of the genus *Enterobacter* and, 3 species were of the genus *Klebsiella*. The results are mentioned as percentages in **Figure 4**.

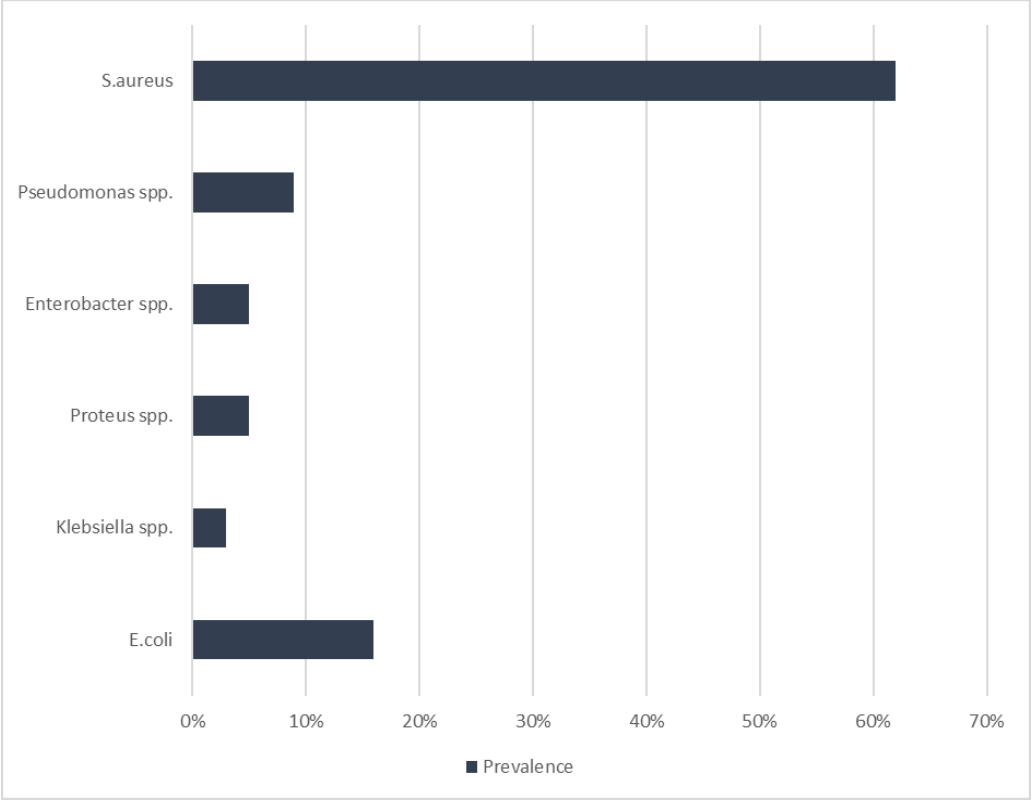

Figure 4: Composition of Skin Microflora of Healthcare Workers

Discussion

Skin is considered as an ecosystem of large number of microbial communities

which play a critical role in maintaining health and immune regulatory functions of the skin (Hoffmann, 2017). During COVID-19 pandemic the health care workers working in hospitals and diagnostic labs were at great risk of getting health-care associated infections (HAIs). The current study is the novel one in the aspect that it investigated the culturable skin microflora of the health workers during COVID-19 pandemic who were following WHO recommended hand hygiene practices which included; hand washing and hand sanitizing. The findings suggested that the frequency of the pathogenic microorganisms was significantly reduced. This is possibly due to strict compliance to guidelines pertinent to periodic hand washing with antibacterial soaps, frequent use of hand sanitizers and bathing after dealing with the infected patients (Christopher et al., 2020; Daye et al., 2020; Esther et al., 2022). The regime had been followed because several studies have shown that, by following specifically the hand hygiene rules, chances of infection can be reduced at a greater level since unhygienic conditions may lead to several diseases (McDonald et al., 2021). Various studies have shown that subungal parts of hands include large number of gram-positive pathogenic bacteria, mostly *Corynebacterium, Staphylococci* and yeast (Nieradko-Iwanicka, 2020). Paramedical staff have high number of pathogens in their subungal areas, and because of this they are asked strictly to follow the hand hygiene strategies. The skin samples collected from the healthcare workers working in the hospitals and diagnostic labs included both gram-positive and gram-negative bacterial species of *Klebsiella* (3%), *Proteus* (5%), *Enterobacter* (5%), *Pseudomonas* (9%), and *E. coli* (16%) while the most abundant strain isolated was *S. aureus* (62%). Similar results were found out in a randomized clinical trial on university students after handwashing with water, plain soap and alcohol-based hand sanitizers (Zefenkey, 2021).

A reduction in number of bacterial isolates from skin of healthcare workers was observed when they followed the hand hygiene especially in those who were working in the diagnostic labs. Frequency of pathogenic bacteria isolated from volar forearm of paramedical staff which can cause fatal infections was 10% in our study. Furthermore, questionnaires about hand hygiene and other hygienic conditions were given to the healthcare workers

working in hospitals, diagnostic labs and pharmacies. According to the data, most of the individuals used 36% hand sanitizer 6 to 8 times, 37% used 4 to 6 times per day and 27% used 2 to 4 time per day. Among all of these self-hygiene regimes, frequencies of using hand sanitizers, which was 36% (6 to 8 times), was found out to be the most accurate to avoid contamination. In another study done on 24 primary schools in Dhaka, Bangladesh, it was shown that the incidence of influenza was 53% reduced in the group who cleansed their hands with hand sanitizers as compared with the controlled group (Biswas et al., 2019).

Hand washing was another hygiene condition asked in the questionnaire which concluded that 27% individuals washed their hands more than 12 times per day, 30% individuals 10 to 12 time a day, whereas 43% washed their hands 8 to 10 time a day. These frequencies proved that healthcare workers follow the hand hygiene conditions regularly to prevent themselves from infections during the COVID-19 pandemic. A similar study was also done on 896 Indonesian citizens over 18 years old which showed that 82.32% of females and 73.37% males reported handwashing practice 8 times or more per day during COVID-19 pandemic (Dwipayanti et al., 2021).

Several reports have previously highlighted the effect of hygiene practices on prevalence of resistant pathogens, particularly MDR pathogens among microflora of humans during COVID-19. For instance, a study reported overall 35% decrease in the proportion of MDR bacteria, 41% decrease in MRSA, and 21% decrease in ESBLs in the post-COVID time as compared to pre-COVID pandemic (Cole & Barnard, 2021). Another study reported that during COVID-19, 4 times increase in the demand of hand sanitizers was observed and consequently, compliance to hand hygiene practices doubled from December, 2019 to April, 2020. Correspondingly, during the same time period, 50% decline in the prevalence of MDR pathogens among HAIs was noticed (Roshan et al., 2020).

The most important aspect of infection prevention is hand hygiene. It is the responsibility of healthcare centers to maintain the hand hygiene regulations. Paramedical staff, nurses, physicians and healthcare professionals throughout the world must get ready to inculcate the efficient, simple and fundamental practices of hand hygiene during their

daily routine for serving as a reference model for succeeding generations.

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Conflict of Interest

None

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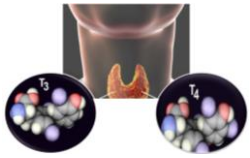

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Brief analysis of Therapeutic Approaches of Type 1 Diabetes Mellitus along with Diagnosis and Screening Methods

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ABSTRACT: *Diabetes is an endocrine system disease which is characterized by abnormal elevated glucose levels. Type I Diabetes Mellitus (T1DM) is an autoimmune disorder with early onset, whereas Type 2 Diabetes Mellitus (T2DM) is non-autoimmune form with late onset. Small and large artery complications are the two main categories of diabetes mellitus long term complications. Overproduction of superoxide by the mitochondrial electron transport chain (ETC), leading to oxidative stress, occurs because of pathogenic effects of hyperglycemia. New vessels are fragile and hyper permeable in case of retinopathy in T1DM. T1DM is known to be occurred by beta cell destruction which leads to hyperglycemia and insulin scantiness. In phase 3 T1DM is normally diagnosed, the stage at which the disorder has led to life threatening condition known as diabetic ketoacidosis. To minimize the possibility of serious complication it is necessary to diagnose autoimmunity which is present during first years of life through early screening or by using diagnostic tools. Measuring fasting blood glucose or standard OGTT's are performed for screening of phase 2 T1DM in the persons which have 1 or more autoantibodies targeting β-cell. The management of type 1 diabetes mellitus is necessary to encourage healthy lifestyle and to control glycaemia conditions in order to avoid severe complication. Pharmacological approaches are the most widely used method for the treatment of T1DM including injectable insulin and sodium glucose cotransporter 2 (SGLT2) inhibitors, Gene therapy and stem cell-based therapies. These are supposed to help in providing life-time freedom from T1DM but there is still a room for debate in this regard.*

Keyword: *Autoimmune disorder, Ketoacidosis, Autoantibodies, Gene*

INTRODUCTION

Diabetes is an endocrine system disease which is characterized by abnormal elevated glucose levels. It is among the most common disease. It is estimated that by 2045 diabetes will affect about

693 million adults globally (Cho et al., 2018). It is a chronic metabolic disease which is the consequence of complete or relative deficiency of insulin. It is divided into two main types: Type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). T1DM is an autoimmune disorder with early onset, whereas T2DM is non-autoimmune form with late onset. Besides this classical classification there are subtypes too which includes monogenic diabetes (may be neonatal diabetes or maturity- onset diabetes of the young MODY), gestational diabetes, and latent autoimmune diabetes (Ahlqvist et al., 2018). T1DM is due to the T-cell mediated self-destruction of β cells (insulin secreting islet) present in the pancreas (Liu et al., 2016) T1DM etiology is complicated. Environment is also found to have a critical role in development of T1DM along with genetics (Jerram and Leslie, 2017). Many factors play a crucial role in type 1 diabetes mellitus progression as shown in Fig. 1. With advancement of technology and continuous efforts of researchers, genes playing a crucial role in T1DM development have been identified. By using gene therapy approach, manipulation of these genes can provide a more comprehensive management of the disease or ever treat T1DM (Cole and Florez, 2020). Stem cell therapy provides a new outlook for T1DM treatment and it can overcome many shortcomings of conventional therapies. They promote the repair as well as regeneration of β cells. But the stem cell therapy has numerous hurdles in its way as tumor genesis, and autoimmune attack (Zhou et al., 2022).

Polydipsia, polyphagia, weight loss, and blurred vision are some common symptoms of hyperglycemia. However, many long-term complications can be the consequence of uncontrolled diabetes.

Long Term Complications

Insulin therapy is shown to be involved in decreasing the likelihood of ketoacidosis and several other metabolic diseases linked with T1DM (Nathan et al., 2014). Microvascular and macrovascular complications are the two main categories of diabetes mellitus long term complications. Neuropathy, retinopathy, and nephropathy fall into the microvascular complications associated with diabetes.

Macrovascular complications demonstrate cerebrovascular disease peripheral artery disease, and coronary

heart disease. These are not only limited to diabetes and can occur due to several other reasons however the individuals suffering from T1DM can develop these conditions.

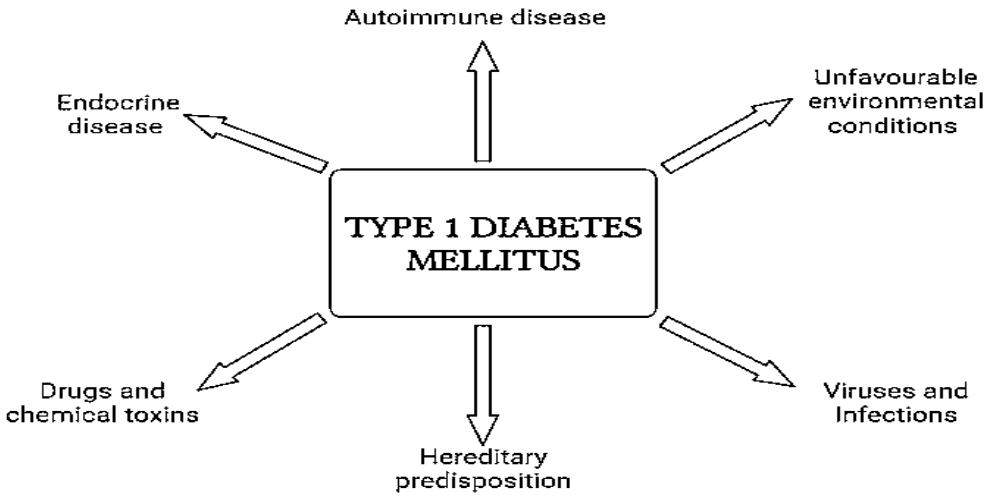

Fig. 1. Different factors shown to have a role in T1DM development (image is created Bio render)

Diagnosis

T1DM is known to be occurred by beta cell destruction which leads to hyperglycemia and insulin scantiness. Rapid appearance of hyperglycemia symptoms includes weight reduction, polydipsia, abdominal symptoms, headaches, and ketoacidosis that normally occur in young children (Care, 2019). More than 95% of recently diagnosed diabetic patients look for medical assistance and help due to appearance of symptoms while a few numbers of diabetic patients are diagnosed and identified by routine screening of glucose levels or through the autoantibodies detection. A diagnostic criterion irrespective of type of diabetes and age of onset according to American Diabetes Association (ADA) 2016 for diabetic individuals was dependent upon evidence of uncommon glucose metabolism (Seo et al., 2020). A diagnosis of autoimmune T1DM conducted if there is existence of autoantibodies targeting β cell. Patients having neonatal diabetes may be affected with type 1 diabetes mellitus but have rare monogenic kinds of diabetes (Flanagan et al., 2014).

Differentiating T1DM and T2DM

Differentiating between individuals having T1DM with those suffering from T2DM is not an uncomplicated and effortless process. T1DM occur in childhood and about 20% to 40% children affected with T1DM are obese or overweight. Family history may be sometimes responsible to determine whether an individual have T1DM. The average Body Mass Index of infants and adults affected with type 1 diabetes mellitus is lesser than infants and adolescents enduring T2DM. The occurrence of ketoacidosis is also higher in T1DM as compared to T2DM. Approximately 30% of African population has ketosis at disease onset due to hyperglycemia-induced βcell toxicity that ultimately leads to decreased endogenous insulin levels and C- peptide. However, level of C-peptide (insulin production marker) may be less at the time of disease T2DM onset.

Screening

During phase 3 T1DM is normally diagnosed, the stage at which the disorder has led to life threatening condition known as diabetic ketoacidosis. To minimize the possibility of serious complication it is necessary to diagnose autoimmunity which is present during first years of life through early screening or by using diagnostic tools. Measuring fasting blood glucose or standard OGTT's is performed for screening of phase 2 T1DM in persons having 1 or more autoantibodies targeting β-cell (Ekoe et al., 2018). Factors responsible for the continuation towards single autoantibodies to multiple autoantibodies, to dysglycemia and from dysglycemia to type 1 diabetes mellitus have been recognized in TrailNet analysis (Xu et al., 2016). In Bavaria and Germany in the month of February a study was commenced on healthy 2-5 years age children using ELISA (an immunological technique) to detect autoantibodies (GAD65, IA2 and ZNT8 autoantibodies) in capillary blood samples. None of the children developed ketoacidosis who were detected with T1DM and suggested through psychological assessment that families of children who were screened have no enhanced distress (Raab et al., 2016).

Prevention

T1DM includes two kinds of prevention. Primary prevention of type 1 diabetes mellitus can be done in infants who have elevated genetic risk through

insulin treatment and diet modification before emergence of islet-targeting autoantibodies. Primary prevention in neonate, who had an increased chance or probability of insulin autoantibodies development, executed by administration of high-dose of oral insulin (Bonifacio et al., 2015). This work accomplished with twenty-five infants having age between 2-7 years were found to show negative results for islet-targeting autoantibodies, and had increase-probability HLA genotypes as well as a family history with T1DM.

Table 1. Secondary prevention trails on nicotinamide, insulin, and immunosuppressive drugs with their phase, and outcome (Cho et al., 2018)

Trails Studies	Drug	Phase	Outcome
DPT-1	Oral insulin	3	No effect
DIPP	Intranasal insulin	3	No effect
ENDIT	Oral modified-release nicotinamide	3	No effect

Management

The management of type 1 diabetes mellitus is necessary and goal is to encourage healthy lifestyle and to control glycaemia conditions in order to avoid severe complications, guidelines have been issued for management as both hypoglycemia and hyperglycemia complications are organ specific (American Diabetes Association, 2016). Management includes therapeutic and pharmacological approaches.

Gene therapy

It has been known that T1DM occur due to the under expression of many genes. It is not feasible to modulate and activate these genes by using surgical instruments therefore, gene therapy seems to be the most feasible way to manage or cure T1DM (Mallol et al., 2017). When the IGF1 coding gene sequence was transferred to non-obese diabetic NOD mice, it was proved that expression of IGF1 gene can lessen T1DM progression (Chellappan et al., 2018). A simple schematic diagram of gene transfer is shown in Fig. 2. For insulin gene delivery in various tissues like muscle, pancreas and liver, viral techniques such as AVV and adenovirus along with non-viral techniques including naked DNA and liposomes are used. For proinsulin processing,

pancreatic beta-cells are important as they form glucose-dependent insulinotropic polypeptide (GIP), and have prohormone convertases which show similarity with intestinal cells. The implantation of K-cells for the reversal of diabetes invitro to produce and generate insulin failed by many researchers. The transgenic mice are modified and altered to express and form insulin by making use of streptozotocin (STZ) so that it can generate diabetes under the influence of GIP promoter, release normal level of glucose. Thus, to maintain a normal glucose homeostasis, these K cells produce insulin in adequate amount. Based on co-expression of glucokinase and insulin genes through AVV, gene therapy has been high lightened as therapeutic management method of diabetes mellitus. It is observable that by using long-term efficient diabetic gene therapy normal glycaemia conditions could be attained without usage of exogenous insulin (Jaén et al., 2017). AVV vectors are considered best candidate for gene therapy as they infect dormant as well as proliferating cells without inserting into the genome of host. In a study, the insulin and glucokinase genes encoded on AVV vectors incorporated in the diabetic dogs and cats. Uptake of glucose in engineered myocytes (muscle cells) is facilitated by the translocation of glucokinase enzyme and GLUT4 accelerated by co-expression of these two genes. In engineered skeletal muscle cells, glucokinase enzymes cause glucose phosphorylation into glucose 6-phosphate (G6P). However, this enzyme can also detect the blood glucose level (Romer and Sussel, 2015). For T1DM management, gene therapy is implemented by humanized liver mouse model usage. PDX1 gene having secreted insulin that is present in the liver is found to have glycaemia control. This was confirmed by the detection method involving the green fluorescent protein (GFP) presence. Elevated glucose level in STZ-induced diabetic mice has decreased when AAV-PDX-1 gene was treated. Inducing genes like glucose 6-phosphatase (G6Pase), phosphoenolpyruvate carboxykinase (PEPCK), S-14, albumin and insulin like growth factor binding protein-1 (IGFBP-1), and liver-type pyruvate kinase (L-PK) in the liver for gene therapy manifested weak discharge of insulin due to less strong activity in promotion as in comparison with less

weak promoters for example cytomegalovirus (CMV) (Handorf et al., 2015). Plasmid DNA carrying tiny patches or fragments of insulin intravenously injected for non-viral introduction showed normoglycemia for seven days and 210 days into STZ-induced diabetic rats' the liver and muscle. As the gene is introduced into host chromosome by making use of DNA transposon system the short time expression of liver injection is solved. However, plasmid DNA with insulin containing furin when co-injected they cause active insulin in muscle.

Stem cell therapy for T1MD

In 1966 Dr. Richard Lillehei successfully performed transplantation of pancreas with the advent of modern technology islet transplantation was introduced and it was first even done in 1974. Unfortunately, due to the scarcity of donors there was hurdle islet transplantation. As human pluripotent stem cells (hPSCs) have emerged as the long-term management tool in many diseases, therefore several studies have been done for islet organoids or insulin producing cells' (IPCs) in vitro generation (Shapiro et al., 2017).

Human embryonic stem cells (hESCs), adult stem cells, human induced pluripotent stem cells (hiPSCs), and differentiated cells taken from developed tissues are used from in vitro generation of islet organoids or IPCs. All these cells are able to undergo trans differentiation into insulin producing cells (IPCs) (Rickels and Robertson, 2019).

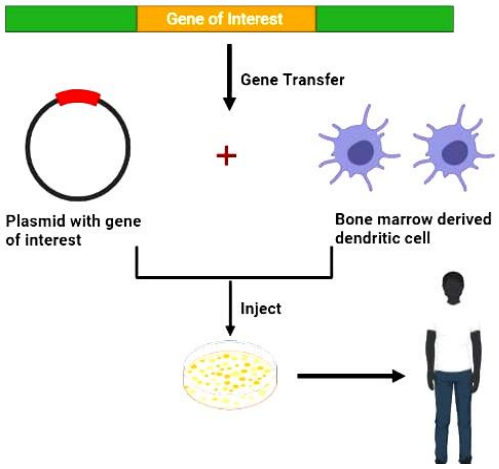

Fig. 2. Basic method of Gene Therapy (image is created Bio render)

Almost all the methods and ways aiming for the generation of IPCs are based on those protocols that imitate the development of normal pancreas. After the generation of IPCs, it is made sure that IPCs express normal β cells' particular biological markers. These markers can identify final differentiation status including MAFA (transcription factor of basic leucine zipper nature, expressed in matured β cells and is not expressed in different pancreatic progenitors), PDX1/NKX 6.1M, and NEUROD1 (Yabe et al., 2017). Moreover, the surety of specific functional characters of β cells is also considered like secretion of C-peptide, and glucose-stimulated insulin secretion (GSIS) (Russ et al., 2015). When IPCs or islet organoids are implanted into immunodeficient diabetic animals or diabetic patients, they should be able to counter changes in blood glucose level and generate suffice amount of insulin and ultimately do the reversal of hyperglycemia (Tao et al., 2019). The results of clinical trials regarding TIDM stem cell therapy is discontented (Hwang et al., 2019). Additionally, many ethical as well as technical queries are still unanswered and are open to debate. Five aspects that contribute to major issue are : (1) method for the in vitro generation of mature and functional β- cells in larger amount from human pluripotent stem cells (hPSCs); (2) protection of introduced insulin producing cells from immune system attack; (3) ways to ameliorate the efficiency of differentiation of insulin producing cells (IPCs) from hPSCs; (4) desired types of cells' sufficient generation for the clinical transplantation; and (5) establishment of thorough independence of insulin (Zhou et al., 2022). In spite of all these hurdles, the utilization of stem cell therapy in T1DM management is the most progressive and up to the minute approach for Type 1 diabetes mellitus.

Pharmacological Approaches

Treatment options for T1DM are limited as injectable insulin is the most used and recommended treatment. But it can induce hypoglycemia which can maximize the chance of heart diseases. Sodium glucose cotransporter 2 (SGLT2) inhibitors are the most advance oral anti-hyperglycemic medications class (Heerspink et al., 2016). The benefit of them is that they do not increase the risk of hypoglycemia. They have been approved by US Food and Drug

Administration (FDA) for the treatment of type 2 diabetes mellitus. It has been proposed that SGLT2 inhibitors can be used for the treatment of T1DM, although FDA has not approved those (Song et al., 2016). Inhibition of SGLT2 in the kidney's early proximal tubules is the primary mechanism of action (Vallon et al., 2017). Several trails about safety and efficacy of SGLT2 inhibitors for the treatment of T1DM are still in progress in different regions of the worlds (Henry et al., 2015). In order to achieve accurate glycaemia control, the diabetic patients may require multiple therapies, while the choice of pharmacological agents to be used by diabetic patients depends upon its, side effects, advantages, price, glucose level decreasing ability as well as dosage. These pharmacological agents include insulin, biguanides, thiazolidinediones, sulfonylureas, analogues of glucagon-like peptide 1 (GLP-1), and inhibitors of dipeptidyl peptidase-4 (DPP4) etc.

CONCLUSION

Diabetes mellitus is the multifactorial disease. Environmental factors in addition to genetic factors have been found to have a role in progress of diabetes mellitus. The disease is divided into several types. Symptoms vary from patients to patients and screening methods mainly include measuring fasting glucose level. In type 1 diabetes mellitus, the main way of treatment and management is injectable insulin whereas several other methods have been developed which includes; Sodium glucose co-transported-2 (SGLT2) inhibitors, thiazolidinediones, Sulfonylureas, biguanides, Dipeptidyl peptidase-4 (DPP4) inhibitors, and glucagon-like peptide 1 (GLP-1) analogues. Gene therapies also made its way in curing and managing type 1 diabetes mellitus as several genes have been discovered that play a role in progression as well as advancement of type 1 diabetes mellitus. Several strategies have been developed and opted in this regard but still there is a room for more advance study and practice. Stem cell-based therapy for type 1 diabetes mellitus management is also the ray of hope. Several clinical trials are still in progress but there is an anticipation that stem cell therapy can provide the insulin independence. Despite this, the whole world is looking to gene therapy and stem cell-based therapies as the most reliable and effective method for curing and regulating of type 1 diabetes mellitus.

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AUTHORS CONTRIBUTION

SR guided and supervised the authors. IF, SA, and RT were involved in data analysis, content editing, and manuscript preparation. All authors have read and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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