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Aims & Scope

The Journal aims to publish research in all fields of clinical, diagnostic, experimental & preventive areas related to medical sciences to disseminate scholastic work among clinicians and scientists around the globe.

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Editorial

ANTI-OXIDANTS AND LUNG CANCER

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Number of clinical studies indicates that antioxidants increase the risk of some malignancies, particularly lung cancer (1, 2). Additionally, study published by Sayin *et al*, suggested that treatment with NAC or vitamin-E accelerated tumour growth in mice with lung cancer caused by a *Ras* gene mutation, although it reduced oxidative stress and DNA damage as would be predicted causing decrease in the activity of the p53 protein associated with the suppression of oxidative stress (3). N-acetylcysteine (NAC), an antioxidant is frequently recommended in chronic respiratory disorders including chronic obstructive pulmonary disease (COPD), due to its anti-inflammatory properties (4). However, the therapeutic efficacy of these treatments has yet to be proven. An expected benefit of antioxidants is their ability to reduce the risk of cancer, by decreasing DNA damage and mutations induced by reactive oxygen species (ROS) (4). A limitation of these studies is the development of experimental animal model of lung cancer induced by the *Ras* mutation, which makes it possible to study the therapeutic impact of antioxidants on tumor progression, however, not on the initiation of tumor. Thus, the potential effect of antioxidants, particularly NAC, on tumor induction and the emergence of lung cancer remained unknown. However, given that NAC is already prescribed to patients with chronic bronchitis, smokers with or without COPD, and particularly those who are at risk of developing lung cancer, this question seems to be of important clinical interest.

Senescence is usually considered as a protection against cancer progression (5). Indeed, the activation of onco-suppressive proteins p53, p21, or p16 is a key step in the engagement of cells in the process of senescence. Thus, senescence in response to a specific stress, such as oncogenic stress, protects against tumor initiation by stopping cell proliferation and promoting their elimination by the immune system. Nevertheless, chronic senescence induced by oxidative stress (6), such as that occurring during aging or that induced by cigarette smoke (6), could have pro-cancerous effects, in particular via the secretory phenotype of senescent cells (senescence associated secretory phenotype, SASP). It is well known, that the majority of human cancers are linked to an escape from senescence, which is due to the inactivation of the onco-suppressive proteins p53, p21, or p16 (5). For most cells, p53 inactivation, bypassing the senescence process, leads to cell death due to excessive replication causing telomere shortening and resulting in genomic instability (5). However, some of these cells can survive, thus escaping senescence and having passed a stage of genetic instability with possible DNA mutations in their key genes, are particularly likely to transform into cancerous cells. This mechanism is relevant to lung cancer

because, in the majority of cases, the development of this cancer is linked to one or more specific mutations in the *p53* gene that result in the protein's loss of function.

A major cause of concern is how these observations may be interpreted in the context of persistent oxidative stress, such as that caused by cigarette smoking. This question has considerable clinical significance given the efficacy of NAC treatment in smoking individuals with chronic bronchitis or COPD who are at risk of developing lung cancer. Indeed, it has been shown that the pathogenesis of respiratory diseases like COPD heavily depends on cellular senescence (7). The lungs of these patients are the site of an accumulation of senescent cells which may become the reason of increase levels of SASP ultimately leading to the development of pulmonary fibrosis, distortion of lung parenchyma causing irreversible damage hence developing pulmonary emphysema, and pulmonary inflammation. Eliminating senescent cell or blocking the mechanism behind cellular senescence, therefore represents a real therapeutic option, which is currently the subject of intense research activity. The use of antioxidants is often cited as one of the possible therapeutic approaches, both preventive as well as curative. However, further research is warranted to confirm its actual role.

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Research article

CORRELATION OF COVID-19 SEVERITY WITH HEMATOLOGICAL, BIOCHEMICAL, COAGULATION AND INFLAMMATORY MARKERS- A CROSS-SECTIONAL STUDY

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ABSTRACT

Coronaviruses cause exceptionally contagious infections and at present they pose a major concern of public health worldwide. This study was conducted to evaluate the severity of Corona Virus (COVID)-19 through hematological, biochemical, coagulation and inflammatory markers. This was a cross-sectional study including 200 COVID-19 patients (97 with non-severe and 103 with severe diseases) admitted to Hayatabad Medical Complex, Peshawar, Pakistan from December 2020 to June 2021. The patients were initially screened through Real-time PCR and positive confirmed patients were further evaluated for serum ferritin, C-reactive protein (CRP), lactate dehydrogenase (LDH), D-dimer, and complete blood count (CBC) by using the standard protocols. Among the 200 COVID-19 positive patients, male positive patients were predominant (n= 138, 69%), and the most prevalent age group was 41 to 60 years (i.e., 49%). CRP was found to be most frequently deranged (95%) followed by D-dimer and LDH levels in 92% of patients. The abnormal levels of neutrophils, lymphocytes, ferritin, and hemoglobin were recorded as 83%, 82%, 79%, and 20%, respectively. All studied inflammatory markers significantly ($p < 0.005$) correlated with the severity of COVID-19 patients.

Key Words: COVID-19, Severity, Inflammatory markers, CRP, Pakistan

INTRODUCTION

Novel Corona virus-2019 (COVID-19) was classified as the sixth public health emergency of international concern (PHEIC) by the World Health Organization (WHO) on January 30, 2020, and later it was declared as a pandemic by WHO on March 11, 2020. Up to April 9, 2020, there were approximately 1436198 positive patients of COVID-19 reported with an overall case fatality rate (CFR) of 5.95%(1,2). Cough, sneezing, talking, and mucus in respiratory droplets, are the most common means of transmission. The risk of transmission is greatest in the early stages when symptoms first occur since viral RNA levels are at their highest. It may, however, transmit throughout the initial stage. The incubation period is usually two weeks after exposure, with the majority of symptoms developing within 4 to5 days(3).

Polymorphonuclear (PMN) cell activation in the immunological response caused by the virus was recently reported. Additionally, neutrophilia has been linked to significant respiratory symptoms and a poor prognosis in COVID-19 patients(4). Lymphopenia, or increased inflammatory cascade activation, is a key feature of COVID-19 disease and has a strong prognostic significance. The fundamental processes, however, are still poorly understood. According to the clinical work, coronaviruses can also directly infect stem cell precursors, leading to defective hemopoiesis, or

triggering an auto-immune reaction to blood cells(5). C-reactive protein (CRP) up-regulation was observed during the 2002 SARS outbreak and was linked to respiratory dysfunction and mortality. Various investigations in COVID-19 patients were done in this regard considering CRP as one of the potential biomolecules connected to the mortality of infected individuals. However, the results of the studies remained conflicting. The CRP was shown to be considerably elevated in the early stages of infection in severe COVID-19 individuals, even before CT results indicated serious findings. Nevertheless, CRP has been linked to the onset of illness and is a predictor of severe COVID-19(6). Lactate dehydrogenase (LDH) is an intracellular enzyme that catalyzes the conversion of pyruvate to lactate in anaerobic glycolysis. Clinically, serum LDH is frequently evaluated in a variety of diseases. Elevated serum LDH levels have been linked to a worse prognosis in a range of infections, including malignancies and inflammation. Patients with severe COVID-19 had increased serum LDH levels, according to previously studies(7). The virus has shown a tendency of mutations in various clinical features. There is limited data available from Pakistan showing pattern of hematological, biochemical, coagulation and inflammatory markers. Thus, this study was designed to determine these markers on the onset and evaluate association with severity of COVID-19 infection in the area and population of Peshawar, Pakistan.

METHODS

This descriptive cross-sectional study was conducted in Hayatabad Medical Complex (HMC) Peshawar, Pakistan from December 2020 to June 2021. A total of 200 patients were recruited randomly from the hospital ward and intensive care unit. Among these, based on the patient's condition/disease severity, 97 patients admitted to the hospital ward were affected with non-severe disease, while 103 patients admitted to the intensive care unit were suffering from severe disease.

Patients more than 20 years, regardless of gender with PCR-confirmed for Covid-19 and having signed informed consent were included in the study. All COVID-19 RT PCR negative individuals, aged less than 20 years and not willing to sign the consent form were excluded from this study.

Specimen for COVID-19 PCR: Nasopharyngeal swab samples were collected from 200 COVID-19 confirmed patients registered at Hayatabad Medical Complex in Peshawar, while their blood samples were taken in EDTA, Sodium Citrate, and Gel Tubes. Blood samples were preserved at 2 to 8 °C temperature till further processing.

Transportation: Nasopharyngeal swabs were transported in a triple zip begs to the PCR section in the Microbiology department and blood samples in EDTA Tube to the Hematology department, Sodium Citrate, and Gel tube to the Biochemistry department were transported at 2 to 8 °C for further processing.

RNA Extraction: The specimens were directly applied to the TAN Beads extractor kit with proteinase K. The extracted RNA was amplified using the nucleic acid extractor. During the process, the silicon dioxide layer coated with the magnetic beads adsorbs and purifies RNA from the sample. In the final stage, the purified RNA was aliquot in a plain tube for further use. Mic PCR thermal cycler was used for amplification of the RNA sample.

Sample Collection for Inflammatory Markers: The blood samples were collected aseptically in three different tubes, Gel Tubes for Serum Ferritin, CRP, and LDH, Sodium, Citrate for D-dimer, and EDTA tube for CBC. After collection hematological and biochemical tests were performed in the pathology department of HMC Peshawar. Samples for D-dimer and serum ferritin were centrifuged at 3000 rpm for 10 minutes except for CBC samples, to allow for examination.

Biochemical analysis of inflammatory markers: Immuno-turbidimetry utilizing Roche kits on the Cobas 601 clinical instrument used for the measurement of ferritin, LDH, and CRP. An antigen/antibody complex was formed when latex-bound antibodies combine with the analyte in the sample. This is measured turbidly following agglutination. The amount of turbidity generated is proportional to the amount of ferritin available, and it is measured at a wavelength of 700nm (primary wavelength). Considering the normal value of ferritin as 30 – 400 ng/mL, LDH as 140 – 280 U/L, CRP as < 10 mg/L. Besides these to check the accuracy of the results one normal and abnormal sample were used as controls.

Analysis of coagulation and inflammatory markers: Latex particles covered with specific antibodies to human D-dimer fragment D form immunological complexes in the presence of D-dimer from the sample. The immune complexes cause an increase in light dispersion, which is directly proportional to the amount of D-dimer present in the plasma sample. The turbidity at 570 nm is used to measure light scattering. The concentration of a sample D-dimer is measured by comparing it to dilutions of a known concentration D-dimer calibrator. Cobas 601 fully automated machine was used for D-dimer analysis. Centrifuged Sodium citrate samples were processed by selecting the D-dimer test on the machine one by one and obtaining the results in 35 minutes. Normal value of D-dimer was < 0.50 µg/dL. In all these procedures one normal and one abnormal sample were used as controls.

Analysis of hematological inflammatory markers: The Sysmex XN-1000 is a quantitative fully automated hematology analyzer used to analyze whole blood tests in vitro. Analyzing the quantitative and/or morphological values of a complete blood count, which involves HB (Hemoglobin), WBC, Platelet count, and other parameters, the differential leukocyte count is important in the identification of illness states such as anemia, leukemia, allergic responses, and viral, bacterial, and parasite infections, according to the reports. To conduct hematology tests, the hydrodynamically focused impedance analysis, the flow cytometry technique (using a semiconductor laser), and the SLS-hemoglobin methodology were used. Cytometry is used to investigate the physiological and biochemical characteristics of cells and other biological particles. Flow cytometry is a technique used for recognizing cells and particles moving through very small flow cells. From the results, the differential leukocytes count i-e Neutrophil (Normal Value: 40 – 75%) and lymphocytes (Normal Value: 20 – 45%) were calculated.

Statistical analysis

The results of the study were statistically analyzed by using Statistical Package for Social Sciences (23.0 version). The data was initially assessed on frequency distribution. For the correlation of inflammatory markers with COVID-19 severity, the simple t-test and one-way ANOVA were applied for continuous variables and chi-square test was applied for categorical variables. A p-value <0.05 was taken as significant.

RESULTS

In the current study, a total of 200 patients meeting the inclusion criteria were selected. Out of which 138 (69%) were males, and 62 (31%) were females. The more affected age group was observed to be 41 to 60 years with 98 (49%) patients, while 61 to 80 years were 56 (28%), 21 to 40 years were 40 (20%), and 81 to 100 years were 06 (03%) patients (Table 1). Among the 200 COVID-19 positive patients, the inflammatory marker CRP was highly associated with severity of disease, 95 % abnormal in COVID-19 disease, followed by D-dimer and LDH in 92 %, neutrophil in 83 %, lymphocyte in 82 %, ferritin in 79 %, and HB in 20 % (Table 1).

The ferritin, D-dimer, LDH and blood inflammatory cells were abnormal in greater proportion of males as compared to females, while higher rate of abnormal CRP was seen in females. The pattern of the markers also showed difference in different age groups. A summary of the pattern of markers according to gender and age distribution is given in Table 2. Statistically, both age and gender showed a significant correlation (p-value <0.001, F=13.16, DF=6, r2=0.6942) with COVD-19 inflammatory markers.

In non-severe disease patients, the mean of ferritin was observed as 518.53 ng/ml (range 11.5-890 ng/ml), while in severe disease the mean ferritin was 1891.84 ng/ml (range 895-16520 ng/ml) (p-value <0.001). In non-severe disease patients, the mean of D-Dimer has observed as 1.06 μg/ml (range 0.2-2.2 μg/ml), while in severe disease the D-Dimer was 9.18 μg/ml (range 2.3-57 μg/ml), (p-value <0.001). The LDH was significantly high in severe cases (mean 857.74 U/L, range 575- 2903 U/L) as compared to non-severe disease patients (mean 404.9 U/L, range 177-573 U/L) (p-value <0.001). Similarly, mean CRP was significantly high in severe cases (mean 19.03, range 7.9-45 mg/dl) while in non-severe cases it was 2.17 mg/dl (range 0.2-7.3 mg/dl) (p-value 0.037). In non-severe disease patients, the mean of neutrophils was observed at 75.54 % with a range (48-87 %), while in severe disease the neutrophils count was 92.04 % with a range (87-97 %), (p-value <0.001). In non-severe disease patients, the mean of Lymphocytes was observed at 16.84 % with a range (09-41 %), while in severe disease the lymphocytes were 4.07 % with a range (01-08 %) (p-value <0.001). In non-severe disease patients, the mean hemoglobin was observed at 15.08 g/dl with a range (13.08-18 g/dl), while in severe disease the hemoglobin was 11.78 g/dl with a range (8.8-13.5 g/dl) (p-value=0.002). A summary of these parameters is given in Table 3.

Table 1. Demographic characteristics and pattern of inflammatory markers in COVID-19 positive patients

Variable	Number (n)	Percentage (%)
Total COVID-19 Positive Patients	200	100%
Gender wise distribution of COVID-19		
Male	138	69%
Female	62	31%
Age wise (Years) distribution of COVID-19		
21-40	40	20%
41-60	98	49%
61-80	56	28%
81-100	06	03%
Pattern of inflammatory markers in COVID-19 patients		
Inflammatory markers	**Normal n (%)**	**Abnormal n (%)**
Ferritin	42 (21%)	158 (79%)
D-Dimer	16 (08%)	184 (92%)
LDH	16 (08%)	184 (92%)
CRP	10 (05%)	190 (95%)
Neutrophil	34 (17%)	166 (83%)
Lymphocytes	36 (18%)	164 (82%)
HB	160 (80%)	40 (20%)

Table 2. Gender wise and Age wise distribution of abnormal inflammatory markers of COVID-19 infected individuals

Inflammatory markers	Gender wise		Age wise			
	Male	**Female**	**21-40**	**41-60**	**61-80**	**81-100**
Ferritin	81.1%	74.1%	85%	77.5%	78.5%	33.3%
D-Dimer	92.7%	90.3%	95%	91.8%	85.7%	100%
LDH	97.1%	80.6%	75%	93.8%	100%	100%
CRP	94.2%	96.7%	90%	95.9%	100%	66.6%
Neutrophil	88.4%	70.9%	20%	87.7%	89.2%	100%
Lymphocytes	86.9%	70.9%	50%	91.8%	89.2%	100%
HB	13%	35.4%	35%	10.2%	28.5%	00%
p-values	<0.001		<0.001			

Table 3. Covid-19 non-severe and severe diseases patient's Inflammatory Markers

Inflammatory markers	Non-severe disease n=97 mean (range)	Severe disease n=103 mean (range)	Standard Deviation	p-Values	Normal reference range
Ferritin	518.53(11.5-890)	1891.84 (895-16520)	1728.201	<0.001	30—400 ng/ml
D-Dimer	1.06(0.2-2.2)	9.18 (2.3-57)	8.452	<0.001	<0.5µg/ml
LDH	404.9(177-573)	857.74(575-2903)	368.219	<0.001	80—235 U/L
CRP	2.17(0.2-7.3)	19.03(7.9-45)	88.418	0.037	<0.5 mg/dl
Neutrophils	75.54(48-87)	92.04(87-97)	11.360	<0.001	40--75 %
Lymphocytes	16.84(09-41)	4.07(01-08)	15.329	<0.001	20--45 %
Hemoglobin	15.08(13.8-18)	11.78(8.6-13.5)	1.972	0.002	11.5--17.5 g/dl

DISCUSSION

COVID-19 pandemic is a significant general health threat now a days that requires a quick action plan for its treatment and control. Notwithstanding the extreme endeavors to discover novel medications for SARS-CoV2, this procedure is tedious with constrained advancement to date. Hence, medicate repurposing has been recognized as the quickest method of figuring out restorative specialists for COVID-19 to meet the desperation of the situation. Males had a greater mortality rate than females in all age groups older than 20 years in Spanish, German, Swiss, Belgium, and Norway(8).

The current study was based on the epidemiological study of COVID-19 and its correlation with hematological, biochemical, and coagulation markers. The current study findings revealed that male patients were predominant as compared to female positive patients. Our study is in close agreement with the study reported previously according to Guan et al. , males were more (58.1%) infected than females (41.9%)(9). Similarly, another study reported from Sahiwal Pakistan by Pervaiz et al. also reported the high prevalence (74.2%) of COVID-19 in male subjects as compared to female subjects (25.8%)(10). In our present study the predominant COVID-19 infected patients age group was 41 to 60 years (49%), followed by 61 to 80 years (28%), 21 to 40 years (20%), and 81 to 100 years (3%). However, the study reported previously showed the age group 51-60 was highly infected(11), this age group is also included in the high prevelant group seen in our study.

nfection with COVID-19 affects entire body causing derangment of biomarkers, such as inflammatory markers. As reported previously COVID-19 patients have higher ferritin levels in their blood. This excess could lead to secondary bacterial infection and intensify COVID -19 infections(12). In the current study the levels of ferritin in the COVID -19 infected patient's serum, was significantly high (79%), while in non-severe disease the mean of ferritin was observed at 518.53 ng/ml and in severe disease was 1891.84 ng/ml. These findings are in agreement with previous studies. According to the study of Arshad et al., the ferritin was high in 51.26% of COVID-19 infected patients(13). In another study, they found that patients infected by bacterial disease had higher ferritin levels compared with infection by the virus the increase of ferritin levels in serum predicts a poor outcome in the hospital with infection by influenza(14).

Biomarkers of inflammation and coagulopathy can help identify hospitalized patients of COVID-19. Ayanian et al. stated in their study that inflammatory marker D-dimer was high in COVID-19 infected individuals(15), the present study results showed agreement with their findings, as per our study, the inflammatory marker D-dimer was 92% high and abnormal in infected individuals of COVID-19,

while in non-severe disease the mean of D-Dimer was observed 1.06 μg/ml and in severe disease was 9.18 μg/ml.

Lactate dehydrogenase is an intracellular enzyme that catalysis the anaerobic glycolysis reaction of lactate to pyruvate. Serum LDH is commonly tested in the clinical practice for assessment of a range of disorders. Serum LDH levels that are elevated have been associated with a poor prognosis in a variety of illnesses, including malignancies and inflammation. According to one study, patients with severe COVID-19 had elevated serum LDH levels (7). In agreement with these previous findings, the current study finding also revealed, that LDH was found to be high in COVID-infected individuals, while in non-severe disease patients the mean of CRP was observed at 2.17 mg/dl and in severe disease was 19.03 mg/dl. This finding is also in agreement with the study of, which stated in their study that LDH was high in COVID-19 infected patients(13).

In the current study, the inflammatory marker CRP was also found to be deranged in COVID-19 patients. Besides these and in agreement with our study, during the 2002 SARS outbreak, CRP overexpression was discovered and was connected to respiratory dysfunctions and patient death. Based on these findings, other studies on COVID-19 patients were conducted, hypothesizing CRP as one of the probable biomolecules positively linked to the mortality. However, the conclusions of the publications remained contradictory. The CRP marker was shown to be significantly raised in the early stages of infection in COVID-19 patients, even before CT scans revealed worrisome abnormalities. CRP, which has been associated with the start of the disease, is also a predictor of severe COVID-19(16) (16). Ayanian et al. also reported that CRP was high in COVID-19 infected individuals and their correlation was significant(15).

Neutrophils are the most frequent immune cells in human blood. Neutrophils have key homeostatic roles and are involved in chronic inflammatory illnesses, as well as being the first line of defense against many infections. Polymorphonuclear cells provide a protective role during bacterial or fungal infections, but their role in viral infections is unknown. Neutrophils have been seen in several lung disorders associated with acute respiratory distress syndrome (ARDS), including influenza and SARS-CoV-1 infections. A bioinformatics study of the SARS condition indicated that neutrophil activation and degranulation are very active processes. Recently, the recruitment of PMN cells in the immune response to SARS-CoV-2 was revealed. Furthermore, in COVID-19 patients, neutrophilia has been associated with substantial respiratory symptoms and a poor prognosis (4). The current study results are also similar to these previous findings, the neutrophil was found abnormal in COVID-19 infected individuals.

According to the current findings, lymphocytes were found abnormal in COVID-infected individuals, while in non-severe disease patients the mean of lymphocytes was observed at 16.84 %, and in severe disease was 4.07 %. In agreement with this finding, Yang et al. (17) reported that lymphopenia was seen in 80 % of severely ill adult COVID-19 patients, whereas Zhao et al. (18) reported only 25% of individuals with moderate COVID-19 infection were found to have the virus(17,18). These findings show that lymphopenia may be linked to the severity of the illness. Furthermore, in the current study the hemoglobin was found abnormal at 20% and 80% normal, while in non-severe disease patients the mean of hemoglobin was observed at 15.08 g/dl and in seere disease was 11.78 g/dl. In this regard, Hassan et al. indicated that both the open reading frame (ORF8) and the surface glycoprotein bind to porphyrin(19). ORF1ab, ORF10, and ORF3a proteins are expected to act at once to break the iron in hemoglobin's 1-beta chain, producing porphyrin and lowering hemoglobin's ability to transport O2

and CO2. The virus's method blocks the normal heme metabolic pathway, resulting in disease symptoms(20).

The study has provided baseline information regarding indicators of the severity of COVID-19, however the small sample size is the limitation. More advanced immunological studies need to explore the mechanism of COVID -19 with inflammatory markers. Genetic level studies of COVID-19 need to explore the mode of action, the activation of the human immune system, and its correlation with inflammatory markers.

CONCLUSION

The current study concluded that among the COVID-19 positive patients, CRP, LDH, D-dimer, and neutrophil-associated inflammatory markers are associated with COVID-19 infection. This study also showed that because an increase in inflammatory markers correlates with disease severity, continuous monitoring utilizing these variables might improve disease outcome and hence could be utilized as important disease prognostic indicators.

ETHICAL CONSIDERATION: The study was approved by ethical committee No. AWKUM/Biochem/Dept/Commit/eth/20 of Abdul Wali khan University Mardan, Pakistan. Informed consent was obtained from all the patients or their attendants in case of severe patients.

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CONFLICT OF INTEREST: Authors declare no conflict of Interest

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Research Article

COMPARISON BETWEEN EFFICACY OF TYGECYCLINE AND MINOCYCLINE IN MULTI DRUG RESISTANT ACINETOBACTER BAUMANII ISOLATED FROM RESPIRATORY TRACT INFECTIONS

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ABSTRACT

Acinetobacter baumannii is gram-negative coccobacilli, widely distributed in environment. It is one of the important infectious agents for nosocomial infections. Multidrug resistance (MDR) is a clinical dilemma in our region. This study was designed to compare efficacy of Tygecycline and Minocycline against MDR *A. baumanii* isolated from respiratory tract infections. All respiratory tract samples including sputum, tracheal aspirate and bronchial lavage were collected, isolated, identified and antimicrobial susceptibility was assessed by using standard protocols. *A. baumanii* isolated from 122 tracheal aspirates and 66 sputum samples. Bronchial lavage showed no bacterial growth. Age group 41-60 years showed 45% isolation of tracheal aspirates, whereas, sputum samples showed predominance (41%) recovered from older age group (>60 years). Sensitivity of Colistan was 10% in tracheal aspirates and 12% in sputum. These samples showed sensitivity of Cotrimoxazole in 6% and 3% in sputum and tracheal aspirates respectively while Beta lactams showed < 5% in both types of specimens. Tygecycline exhibited antibiotic sensitivity 34% from tracheal aspirates and 30% from sputum. Minocycline was found to be sensitive in 27% in tracheal aspirates and 23% from sputum. Our study concluded that Tigecycline found to be more effective as compared to Minocycline for the treatment of respiratory tract infections caused by multidrug resistant *A. baumanii*. Further studies are required to confirm these findings and large clinical trials will be required to make evidence based management guidelines in this regard

Key Words: Minocycline, Tigecycline, *Acinetobacter baumannii*, Multi Drug Resistant

INTRODUCTION

Acinetobacter baumannii is gram-negative coccobacilli, widely distributed in environment (1). It is one of the important micro-organisms causing nosocomial infections, including cardiovascular infections, superficial and deep wound infections, mening-encephalitis, urinary tract infections, sepsis and ventilator associated pneumonia particularly in intensive care units (2). The peculiar ability of nosocomial outbreaks is linked with biofilm formation (3). The antimicrobial resistance of *Acinetobacter baumannii* is on surge and becoming a clinical dilemma. It is getting resistant to almost all antibiotics, including Aminoglycosides, Fluoroquinolones, β-lactams and Carbapenems (4). Major contributing factors of the antibiotic resistance include prolonged hospital stay, urinary catheterization, and different invasive procedures (5). The World Health Organization (WHO) has marked Acinetobacter species as one of the top priority that require development of new antibiotics (6). Moreover, multidrug-resistant (MDR) *A. baumannii* with bacteremia is linked with high mortality rates (i.e. 56.2%), in comparison with non –MDR *A. baumannii* strains (4.7%) (7, 8).

Minocycline is a bacteriostatic semi-synthetic derivative among class of antibiotic Tetracycline with activity against both aerobic, anaerobic gram-positive and gram-negative bacteria. It acts by inhibiting protein synthesis in bacteria (9). Tigecycline is a glycylcycline, derivative of Minocycline. It was

established to overcome emerging antimicrobial resistance with broad spectrum activity against gram positive and gram negative bacteria (10). Both Minocycline and Tigecyclin belong to the same group of antibiotics with encouraging clinical outcome against MDR *A. baumannii* infections (11). Therefore, this study was designed to compare the efficacy of Tygecyclin and Minocycline against MDR *A. baumanii* isolated from respiratory tract infections.

METHODS

This was a comparative cross-sectional study conducted at Dow Diagnostic Research laboratory, Dow University of Health Sciences, Karachi, Pakistan. The study was conducted during the period from 1 December 2021 till 30 May 2022. All respiratory tract samples including sputum, tracheal aspirate and bronchial lavage were collected from patients suffering from *A. baumanii* infections. Clinical samples were inoculated on Sheep blood agar plate, Chocolate agar plate (aerobic with 5% CO2) and Mac Conkeys according to standard microbiological protocols. Identification of bacterial growth, was performed by specific methods, followed by confirmation by API 20NE (bioMerieux France). Mueller Hinton agar (MHA) (oxoid Ltd, England) used for antimicrobial susceptibility testing by modified Kirby Bauer's disc diffusion.

Statistical analysis

Data was collected and analyzed on Statistical Package for Social Sciences (SPSS) version 21.0. Descriptive data was presented as frequency distribution in number and percentage.

RESULTS

A. baumanii isolated from 122 tracheal aspirates and 66 sputum samples, whereas, bronchial aspirates showed no growth. Age group 41 to 60 years showed (45%) isolation of tracheal aspirates followed by (34%) from greater than 60 years and then (13%) from 21 to 40 years. Sputum samples showed predominance (41%) recovered from age group greater than 60 years, followed by (32%) from 41 to 60 years (Figure 1). Majority of tracheal aspirates collected from high dependency units (HDU) (45%), followed by medical Intensive Care units (ICU) (37%) and surgical ICU (16%). Predominance of sputum was found in HDUs (52%), then medical ICUs (30%) and surgical ICUs (12%) (Table 1).

A. baumanii showed highest sensitivity towards Tygecycline (34%) from tracheal aspirates and (30%) from sputum. Minocycline was sensitive (27%) in tracheal aspirates and (23%) from sputum. Colistan was found to be sensitive in (10%) and (12%) in tracheal aspirates and sputum, respectively. Beta lactams including Ceftriaxone, Cefoperazone salbactum, Tazobactam, Meropenem were about to be least sensitive among all antibiotics (less than 5%) in both sputum and tracheal aspirates. Amikacin (7%) and Gentamicin (5 %) were sensitive in tracheal aspirates, whereas, (6%) and (5%) in sputum, respectively. Cotrimoxazole showed (6%) sensitivity in sputum and (3%) in tracheal aspirates (Figure 2). Tygecycline was more sensitive than Minocycline in age groups 41 to 60 years from sputum and all age groups from tracheal aspirates. Higher frequency of Minocycline was observed from 0 to 20 years and greater than 60 years in sputum (Figure 3, 4).

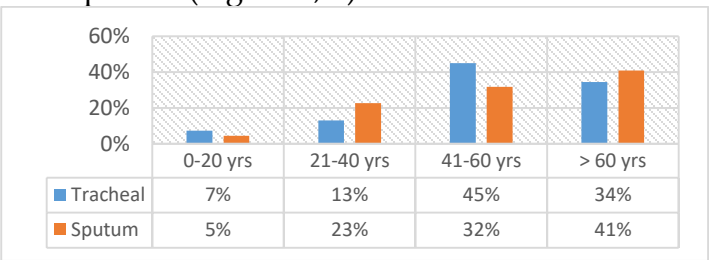

Figure 1 : Age wise Distribution of Clinical Samples

S.No	Hospital Ward	Trachea	Sputum
1	High Dependency Unit (HDU)	45%	52%
2	Medical Intensive Care (ICU)	37%	30%
3	Surgical Intensive Care (SICU)	16 %	12%
4	Private Ward	2%	6%

Table1: Frequency of Respiratory Tract Samples isolated from Different Hospital Wards

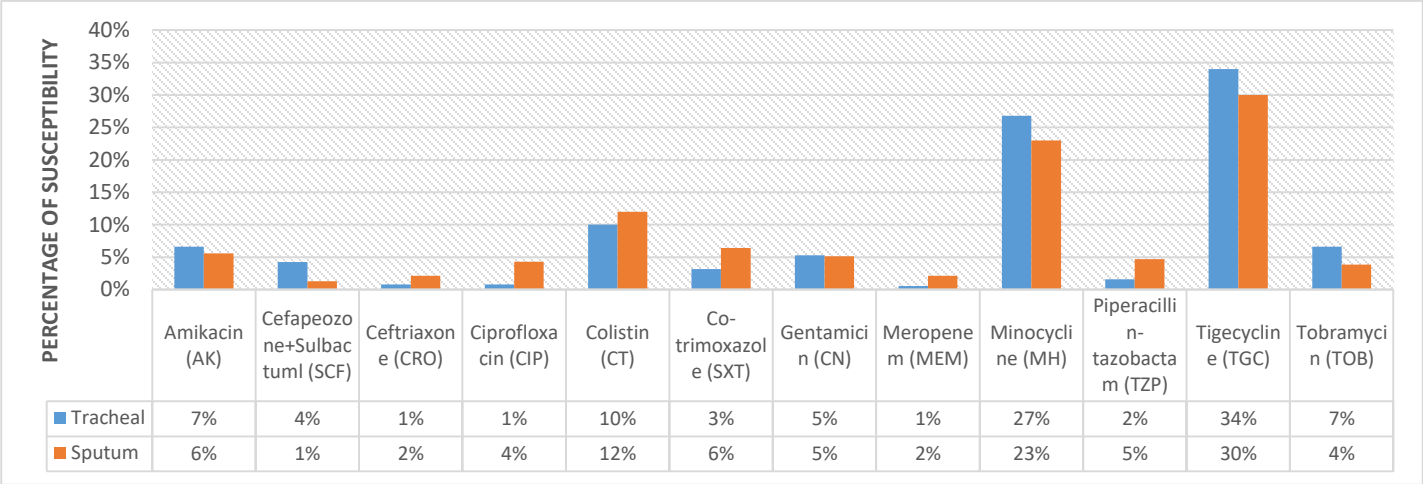

Figure 2 : Antibiotic Susceptibility Pattern of *Acinetobacter baumanii* isolated from Respiratory Tract Samples

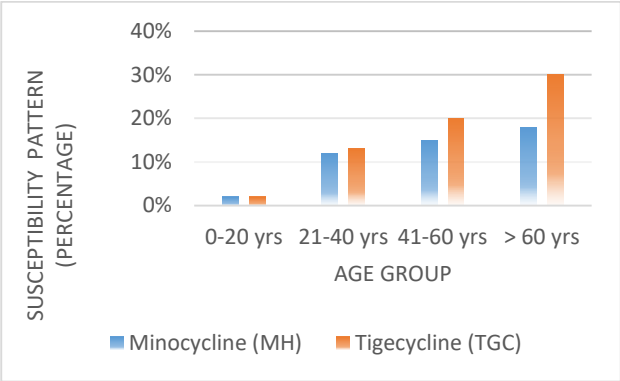

Figure 3: Comparison of Antimicrobial Susceptibility Pattern of Tygecycline and Minocycline isolated from Sputum among Different Age groups

Figure 4: Comparison of Antimicrobial Susceptibility Pattern of Tygecycline and Minocycline isolated from Tracheal Aspirates among Different Age groups

DISCUSSION

Respiratory tract infections are amid most prevalent and significant concerns in Medicine. Acute respiratory tract infections are most common ground for antibiotic prescription worldwide. *Acinetobacter baumannii* is a gram-negative aerobic bacillus notorious for hospital acquired pneumonia. Its multidrug resistant strains are emerging rapidly and gaining the focus of research to find effective antibiotic against this pathogen. Current study observed high prevalence of *A. baumannii* in age group 41 to 60 and greater than 60 years from tracheal aspirates and sputum respectively, endorsed by other study (12). Present study reported that majority of patients were admitted in HDU and Medical ICU, which is consistent with the studies reported earlier (13). Medical and surgical interventions including intubation, urinary and central venous catheterization are probable risk factors associated with Acinetobacter baumannii infection in patients admitted in ICU and HDU. Our study observed male predominance, is also in line with a previously reported studies where the number of male patients was more than females (13). Present study has found high resistance to Ceftriaxone and Carbapenems, as reported by other study (14). Acinetobacter baumanii has intrinsic resistance towards Cephalosporins and Penicillin. Carbapenems were ideal choice but their injudicial use has led to the development of resistance. The most common acquired mechanism to carbapenem resistance in *A. baumannii* is the production of enzyme oxcillinase. A number of studies reported the emergence of Carbapenem-resistant *A. baumannii* has increased in the past two decades (15, 16).

The quick upsurge of antibiotic resistance has multiple factors, including spread of resistant clones among patients, transfer through asymptomatic colonised patients and health workers, travellers and refugees from high antimicrobial resistance prevalent areas (17). Ciprofloxacin is second highest resistant antibiotic in present study. Studies endorsed our results and discovered that mutations (gyrA/parC) could be responsible for Ciprofloxacin resistance (18, 19). Resistance of Acinetobacter baumanii is also observed against Aminoglycosides including Amikacin and Gentamicin in present study. Aminoglycosides are important substitute for the treatment for MDR *A. baumannii* infections. Resistance mechanism developed through Aminoglycoside-modifying enzymes and target modifications (20).

Our study reported resistance against Colistan, in line with other study (21). Colistin is being used as a "last-resort" treatment option after Carbapenem resistance against MDR *A. baumannii*. Colistan plays important role alone or in combination with other drugs against resistant bacteria especially *A.baumanii*. Plasmid mediated resistance due to mobile genetic elements is reported to be responsible in the distribution of Colistan resistance (22). Due to this worsening scenario of emergence of MDR, XDR and pan DR *A. baumanii* with paucity of new antibiotics, few options have been left.

Reinstituting the use of older antimicrobials has now become a choice. The role of an antibiotics like Minocycline and Tygecycline in the treatment of *A. baumannii* is still being discovered. The promising safety profile and low cost, make Minocycline an attractive therapeutic option. However, studies have encouraged the use of Tygecycline in eradicating *A. baumannii* in the ICU(23).

The current study observed the comparison between therapeutic effectiveness of Tygecycline and Minocycline. The comparison showed high susceptibility of Tygecycline in all age groups except 41 to 60 years in sputum samples, whereas, in tracheal aspirates it was found to be more sensitive than Minocycline in all age groups, studies have contrasting results(24, 25). The most important mechanism for attaining resistance in tetracycline is by efflux pumps (25). However, Tigecycline has the unique capability to overwhelmed most of the efflux pumps but other mechanisms of resistance can be seen in *A. baumanii* (26). WHO has suggested infection prevention approaches; including hand hygiene, investigation for Carbapenem resistant bugs, contact and airborne precautions, environmental hygiene and patient cohorting. These measures have to be strengthened by auditing of strategies and health care surveillance system (27).

CONCLUSION

Our study concluded that Tigecycline found to be more effective agent as compared to Minocycline for the treatment of respiratory tract infections caused by multidrug resistant *A. baumanii*. The development of resistance against Tigecycline and Minocycline is a matter of concern. The judicious use of these lifesaving antibiotics with infection control measures is urgently needed.

ETHICAL CONSIDERATION: This study was approved by local Research Ethics committee.
FUNDING SOURCE: This study required no additional funding
CONFLICT OF INTEREST: Authors declare no conflict of Interest

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Research Article

FABRICATION AND CHARACTERIZATION OF ALOE VERA AND TEA TREE OIL LOADED ANTIMICROBIAL NANOFIBEROUS DRESSING

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ABSTRACT

Skin infections due to microbes are reported with high spread and reportedly affected one –third of the world population. Connective tissue cells produce collagen and connective tissue fibers to heal wounds of skin and white blood cells produce antibodies to fight against harmful microbes. This study presents the process of synthesis of nanofiberous dressing loaded with Aloe vera and tea tree oil. Aloe Vera and Tea tree oil contain glucomannan and terpinin-4-ol, which help in the production of collagen and kill microbes. The surface morphology of prepared dressing was studied using Scanning Electron Microscope micrographs. The average diameter of nanofibers was measured to be 137.32nm. Fourier transform infrared spectroscopy of nanofiberous dressing confirmed the loading of aloe vera and tea tree oil. Drug release profile was analyzed by UV-vis spectroscopy. It was observed that 100% drug released within 12 minutes. The antimicrobial properties of prepared fibers against Aspergillus Niger (fungi) and Escherichia coli (bacteria) were systematically evaluated. The results showed that aloe vera- tea tree oil composite fibrous dressing was found to be effective for antimicrobial activity.

Key Words: Aloe vera; Tea Tree oil; Skin infections; Nanofiberous

INTRODUCTION

Infectious diseases caused by microorganisms are leading cause of morbidity all around the world. These organisms can grow almost anywhere and multiply rapidly when exposed to moisture and conducive temperature(1). Diseases caused by fungi are severe threat to the human body (2,3) specially in immune-compromised patients. Bacterial cohabitation is a crucial problem during fungal infection, which increases inflammation and hampers therapy(4).As a result, it usually requires antibiotic and antifungal treatment(5) and increases the side effects due to dual therapies. Aloe vera and tea tree oil are traditionally known to help in healing process with the aid of their antimicrobial properties. Aloe vera is well-known for health benefits(6). It has been utilized for quite a long time for its therapeutic, curative, and skin nourishment properties. Moreover it is biocompatible, biodegradable, and non-toxic (7, 8, 9, 10). The hydro alcoholic part of aloe-vera has antifungal action against numerous kinds of fungi [7]. Aloe-vera has antiseptic property; it has six different antiseptic elements including lupeol, salicylic acid, urea nitrogen, cinnamon acid, phenols, and sulfur. They all have antimicrobial properties against fungi, bacteria and viruses (10, 11). Due to active biological ingredients in aloe-vera integrating it into nanofiber would further improve its effectiveness(12).

Many plants from the aromatic plants family have been investigated for essential oil contents (13). Essential oils are popular these days because of their antibacterial, antifungal, antiviral, emollient,

regenerative, and fragrant characteristics(14, 15). Tea tree oil (Melaleuca alternifolia) is mainly used in medicine for its antibacterial, antifungal , anti-allergic, analgesic, and antioxidant activities(16). Terpnen-4-ol, the primary active component in tea tree oil exhibits antimicrobial properties (13). Luckily, electrospinning is a modern technique that can uphold the bioactivity of these chemical components by converting them into nanofibers (13, 15, 17). It is a technique for producing high-quality polymer-based nanofibers, in the textile industry, wound dressings, tissue engineering, drug delivery systems and medical coating methods by using a high voltage supply(5, 14). Electrospun nanofibers not only encapsulate bioactive substances but also uphold and increase their therapeutic potential by offering a substantial surface area to volume ratio (15).

Since 1970s, beta-cyclodextrin (β-CD) water-soluble oligosaccharides have been used in formulations to increase the stability (13, 18, 19) and delivery of active chemicals in a variety of applications, including food preservation and drug delivery. β-CD is a conical shaped substance with an outer surface hydrophilic and a lipophilic cavity in the middle (18). This form allows hydrophobic molecules to be encapsulated and increase their water solubility (20). β-CD is proved to be effective for protecting essential oil molecules and functional properties by encapsulating hydrophobic essential oils inside the lipophilic cavity(13).

The study aimed to prepare antifungal and antibacterial nanofiber film, which can be used for medical applications. For this purpose, aloe vera and tea tree oil loaded Polyvanyl Alcohol (PVA) nanofibers were prepared by electrospinning process; due to the hydrophobic nature of tea tree oil, β-CD used to encapsulate tea tree oil into its lipophilic cavity. Functional group testing of aloe vera- tea tree oil nanofibers was analyzed through Fourier Transform Infrared (FTIR), and surface morphology was analyzed by Scanning Electron Microscope (SEM). The drug release profile was observed by UV-vis spectrometry. Antifungal activity against Aspergillus Niger and antibacterial activity against Escherichia coli were analyzed using the shake flask method.

MATERIALS AND METHODS

Materials

Polyvinyl alcohol(CAS-NO:9002-89-5), beta-cyclodextrin(CAS-No:7585-39-9)were purchased from Al-Beruni scientific store Hyderabad. Aloe vera gel was obtained from aloe vera leaf, tea tree essential oil was purchased online.

Methods

Preparation of polyvinyl alcohol nanofibers

Polyvinyl alcohol nanofibers were prepared through electrospinning. To prepare pure PVA nanofibers sheet 0.3gm of PVA (10% of total weight) was dissolved into the 2.7ml of distilled water. The solution was stirred on a magnetic stirrer for 3hours. Once the solution became homogenous, the polymer solution was filled into the syringe. A high voltage (14 kV) was applied to the solution containing syringe, due to electric field charged fibers spurt out of syringe. The fibers were collected on aluminum foil at a stationary collector 7inches apart from a polymer solution syringe.

Preparation of aloe vera loaded polyvinyl alcohol nanofibers

To prepare the aloe vera loaded PVA nano fibers sheet same procedure was followed by setting concentration of solution as 8% PVA, 7% AV and 85% solvent distilled water. After preparing a homogenous solution, the fibers were prepared through electrospinning.

Preparation of tea tree oil loaded polyvinyl alcohol nanofibers

To prepare tea tree oil loaded PVA nanofibers, the wall material β-CD used to load essential oil due to its hydrophobic nature. The solution was prepared by taking amounts as 8% PVA, 3% tea tree oil, 3%-ß-CD

and 86% distilled water. Once the solution became homogenous then the fibers were prepared by electrospinning.

Preparation of aloe vera- tea tree oil loaded polyvinyl alcohol nanofibers

To prepare nanofibers, PVA (8%of total weight) was dissolved into distilled water (76% of total weight), aloe-vera gel(10% of total weight) was added and stirred on a magnetic stirrer for 30mins, then 3% of β-CD and 3% of tea tree essential oil were added into the solution. The solution were stirred 30 min further to form homogenous solution, following the protocol of electrospinning nanofibers were prepared at 18kV and collected on grounded aluminum foil plate. Once the process was completed, the nanofiber sheet formed and was kept for drying at room temperature in the drying room. The dried fibers sheets were kept in a zipper bag till further analysis. Figure 1 presents preparation process.

Figure 1. Schematic presentation of aloe vera- tea tree oil loaded polyvinyl alcohol nanofiber preparation

Antibacterial and Antifungal testing

Antimicrobial activity aloe vera nanofibers were investigated against Escherichia coli (Mac Conkey's ager) bacteria and antifungal activity against Asparagus Niger (potato dextrose agar). Common shake flask protocol was used to examine antimicrobial action(1, 21) of aloe vera nanofibers. Asparagus Niger (potato dextrose agar) and Escherichia coli (Mac-Conkey ager) were cultured in nutrient broth for 19 hours at 37 °C (98.6° F), and the broth dilution method was used to examine the prepared solution. Bacterial cells reached to 1×10^9cfu/ml. It was adjusted at 3×10^5 CFU/ml to 4×10^3 CFU/ml by serial dilution with 0.03 mol/L Phosphate Buffer Saline (PBS). After getting the desired growth of bacterial and fungal cells, 50mg of aloe vera-tea tree oil fibers (sample) were added to the conical flask holding 65mL of 0.3mM PBS solution and 5mL of the prepared solution containing bacterial/fungal growth. The solution was then shaken for 18 hours at 37° C on shaking machine. Then the procedure was repeated three times for serial dilution by mixing 1mL of solution from the flask and 9mL of 0.3mM PBS. Finally, 1ml of the solution of bacterial growth with different concentrations was taken and placed onto an agar plate. After 24 h of incubation at 37.8 °C, microbial colonies formed were visually counted on the agar plates (Figure 2). Furthermore, based on acquired results, the antibacterial and antifungal activities were determined and bacterial /fungal reduction was calculated using the equation below:

$$R = \frac{(B - A)}{B} \times 100$$

Here R is the reduction percentage, B and A is the number of microbe's colonies before and after being treated with aloe vera- tea tree oil nanofibers.

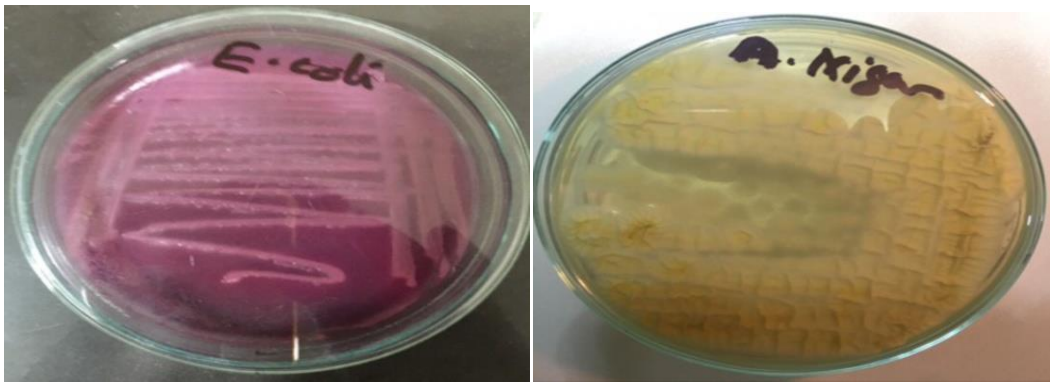

Figure 2 Fresh Culture of Escherichia coli and Fresh Culture of Aspergillus Niger

Characterization

Surface morphology of all nanofibers was analyzed by using SEM. Average diameter of nanofibers was measured by taking 80 different measurements using image J software and represented graphically. Functional groups of materials were analyzed through FTIR (FT-IR spectrometer, PerkinElmer). Drug release behavior was analyzed through UV-vis spectroscopy, and samples were analyzed through (UV/VIS Lambda 365, PerkinElmer).

RESULTS

Chemical Analysis

FTIR spectrum of neat polyvinyl alcohol nanofibers shown in Figure 3 has the characteristic bands at 3419cm-1, 2932cm-1, 1706cm-1, 1419cm-1, 1257cm-1, 883cm-1 is related to the OH stretch, CH stretch, C=O stretch, OH bending ,CO stretch CH bending. FTIR spectrum of aloe vera loaded polyvinyl alcohol nanofibers shown in Figure 4 has the characteristic bands at 3417cm-1, 2929cm-1, 1769cm-1 ,1644cm-1, 1425cm-1, 1250cm-1 ,1064cm-1, 87cm-1 is related to the OH, CH, C=O, C=N, CO, SO, stretching and OH, CH bending. The band of aloe vera appeared at 1644 and1064 in the loaded sheet, which expresses the successful loading of aloe vera. FTIR spectrum of tea tree oil/ β-CD loaded polyvinyl alcohol nanofibers shown in Figure 5 has characteristic peaks at3433cm-1, 2922cm-1, 1737cm-1,1631cm-1, 1428cm-1, 1397cm-1, 1257cm-1, 1100cm-1, 1031cm-1, 875.2 are related to OH, CH, C=O, C=C stretching, O-H bending, C=C, C-O, C-O, CO-O-CO stretching, CH bending and has vibrations between 620-1000 cm-1due to C-O-C aromatic rings of tea tree oil. Peaks at1100 cm-1 and 1397 cm-1 are due to functional group of tea tree oil and peaks. FTIR spectrum of aloe vera- tea tree oil β-CD loaded polyvinyl nanofibers shown in figure 6 has characteristic band as OH stretch-3426cm-1, CH stretch – 2928cm-1,C=O stretch- 1742cm-1,C=N stretch- 1646cm-1,O-H bending-1436cm-1,C=C stretch- 1388cm-1,C-O stretch- 1272cm-1,C-O stretch- 1101cm-1,S=O stretch- 1060cm-1,CO-O-CO stretch- 970cm-1,CH bending-879and has vibrations between 620-1000cm-1 due to C-O-C aromatic rings of tea tree oil. This shows the proper encapsulation of aloe vera and tea tree oil using β-CD as wall material for tea tree oil in PVA.

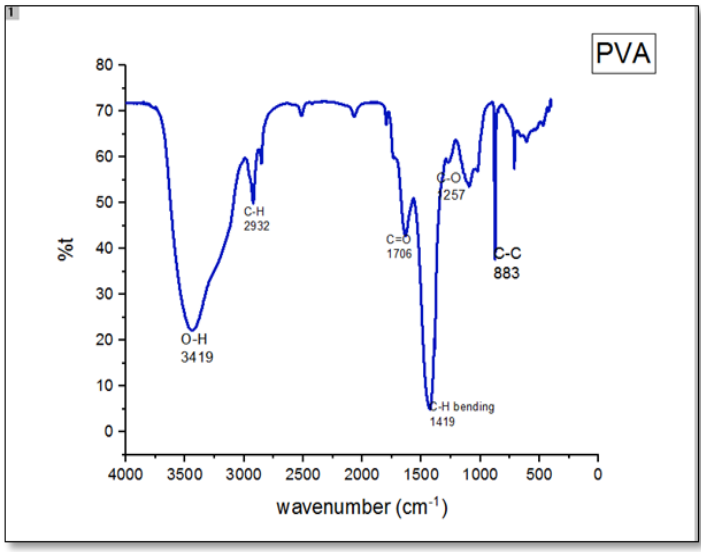

Figure 3. Fourier transform infrared spectroscopy spectrum of Polyvinyl Alcohol (PVA)

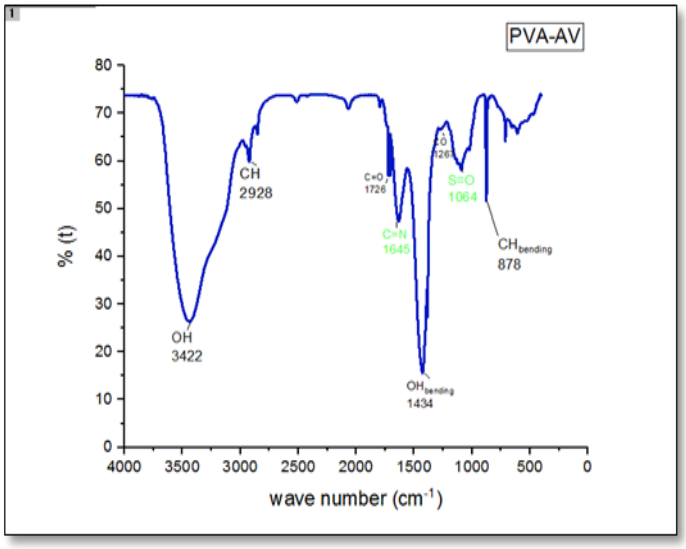

Figure 4. Fourier transform infrared spectroscopy spectrum of Polyvinyl Alcohol (PVA)-aloe vera (AV)

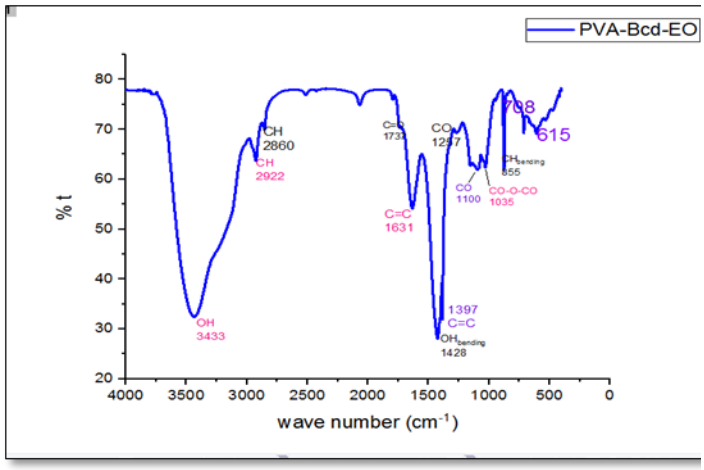

Figure 5. Fourier transform infrared spectroscopy spectrum of Polyvinyl Alcohol (PVA)-β-CD-Tea tree oil(TTO)

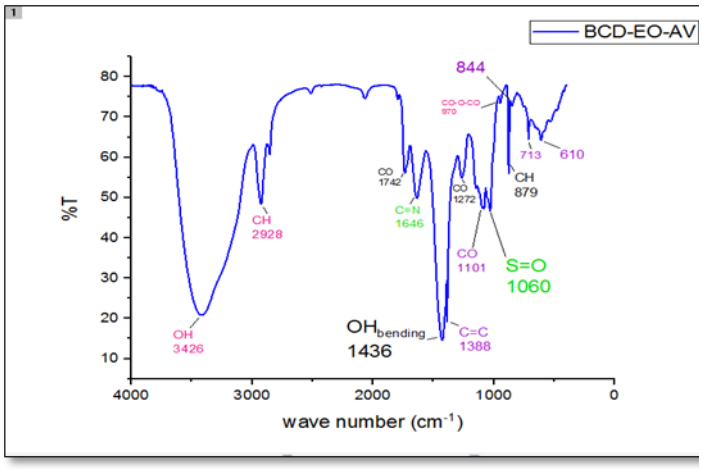

Figure 6. Fourier transform infrared spectroscopy spectrum of Polyvinyl Alcohol(PVA)-Tea Tree Oil(TTO)-Aloe Vera (AV)

Surface morphology of nanofibers

Surface morphology of all these fibers was studied from SEM images. It was observed that fibers were smooth and the average diameter of nanofiber was 200.67nm, 113.53 nm, 272nm, and 137.32nm for aloe vera- polyvinyl alcohol, tea tree oil- polyvinyl alcohol and aloe vera- tea tree- polyvinyl alcohol fibers respectively shown in Figure 7. The average diameter of fibers was calculated by taking 80 distinct measurements from SEM images by image j software.

Release behavior of nanofiber

Drug release profile of aloe vera- tea tree oil nanofibers was observed by UV–vis spectrophotometer. PBS was prepared by dissolving NaCl, KCl, NA2HPO4, KH2PO4 (8gm, 0.2gm, 1.44gm, 0.24gm respectively) in 1-liter of distilled water. Aloe vera- tea tree oil loaded polyvinyl alcohol nanofibers were peeled off from aluminum foil weighted carefully and immersed into PBS solution and stirred

slowly. The solution was analyzed under UV-vis spectrometer under multiple time spans. Figure 8 shows the UV-vis spectrum of drug released in PBS solution, it was observed that the drug was completely released within 10 minutes.

Antimicrobial activity

The antimicrobial properties of aloe vera- tea tree oil nanofibers against Escherichia coli (bacteria) and Aspergillus Niger (fungi) were systematically evaluated. Neat polyvinyl alcohol electrospun nanofibers were examined as a control experiment. The bacterial and fungal colonies were incubated in growing medium with aloe vera- tea tree oil nanofibers and antibacterial property were examined by standard shake flask protocol. Aloe vera- tea tree oil nanofibers have played a vital role for antimicrobial activity. Total density of microbial colonies for neat polyvinyl alcohol was high (Figure 9, Figure 10 (A, B)), and has nearly zero reduction in colonies. The highest reduction was observed in bacterial and fungal colonies treated with aloe vera- tea tree nanofibers. The results shows that aloe vera- tea tree oil has 99.9% reduction rate and calculated in Figure 9.These results suggest that neat polyvinyl alcohol did not show any bacterial reduction and addition of aloe vera- tea tree oil fibers were the reason behind the reduction of fungal and bacterial colonies.

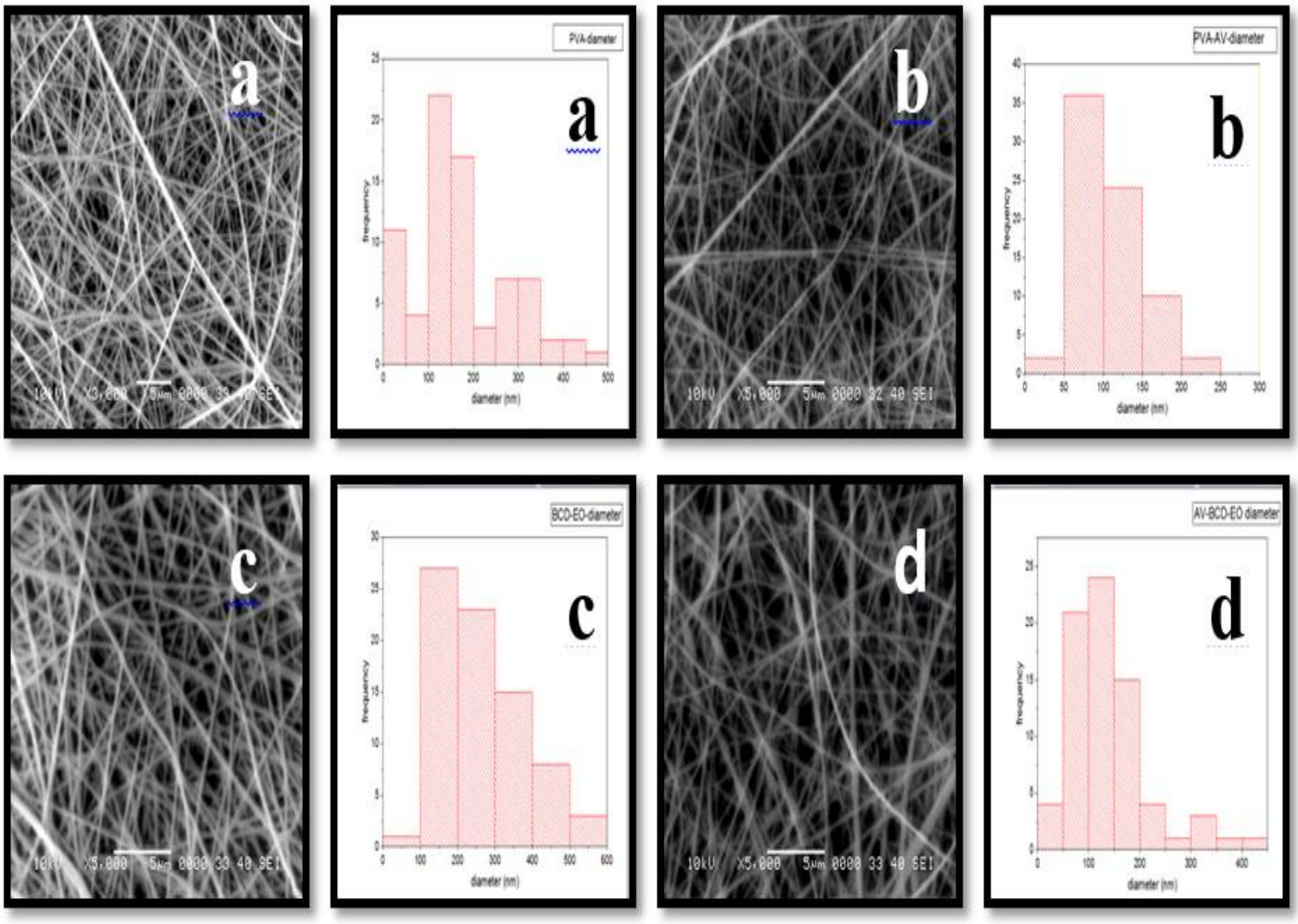

Figure 7. Scanning Electron Microscopics images and graphical representation of (a) Polyvinyl alcohol nanpfiber, (b) Polyvinyl alcohol- Aloe vera nanofiber (c)Polyvinyll alcohol-β-CD-Tea Tree Oil, (d)Polyvinyl Alcohol –β-CD- Tea Tree Oil nanofibers.

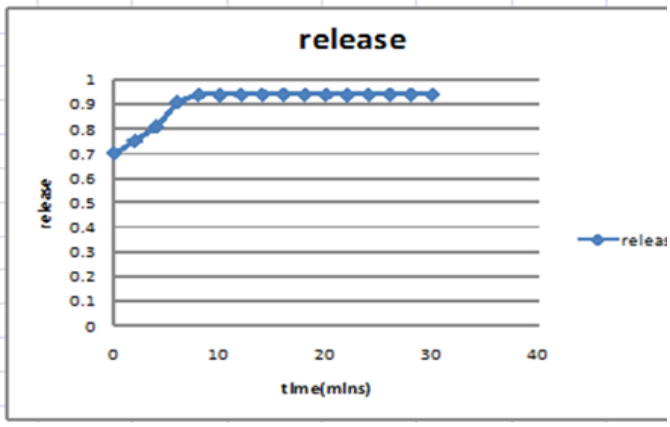

Figure 8. Drug release behavior of nanofibers

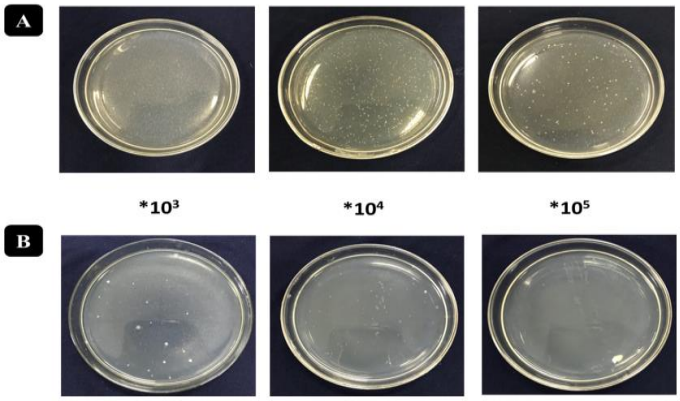

Figure 9. Aspergillus Niger colonies (A) treated with neat polyvinyl alcohol nanofibers (B) treated with aloe vera- tea tree nanofiber

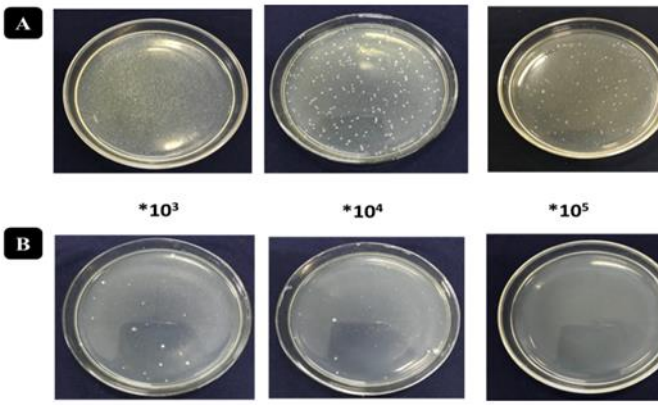

Figure 10. Escherichia coli colonies (A) treated with neat Polyvinyl alcohol nanofibers (B) treated with aloe vera- tea tree oil nanofibers

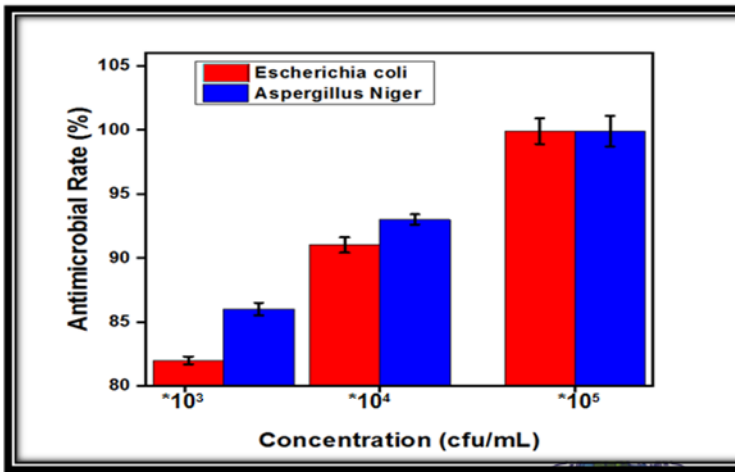

Figure 11. Bacterial and Fungal growth reduction rate

Table 1. Bacterial and Fungal reduction rate with different nanofibers

Sample	Antibacterial percentage		Antifungal percentage	
Nanofibers	Polyvinyl Alcohol	Aloe Vera-Tea Tree Oil	Polyvinyl Alcohol	Aloe vera- Tea Tree Oil
1(*10 3)	0	82	0	86
2(*10 4)	0	91	0	93
3(*10 5)	5	99.9	5	99.9

DISCUSSION

Fungal skin infections pose a severe threat to human health. Fewer standard therapies, a dearth of antimycotics for addressing fungal contamination, and the impediment of bacterial cohabitation in the treatment all decreases wound healing effectiveness resulting in raising cost of treatment and

morbidity. In addition to these conventional treatments, which mostly consist of gauze dressings, limited ability to load medicament of choice while nanofibers are ideal for use in wound dressings because they are light weight, have tiny diameter, controlled porosity structures, and cover a larger surface area with a small amount of medicine or substance as compared to conventional fibrous dressings as drug carriers. In our study, we prepared antimicrobial nanofibrous scaffolds to treat superficial skin infections caused by pathogenic fungi and bacteria by utilizing the antifungal and antibacterial potency of tea tree oil and aloe vera brought on by phytochemicals. Since Aspergillus Niger and Escherichia coli cause widespread skin infections, the effectiveness of the prepared nanofiberous scaffold offers new possibilities for antifungal and antibacterial wound dressing that will help treat and eradicate these infections. Ogidi, 2021 in this study found phytochemicals and bioactive components in turmeric essential oil and aloe vera gel. It also evaluated the antifungal efficacy and synergistic effects of antifungal creams, turmeric essential oil and aloe vera gel using the agar diffusion disc method. The study came to the conclusion that unique product made with bioactive ingredients from plants and commercially available antifungal creams will be a possible alternative therapy for treating dermatological infections (22). Several similar studies were conducted in the study in 2017 by Orchard, van Vuuren and reported that for dermatological infections the development of antimicrobial medicines the accessibility of essential oils or plant extracts are an optional therapy. Essential oils aromatic compounds and plant extracts in the formulation of topical antifungal creams to obtain best antifungal efficacy (23). According to Yue et al, tea tree oil compounds were created using the co-precipitation method, and their antifungal effectiveness against botrytis was tuned. It was also found that various types of wall materials affect tea tree oils volatility and oxidation, and that tea tree oil complexes also have antifungal properties. When compared to Fluconazole, itraconazole, and voriconazole, essential oils of oregano, pine, thymus vulgaris (thyme red), melaleuca alternifolia, and their components showed antifungal effectiveness against Cryptococcus neoformans strains. Potential synergistic interactions between two or more antibiotics can decrease the emergence of resistant mutations, boost their potency against pathogens, and function as an effective substitute for traditional therapy for a number of fungal infections. Nazzaro, Fratianni, Coppola, and Feo claimed that elements in essential oils act as antifungal agents (fungistatic and fungicidal) against fungi and disrupt the structure and function of fungal cell membranes by inhibiting extracellular/intracellular enzymes and reducing nuclear material or protein production (24). Khan, et al reported that due to their high surface-to-volume ratio, durable elasticity, and particular strength, nanofibers are essential in the creation of textiles and biomedical products. Due to its smaller diameter, less weight, and higher surface to volume ratio, nanofiber is significantly more important than conventional dressing (25). According to the above mentioned studies aloe vera and tea tree oil have antifungal and anti bacterial properties. In our study tea tree oil and aloe vera are used as therapeutic agents to fabricate effective dressing for skin infections caused by microorganisms. Antifungal activity against Aspergillus Niger and antibacterial activity against Escherichia coli of prepared nanofiberous scaffold were analyzed. Nanofiberous scaffold containing tea tree oil and aloe-vera have shown 99.9% efficiency against bacterial and fungal culture, which is remarkable and will serve great in microorganism's growth reduction. Further large scale clinical trials will be required to prove its effecacy in human subjects.

CONCLUSION

In this study, synthesis route of aloe vera- tea tree oil fibers via electrospinning has been introduced in order to report antibacterial and antifungal properties. The average diameter of nanofibers measured was 137.32nm. Fourier transform infrared spectroscopy of nanofiberous dressing confirmed

the loading of aloe vera and tea tree oil.The current study confirmed the antimicrobial effect of aloe vera- tea tree oil nanofibers and it can be used as promising antibacterial and antifungal material for medical application. Large scale clinical trials are recommended to produce high level evidence for use of nanofibers in clinical practice.

ETHICAL CONSIDERATION: The study was approved by local ethical committee, there was no ethical concern identified in this study.
FUNDING SOURCE: This study required no additional funding
CONFLICT OF INTEREST: Authors declare no conflict of Interest

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Research Article

EVALUATION OF SPECTRUM OF LABORATORY HEMATOLOGICAL MANIFESTATIONS IN DENGUE FEVER

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ABSTRACT

Dengue virus belongs to flavivirus family which gains entry into the host organism through skin following an infected mosquito bite. Humoral, cellular, and innate host immune responses are involved in the progression of the disease. Dengue fever is getting common in Pakistan, and at times shows high mortality, but there is limited literature available. Therefore, this study was designed to evaluate hematological parameters in patients with dengue fever. This was a retrospective cross-sectional study conducted in the Department of Microbiology, Dow Diagnostic Reference and Research Laboratory, Karachi, Pakistan from 1 January 2021 till December 2021. A total of 6140 were collected, out of which 1746 were found positive. Dengue infection was confirmed by rapid screening NS1 antigen by ICT method. IgM antibodies were detected by Enzyme linked immunosorbent assay (ELISA). Hematological analysis was performed on Sysmex analyzer. Among the 1746 positive samples, 1036 (60%) were males and 710 (40%) were females. More than 10 hemoglobin and 45 hematocrits were found in greater than 40% of cases. Leucopenia less than 4000 was observed mostly in age group 0-20 years. Eosinophilia, basophilia, lymphocytosis and atypical lymphocytosis were shown equally in all age groups. Our study found greater incidence of Dengue fever among 21-40 years of age group with male predominance. Hematological spectrum revealed thrombocytopenia, lymphocytosis, high hematocrit, eosinophilia, basophilia and monocytosis at the time of diagnosis.

Key Words: Dengue fever, Incidence, Hematological parameters

INTRODUCTION

Dengue virus is a member of flavivirus family which gains entry into the host organism through the skin following an infected mosquito bite. Humoral, cellular and innate host immune responses are involved in the progression of the illness and the more severe clinical signs occur following the rapid clearance of the virus from the host organism. It has an acute onset and is often self-limiting in most cases(1). According to the World Health Organization (WHO) report dengue has emerged as a major global public health challenge especially in the tropic and sub-tropic nations with almost 30-fold upsurge worldwide between 1960 and 2010, due to increased population growth rate, global warming, unplanned urbanization, inefficient mosquito control, frequent air travel, and lack of health care facilities providing unhindered opportunities for mosquito breeding(2).

The clinical presentation of dengue fever is tri-phasic with the febrile phase typically characterized by high grade fever, headache, myalgia, body ache, vomiting, joint pain, transient rash and mild bleeding manifestations such as petechiae, ecchymosis at pressure sites and bleeding from venipunctures (3). In the next critical phase, there is a heightened risk of progression of the patient to severe dengue which can result in shock or fluid accumulation such as ascites or pleural effusion with or without respiratory distress, severe bleeding, and/or severe organ impairment. The risk of severe bleeding in dengue is much

higher with a secondary infection and is seen in about 2–4% of cases. Atypical presentations are also encountered with acute liver failure, encephalopathy with seizures, renal dysfunction, lower gastrointestinal bleeding. Several studies have previously analyzed the clinico-epidemiologic profile of dengue infection (4).To differentiate them on clinical findings is troublesome and to reach a definitive diagnosis on the basis of serology is difficult in the areas of limited resources. NS1 Antigen, IgM antibodies and complete blood examination is a routine initial investigation performed at many laboratories and most frequently suggested initial investigation by the physicians(5).

Hematological parameters in various situations are helpful in giving a clue about dengue and these parameters are also useful in the management of the disease. A few findings such as presence of plasmacytoid lymphocytes and thrombocytopenia are associated with dengue infection so these CBC parameters can be utilized as diagnostic clue in area where lab facilities are scarce and patient cannot afford to go for serology. In addition evaluation of other CBC parameters can be evaluated for a potential help in diagnosis of dengue(6).

Therefore, this study was designed to evaluate different hematological parameters in samples of patients with confirmed diagnosis of dengue fever. The CBC findings as identified in this study will serve as a surrogate marker in helping the clinicians to diagnose dengue less developed settings.

METHODS

This was a retrospective study conducted at the Department of Microbiology, Dow Diagnostic Reference and Research Laboratory, Karachi, Pakistan. All patients who presented with acute fever from 1st January 2021 till December 2021 were included in this study.

Collection of clinical samples:

A total of 6140 blood samples were collected from different areas of Karachi in collaboration with Dow Diagnostic Reference and Research Laboratory. All these patients had history of fever of acute onset. A 5ml venous blood was received in yellow gel tube then tubes were centrifuged for 10 minutes and the serum was separated into another tube and tested for immunoglobulin M (IgM) serum dengue antibodies.

Screening for dengue antigen/ antibodies:

Currently the two serological tests are being used in the laboratory to confirm the diagnosis of dengue. The detection of NS1 dengue antigen, its sensitivity is 76% and specificity is 98% and another one is dengue IgM antibodies by Enzyme linked immunosorbent assay (ELISA), it reported specificity is 93% and sensitivity is 90% (23). Initially, the diagnosis of dengue infection was confirmed by rapid screening NS1 antigen was detected by ICT method using NS1 dengue antigen kit. It's the rapid qualitative dengue virus detection test which can be done from both serum and plasma. Further, confirmation was done by detection of antibodies in serum through IgM a micro titer plate technique. This technique identify IgM antibodies through ELISA. In this techniques 96well plates were used, coated with specific antigen for dengue antibodies. After antigen antibody's reaction the color changes that was read on ELISA reader. The absorbance of every sample was noted and compared with cut off values to differentiate positive and negative tests for dengue.

Hematological analysis:

Hematological analysis was performed on Sysmex XN-1000 which is calibrated after every six months and the quality control done on regular basis. 2ml of blood collected into EDTA containing purple top tubes. Complete blood count was done by Sysmex analyzer and different parameters such as hemoglobin (Hb), hematocrit (HCT), white blood cells count, differential percentages of the white blood cell counts and platelets. Sample was loaded on the analyzer which displays their result on screen. All the results were recorded.

Statistical analysis

The data was analyzed using Statistical Package for Social Sciences (SPSS) version 25.0. Demographic data and laboratory data were presented as descriptive statistics including frequency distribution and percentages.

RESULTS

A total of 6140 samples were received for the dengue antigen test in which 1746 (28%) were positive, 4709 (76.6%) were negative and 634 (10.28%) samples were rejected due to errors. The patients were both males and females ranging from 2 months to 104 years of age. Among the 1746 positive samples, 1036 (60%) were males and 710 (40%) were females (Figure1).

In this study, there were 471 patients of age group from 0 years to 20 years in which hemoglobin level below and above 10 was seen in 196 (41.6%) and 275 (58.38%) patients, respectively with Hematocrit above 45% was observed in 287(60.93%) patients. Leucopenia was most commonly seen in the age group 0 years to 20 years in 206 (43.73%). Lymphocytes were increased above 45 was detected in 252 (53.5%) patients whereas atypical lymphocytes were seen in 398(84.5%).

In age group between 20 to 40 years 853 patients were positive for Dengue antigen, hemoglobin level below 10 and above 10 was seen in 337(40%) and 516 (60.49%) of patients respectively. In the age group 21-40 (n=853) 620 (72.6%) of patients' showed hematocrit above 45. WBCs were investigated below 4000 in 152 (17.8%), and >11000 in 280(32.8%) patients. Lymphocytes above 45 and atypical lymphocytes were evaluated in 551 (64.5%) and 666 (78.07%) respectively. Dengue antigen was positive in 334 patients of ages between 40 to 60 years in which hemoglobin >5, between 5 to 10 and above 10 were detected in 127 (38%) and 207 (61.9%) respectively. In 218(65.26) of patients' hematocrit above 45 was seen. WBCs were evaluated above 4000 in 270 (80%), and >11000 in 64 (19.16%) patients. Lymphocytes above 45 and atypical lymphocytes were evaluated in 224 (67.06%) and 188 (56.2%), respectively.

In adult age group above 60 years, 88 patients were detected positive for dengue antigen in which hemoglobin level below and above 10 is seen in 50 (56.8%) and 38 (37.5%), respectively. In 23 (26%) of patient's hematocrit above 45 was seen. WBCs were perceived below 4000 in 15 (17%), and >11000 in 20 (22.72%) patients. Lymphocytes above 45 and atypical lymphocytes were evaluated in 52 (59%) and 61 (69.31%), respectively. A summary of hematological indices are shown in Table 1. Figure 2 shows platelet pattern in Dengue fever.

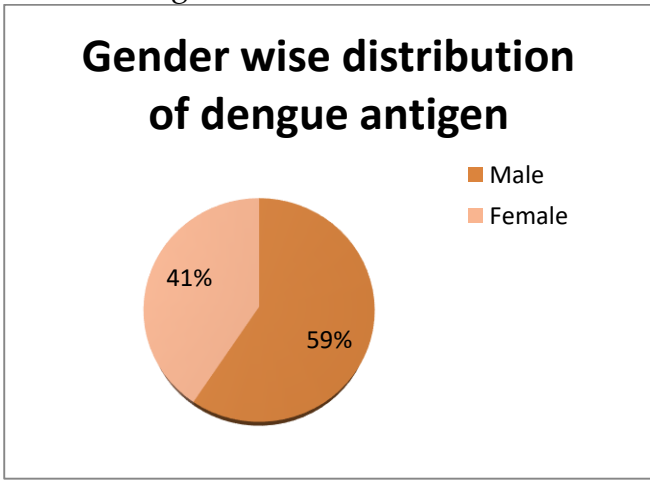

Figure 1: Gender wise distribution of dengue antigen. Light orange color shows the female percentage and dark brown shows the frequency of the males.

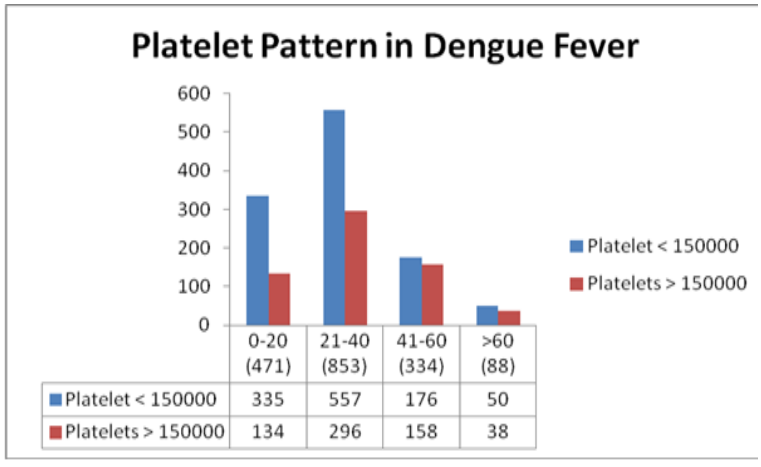

Figure II: Platelet shows thrombocytopenia in majority of patients of all age groups shown in.

Table 1: Hematological parameter (Hemoglobin, Hematocrit, WBCs, lymphocytes, atypical lymphocytes, Eosinophils and Basophils) among patients:

Age groups	Sample size	Hemoglobin >10 n(%)	HCT >45 n(%)	WBC <4000 n(%)	Lymphocytes >45% n(%)	Atypical lymphocytes n(%)	Eosinophils >5 n(%)	Basophils >2 n(%)
0-20	471	196(40)	287(60.93)	206 (43.73)	252(53.5)	398 (84.5)	15(3.18)	186(40)
21-40	853	220(40)	620(72.6)	152(17.8)	551(64.5)	666 (78.07)	119(13.9)	398(46.6)
41-60	334	66 (38)	218 (65.26)	41(12.2)	22467.06)	188(56.2)	24(7.1)	169(50)
>60	88	50(56.8)	23 (26)	15(17)	52(59)	61(69.31)	7(8)	38(43)

DISCUSSION

Dengue is a mosquito-borne disease and is one of the major health threat (7). The fast rate of globalization, growing burden of population, poor waste disposal and accumulation of water result into breeding habitats for mosquitos resulting in increasing number of mortality and morbidity (8).

A dengue is a hemorrhagic viral fever with grave magnitudes and can turn out to be fatal. Hence, this study was designed to analyze laboratory dynamics in order to increase the likelihood of prompt diagnosis (9). Serological diagnosis of dengue virus infection is normally performed by observation of the NS1 antigen and dengue IgM antibodies in the patients. Our study reported male predominance and is in harmony with the other study reported previously(10). The incidence of dengue fever in the current study was higher in the group aged 21-40 years, which was consistent with a previous study by Rabbani et al (11). Hematologic parameters appear to be useful in differentiating dengue infection from other infections by examining the complete blood picture.The most important finding of present study is thrombocytopenia which is in agreement with other studies reported previously(12, 13). B lymphocytes are commonly infected by dengue virus. The excess production of B cells, accompanied by IL – 6 stimulate an abnormal development of plasma cells and atypical lymphocytes that further lead to the formation of autoantibodies against platelets resulting in thrombocytopenia. Presence of these anti-platelet antibodies also hinders ADP brought aggregation of platelets. Thrombocytopenia also results from infection and suppression of the bone marrow megakaryocytic precursors. It has been reported in the literature that overproduction of cytokine and atypical lymphocytosis are contributing factors to the pathogenesis of dengue fever (14).

Our study showed leucopenia, in line with the findings of other study (15). A hypothesis is related to the incidence of the leucopenia was due to the destruction of myeloid progenitor cells because bone marrow investigations exhibited initial hypocellularity then normal cellularity(6). Leukocytosis is uncommon finding usually associated with the superimposed bacterial infections.

Lymphocytosis was observed in current study especially higher in age group 40-60 years. The lymphocytes in the differential leukocyte count are good predictor of the length of hospital stay. The faster recovery and short duration of stay in the hospital is directly proportional to the higher lymphocyte count (16). Elevated lymphocytes with atypical morphological features have been observed in some studies, which is similar to our study (17). Atypical lymphocytes have increased amount of cytoplasm with basophilic cytoplasmic edges , scanty nucleoli may be found but non-neoplastic in nature(18).Severe dengue infection is related to elevated lymphocytes with atypical features, which specifies the role of immunological response, including cytokines production during initial and subsequent exposure of the disease (19). Moreover, exposure of secondary viral antigen resulted in aggressive form of disease could be due to the amplified immune response ,also called as "the original antigenic sin"(20).

High hematocrit points towards plasma leakage from blood capillaries. Our study demonstrated greater than 45 hematocrit in all age groups which is in alignment with other study(21). Hematocrit values are quite helpful in monitoring amount of intravenous fluid and also direct us about the use of blood products if needed in dengue patients. The high hematocrit along with leucopenia and thrombocytopenia are substantial in the progress of severe dengue infection. Eosinophilia was observed in present study. Eosinophil count lowers in initial phase of study due to the inflammatory markers but it rises during the convalescent stage of diseases, also reported by other study (15). Monocytosis found riased in patients in our study, concomitant with the findings of study. Monocytes are mostly raised in dengue hemorrhagic fever, thus indicating the severity of disease. Moreover, it is speculated that monocytes increases in early phase of disease because they are a part of innate immunity helps in phagocytosis during the prodromal period (15). Basophilia specifies salvage from suppression of bone marrow during convalescence serves as a recovery marker in Dengue. It is usually seen in inflammation and myeloid leukemia. Current study observed basophilia, results are endorsed by other study(22).

The study has presented hematological pattern of dengue in a large population. However further studies to confirm these findings will be required.

CONCLUSION

Dengue is one of the most important public health problems in resource limited countries like Pakistan. Our study found greater incidence of dengue fever among 21-40 years of age group with male predominance. Hematological spectrum revealed thrombocytopenia, Lymphocytosis, high hematocrit, eosinophilia, basophilia and monocytosis. The infection control practices, proper vector control, surveillance program and prompt diagnosis would be helpful in reducing mortality and morbidity.

ETHICAL CONSIDERATION: This study was approved by local Research Ethics committee.

FUNDING SOURCE: This study required no additional funding

CONFLICT OF INTEREST: Authors declare no conflict of Interest

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Research Article

VENOUS THROMBOEMBOLISM AND ITS OUTCOME IN PEDIATRIC PATIENTS AT A TERTIARY CARE HOSPITAL OF KARACHI

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ABSTRACT

Venous thromboembolism (VTE) was once considered a rare finding in pediatric population but it has been increasing with recent advances in medical care and technology to diagnose it. This study was conducted to determine the clinical presentation, associated factors and outcome of VTE in hospitalized children in a tertiary care children hospital of Karachi. This descriptive cross sectional study was conducted at National Institute of Child Health, Karachi from December 2020 to January 2022. All patients hospitalized with VTE or who were diagnosed to have DVT while at hospital were included in study. Demographic characteristics, clinical presentation, medical history and laboratory workup was recorded in a semi-structured proforma. Patients were managed by treating physicians as per hospital protocol. Treatment details were recorded along with outcome. A Total of 36 children were diagnosed with VTE. Nine (25%) patients were admitted with clinical features suggestive of DVT while remaining 27 (75%) developed DVT during hospital stay. Median hospital stay of those who developed DVT at hospital was 15 days (IQR 10-30days). Infectious etiology (n=21, 58.3%) was the most common admitting diagnosis followed by central nervous system disorders (n=4, 11.1%). Common clinical features among VTE children were fever, seizures and edema of limbs. Prolonged hospital stay with immobilization and central venous catheterization particularly due to infectious etiology are common factors among children who develop VTE and such patients need to be considered for VTE prophylaxis and treatment.

Key Words: Venous thromboembolism, cerebral sinus thrombosis, anticoagulation

INTRODUCTION

Venous thromboembolism (VTE) is an increasing source of concern in hospitalized children(1). The children's hospitals solutions for patients safety national children's network, comprised of more than 100 pediatric hospitals in united states has shown deep venous thrombosis (DVT) and pulmonary embolism (PE) as one of the eleven preventable hospital acquired conditions(2). Although it carries low risk of mortality but there is significant risk of long term sequelae associated with VTE(1). It is comprises of both DVT and PE. Its incidence varies from 0.07-0.14 per 10,000 children in general population, 5.3 per 10,000 hospitalized children, 0.24 per 10,000 neonates and 0.51 per 10,000 live births with peak incidence in neonates and infants(3). About two thirds patients present with DVT and one third patients present with PE with or without DVT or less frequently in other veins like cerebral veins, portal, mesenteric renal or veins of upper extremities.

The VTE can present with a wide range of clinical symptoms including fever, localized tenderness, pain, edema, headache, seizures, focal neurological deficits or altered level of consciousness(4). It is secondary to certain risk factors in 95% of children. Different studies have identified central venous catheter, infections, congenital heart diseases, trauma or prematurity to be associated with high risk of DVT in hospitalized children(5,6). Whereas prolonged duration of mechanical ventilation and ICU stay are also

recognized risk factors for DVT(7). Congenital prothrombotic conditions like protein C and protein S deficiency are shown to be associated with VTE in almost 5% of pediatric patients(7).

In adult population extensive data on risk factors are available and hence recommendations based on randomized clinical trials are also available for prevention of the hospital acquired DVT (8). In children such data and recommendations are limited due to relatively less common occurrence of DVT in pediatric population(9). Hence, guidelines for prophylaxis and treatment of pediatric patients have not been established and are mainly drawn from the data driven from adult population

Thus this study was conducted to report clinical presentation, risk factors, treatment patterns and outcome in children presenting with VTE so that the disease pattern can be understood better and provide basis for guidelines to anticipate prophylactic anticoagulant treatment.

METHODS

This cross-sectional study was conducted at National Institute of Child Health (NICH), Karachi, Pakistan over a period of two years from December 2020 till January 2022. A sample size of 36 patients was calculated on the basis of previous study having only 9.7 per 10,000 hospital admissions (99 DVT in 102,502 admissions) with deep venous thrombosis (9). Sample size was calculated on 95% confidence interval with 4% precision using OpenEPI software (6). Non probability consecutive sampling technique was followed to include all patients diagnosed with DVT during the study period. A questionnaire was developed comprising of 26 questions including demographic characteristics of patients, their clinical presentation, laboratory workup, risk factors, management and outcome.

Patients from all medical units and ICU with age ranging from one month to twelve years diagnosed as having DVT. Venous thromboembolism was defined on either duplex ultrasonography, computed tomography, magnetic resonance imaging or computed tomography angiogram showing thrombus in any of the deep veins. Demographic characteristics, clinical presentation, laboratory workup, risk factors, treatment received in hospital and outcome after management was recorded in a predesigned proforma. Details regarding any prophylaxis (anticoagulants) used in admitted patients were also recorded.

Statistical analysis

Data was entered and analyzed using Statistical Package for Social Sciences (version 23.0). Frequency and percentages were calculated for categorical variables while Mean ± standard deviation (SD) or median and inter quartile range were calculated for continuous variables.

RESULTS

A total of 36 children with VTE were hospitalized during the study period including 34(94.4%) children with only DVT and 2(5.6%) with DVT and PE. There were 20 (55.6%) males and 16 (44.4%) females. Mean age of patients was 64.53, SD± 45.55 months (range 5-180months). A total of 9 (25%) patients were admitted with presenting clinical features suggesting DVT at admission while remaining 27 (75%) developed DVT during their hospital stay. Fever was the most common presenting feature in children followed by seizures, edema of limbs and loose stools. A summary of presenting features is given in Table 1. Mean hospital stay was 22.64 days (SD± 26.82, range 2-120 days). Intensive care unit (ICU) admission was given in 5 children with mean duration of stay 2.72 ± 7.592 days (±2.72, range 0-30 days). Regarding admitting diagnosis (Table 2) most patients 21 (58.3%) were admitted with infectious causes followed by central nervous system diseases 4 (11.11%), cardiovascular disease 4 (11.1%) and other less common causes. Table 2 presents further details regarding admitting diagnosis. Diagnosis of VTE was confirmed by ultrasound Doppler in 27 (75%) cases, Computed Tomography (CT) in 7 (19.44%) cases and magnetic Resonance Imaging (MRI) in 2 (5.6%) cases. Twenty three (63.8%) cases were found to

have DVT of legs followed by cerebral sinus thrombosis case (n=11, 30.5%), internal jugular vein thrombosis secondary to empyema thoraces (n=1, 2.8%) and 1 (2.8%) patient had portal vein thrombosis secondary to recurrent pancreatitis.

Frequency of DVT with respect to site of thrombosis is presented in Table 3. None of our patients had received prophylaxis for venous thromboembolism. International normalized ratio (INR) was found normal in 33 (91.7%) cases and prolonged in 3 (8.3%) cases. Thirty two (88.9%) patients were given anticoagulant treatment at admission including LMWH (low molecular weight heparin), in 28(77.8%) cases and unfractionated heparin in 4 (11.1%);

Table 1. Presenting clinical features of children in hospital with venous thromboembolism

Clinical feature	Frequency n (%)
Fever	21 (58.3)
Seizure	15 (41.66)
limb swelling	7 (19.44)
Loose stools	5 (13.88)
Hemiplegia	4 (11.11)
Lower limb weakness	2 (5.55)

whereas 4 (11.1%) patients were not given any treatment at admission including 3 (8.3%) due to prolonged INR i.e. deranged coagulation profile and 1 (2.8%) patient with empyema thoraces in whom thrombus was resolved following resolution of infection. Of those who received treatment at admission; 27 (75%) patients were switched to rivaroxaban, 4(11.1%) to warfarin and 1 (2.8%) patient died before 2 weeks follow up. Regarding outcome of children under treatment; 14 (38.9%) children recovered at 2 weeks, 6 (16.7%) recovered at 3 months and 5 (13.9%) children recovered at 6 months. Mortality was 13.9% (n=5) children at 2 weeks of treatment and 2 (5.5%) children at 3 months. 2 (5.6%) had persistent DVT on 6 months' follow up. During the study 2 (5.6%) children were lost to follow up.

Table 2. Admitting diagnosis

Admitting diagnosis		Number n(%)
Infections	Sepsis	8 (22.2)
	Tuberculosis	4 (11.1)
	Tetanus	3 (8.3)
	Enteric fever	1 (2.8)
	Measles	1 (2.8)
	Septic arthritis	1 (2.8)
	Empyema thoraces	1 (2.8)
	Meningoencephalitis	2 (5.6)
Central nervous system disorders	Seizure disorder	1 (2.8)
	Stroke	3 (8.3)
Cardiovascular disorders	Tetrology of fallot	2 (5.6)
	Tricuspid atresia	1 (2.8)
	Rheumatic heart disease	1 (2.8)
Others	Lymphoma	2 (5.6)
	GBS	1 (2.8)
	Transverse myelitis	1 (2.8)
	Recurrent pancreatitis	1 (2.8)
	Gaucher disease	1 (2.8)
	Nephrotic syndrome	1 (2.8)

Table 3. Site of thrombosis in children

Site of thrombosis		Frequency N (%)
Lower limb thrombosis	Total cases	23 (63.8)
	Left femoro-popliteal system	10 (43.4)
	Right femoro-popliteal system	10 (43.4)
	Bilateral femoro-popliteal system	3 (13.04)
Cerebral sinus thrombosis		11 (30.5)
Internal jugular vein thrombosis		1 (2.8)
Portal vein thrombosis		1 (2.8)

Table 4. Risk factors associated with VTE

PARAMETERS		Frequency n (%)
Risk factors	Sepsis	11 (30.6)
	Congenital heart disease	4 (11.1)
	Dehydration	4 (11.1)
	Tuberculosis	4 (11.1)
	Tetanus	3 (8.3)
	Recent surgery	3 (8.3)
	Protein C deficiency	2 (5.6)
	Central venous catheter	2 (5.6)
	Malignancy	2 (5.6)
	Empyema thoraces	1 (2.8)
	Septic arthritis	1 (2.8)
	Nephrotic syndrome	1 (2.8)

DISCUSSION

Venous thromboembolism is rare in pediatric population but its incidence is increasing due to advancement of diagnostic approaches. Guidelines for prophylaxis and treatment have not been established and recommendations for treatment are mainly drawn from adult population(10). Prolonged hospital stay is an established risk factor for DVT as shown by literature and our study also confirmed that. Median duration of hospital stay in these patients was 15 days. In a study by Atchison et.al. it was identified that hospital stay for more than 4 days is most important risk factor for hospital acquired DVT and these patients must be considered for prophylaxis(11). Similarly, other studies by CM Witmer et. al and Takemoto et. al. also identified prolonged hospital stay as a risk factor for hospital acquired VTE(12,13). Most common site of DVT was observed to be femoro-popliteal venous system of legs. Similar findings have been reported by various studies where femoro-popliteal venous system was the most common site of DVT in pediatric population(9).

Regarding clinical symptoms most common presenting feature was fever reported in 18 (50%) patients, followed by seizure, edema of limbs, loose stools, hemiplegia, and lower limb weakness. It was consistent with previously reported studies(14). Study by Turpie et. al. showed that most of the patients with venous thromboembolism present in a hospital setting with complains of edema and tenderness of limbs(15). In a study by Blann et. al. edema and tenderness was the most common presenting clinical features(16). CVST was found to be most commonly related to sepsis followed by congenital heart disease. Vieira et al. have also reported infection as the most common cause of VTE (17), while Branchford et al. found that most common factor leading to VTE was mechanical ventilation followed by systemic infection and hospitalization for more than 5 days(18). A study by Ozcan et al. also showed that infection is the main cause of CVST(19). We had 4 (11.1%) children with limb VTE having tuberculosis which suggests that tuberculosis patients are also at risk of VTE and may be considered for prophylaxis(20). This is explained by the fact that infections result in activation of coagulation pathway and platelets which in turn lead to thrombosis(20). Contrary to this study by Wagner et al. who found traumatic head injury as most common cause for VTE(21). As we did not include surgical patients in our study so we could not find the relation of trauma and VTE.

We had 1 child with nephrotic syndrome with diffuse venous thrombosis involving inferior vena cava, iliac, femoral and popliteal veins. Nephrotic syndrome is well established cause of venous thromboembolism(22). In study by Carpenter et. al. VTE was found in 3% children with nephrotic syndrome(23). Regarding treatment none of our children received prophylaxis even in diseases with prolonged hospital stay like tetanus or GBS etc. All patients after documentation of VTE were started on anticoagulation with LMWH in 28(77.8%) cases and unfractionated heparin in 4 (11.1%). Regarding treatment duration we have given for 3-6 months in all children in which primary risk factor was treated and for lifelong in children with prothrombotic conditions. Similar has been reported previously(2).

Mostly our children received LMWH which has now become treatment of choice in pediatric population due to easy subcutaneous twice or once daily dosing whereas unfractionated heparin is given in infusion in a hospital setting. It was followed by oral anti-factor Xa like rivaroxaban in 27 (75%) patients and vitamin K antagonists i.e. warfarin in 4 (11.1%). Mostly in our children rivaroxaban was used instead of warfarin because of less need of frequent monitoring and minimal risk of bleeding(24).

No treatment complication such as bleeding was observed in children treated with rivaroxaban while one child expired due to bleeding secondary to deranged INR of 9.0 following treatment with

warfarin. Study by Chan et. al reported increased incidence of bleeding complications secondary to warfarin and emphasized on closed monitoring while using anticoagulation(25). Similarly study by Clarke-Pearson identified significant risk of bleeding following start of anticoagulation therapy(26).
Almost 70% of our patients recovered within 6-months of treatment while 14% died. Similar findings were noted in study by Wright, in which he stated that 70% of the children with venous thromboembolism recovered while 14% patients had persistent DVT and 9% died(27). Goldenberg in his study discussed that although recurrence of VTE in children is rare but it is associated with increased morbidity and mortality and it can severely affect the quality of life(28).
The study provides a short term follow-up data from a single centre with a good number of patients showing relatively rare disease presentation known. Though due to time constraints the patients were not followed up beyond 6 months with persistent VTE, thus it is considered as the limitation of the study. Further studies are needed for prolonged follow-up to show the long term outcome of patients with persistent VTE.

CONCLUSION

Longer hospital stay is established risk factor for VTE and such children should be considered for prophylaxis. The problem is not that rare, therefore large scale studies and clinical trials are recommended to establish prophylaxis guidelines in paediatric patients admitted in hospitals.

ETHICAL CONSIDERATION: The study was approved by ethical committee.
FUNDING SOURCE: This study required no additional funding
CONFLICT OF INTEREST: Authors declare no conflict of Interest

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Research Article

LUMBER PUNCTURE FOR SUSPECTED CENTRAL NERVOUS SYSTEM INFECTIONS- EVALUATION OF REFUSAL RATE AND ASSOCIATED FACTORS

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ABSTRACT

Lumber Puncture (LP) can be performed for both diagnostic and therapeutic purposes. Failure to perform LP is associated with greater morbidity and mortality due to delayed diagnosis and improper management. This study was conducted to evaluate frequency of LP refusal and its associated factors among parents of children hospitalized with suspected Central Nervous System **(**CNS) infections. This cross-sectional study was conducted from October 2021 to April 2022 at National Institute of Child Health (NICH), Karachi, Pakistani. Parents/attendants of all pediatric patients hospitalized with suspected CNS infections and advised LP by the treating physicians were included in the study. A semi-structured questionnaire was used to assess the perception and attitude of patients' towards the procedure and if they agreed for their child to undergo LP. Parents of 338 children were enrolled, out of which 203(60.1%) consented for LP. Majority refused because of fear of complications followed by those who believed LP was not required (29%). Common misconceptions regarding LP complications were risk of death (13%), epilepsy (11%) and paralysis (7%). Significantly lower refusals were observed when no other course was offered as alternate to LP (p-value <0.001). There was high frequency of LP refusal among parents of pediatric patients presenting with suspected CNS infections, most important cause of refusal was fear of complications.

Key Words: Lumbar puncture, central nervous system infection, consent

INTRODUCTION

Lumber Puncture (LP) is an invasive procedure that can be performed for both diagnostic and therapeutic purposes and it is important to rule out different forms of meningitis and encephalitis(1-3). For lumber puncture a needle is inserted into the tissues of lumber region to reach the spinal canal to obtain the cerebrospinal fluid (CSF) (4-7). In pediatric population it is performed to collect CSF for establishing the diagnosis and management of neurological diseases including CNS infections(6, 8,9,10). It is a safe procedure if performed in the absence of raised intracranial pressure. Complications are rare, but include minor discomfort, headache, backache and local bleeding (2). Obtaining informed consent from patients or their family prior to performing LP is a universal recommendation(8). However, refusal to LP is the common issue encountered throughout the world. However, reported refusal rates vary in different countries, where Unites States of America has 5%, Malaysia in 25%, while 62% in Iran and 80% in Kuwait(11,12,13).

Failure to perform LP, especially in resource limited settings is associated with greater morbidity and mortality due to delayed diagnosis and improper management (14).The refusal often results in hospital admission for empirical intravenous antibiotics that besides increasing use of hospital resources, also increase duration of hospital stay, further exposing patients to nosocomial infections leading to further risk of rising antibiotic resistance (15). As the diagnosis in patients with LP refusal is usually delayed

hence risk of incomplete treatment, and longer duration of hospital stay, increasing further risk of complications (16). From epidemiological point of view refusals to LP results in underestimation of laboratory proven cases of meningitis (17). It is important to identify the perceptions and attitude of patients or their attendants towards LP in order to address the issue of refusals. Many studies have shown that usual reason for refusal of LP were fear of paralysis, mental retardation, child death and painfulness of the procedure and parents had these misconceptions because of their insufficient guidance for LP(16,18). Lumber puncture refusal has also been observed as a common issue in Pakistan but the data regarding its frequency and associated factors is limited in particular with reference to pediatric patients. Hence, this study was aimed to evaluate the frequency of LP refusal and its associated factors among parents of children hospitalized with suspected CNS infections at a leading tertiary care dedicated service for children in Pakistan.

METHODS

This cross-sectional study was conducted from October 2021 to April 2022 at National Institute of Child Health (NICH), Karachi, which is one of the largest tertiary care children hospitals of Pakistan. As per NICH policy, LP can only be performed after written consent is obtained from the parents of the child. Parents/attendants of all patients between ages 1 month to 12 years hospitalized with suspected CNS infections in any of the three medical units of NICH and advised LP by the treating physician were included in the study. Purposive sampling technique was used to enroll all patients, meeting the inclusion criteria. Patient's demographic information as well as pertinent clinical and laboratory data were recorded from their medical files after taking informed consent from patients' parents or attendants. Parents of these patients were then interviewed using a semi-structured questionnaire regarding their perceptions and attitudes towards LP and whether they agreed or not for their child to undergo LP.

Statistical analysis: Data were entered and analyzed using Statistical Package for Social Sciences (SPSS) version 25. Frequencies and percentages were calculated for categorical variables. Comparisons between parents who consented and those who refused were done with respect to demographic & clinical features and parent's awareness regarding LP. A p-value <0.05 was considered significant for all statistical tests applied.

RESULTS

A total of 338 children were enrolled including 185 (54.7%) male and 153 (45.3%) female patients. Majority (n=115, 34.0%) of patients were up to 1 year of age followed by 111 (32.8%) in the age group between 1 to 5 years, 66 (19.5%) in age group between 6 to10 years and only 6 (1.8%) patients above 10 years of age. Clinical signs and symptoms of majority (n =223, 66%) of patients were suggestive of meningitis followed by encephalitis (n=45,13.3%), febrile fits (n=40,11.8%), Subacute sclerosing panencephalitis (SSPE) (n=22,65.1) and Guillain -Barr'e Syndrome (GBS) in 8 (2.4%) patients. Out of total 338 patients who were advised LP, parents of only 203(60.05%) children consented while 135 (39.9%) refused for LP. The majority of parents (n=66, 49%) refused because they feared any complications followed by those who believed that LP is not required 39(29%) while 19 (14%) preferred their family opinion and 11 (8%) consulted some other physicians.

The parents' perception regarding LP complications, majority 199 (58%) did not think that it may lead to any complications. However common LP complications perceived by parents included death (n= 43 ,13%) followed by epilepsy (n=38, 11%), paralysis (n=22,7%) mentally handicapped (n=20, 6%) and developmental delays(n=16 , 5%). A summary of the comparison of consenting and non-consenting parents is given in Table 1.

Table 1. Comparison of demographic features of consenting and non-consenting parents

Variable	Categories	Consent Given for LP		p-value
		Given n (%)	Not given n (%)	
Gender	Male 186(55%)	102 (54.8)	84 (45.2)	0.030
	Female 152 (45%)	101 (66.4)	51 (33.6)	
Total	338 (100%)	203(60%)	135(40%)	
Patients' Age	Up to 1year	68 (43.9)	87 (56.1)	<0.001
	1-5 years	80 (72.1)	31 (27.9)	
	>5year	55 (76.4)	17 (23.6)	
Father's age	<30 years	95 (56.2)	74 (43.8)	0.009
	31-40 years	100 (62.1)	61 (37.9)	
	>40 years	8 (100)	0 (0.0)	
Mother's Age	<30 years	160 (58.6)	113 (41.4)	0.018
	31-40 years	39 (73.6)	14 (26.4)	
	>40 years	4 (33.3)	8 (66.7)	
Fathers' Education	Illiterate	66 (53.2)	58 (46.8)	0.001
	Up to Primary	68 (62.4)	41 (37.6)	
	Secondary	65 (73.0)	24 (27.0)	
	Graduate	4 (25.0)	12 (75.0)	
Mothers' Education	Illiterate	118 (58.7)	83 (41.3)	<0.001
	Up to Primary	55 (52.4)	50 (47.6)	
	Secondary	24 (92.3)	2 (7.7)	
	Graduate	6 (100)	0 (0.0)	

Comparison between patient's clinical features with respect to parents' consent is shown in Table 2. Parents of children with SSPE or febrile fits were observed to have higher consenting rates as compared to others (p-value <0.001). While parents of only 199 (59.6%) patients with fits consented for LP however, consent rate was significantly higher (p-value <0.001) in parents whose children presented with focal or myoclonic fits as compared to those with tonic or generalized tonic fits (Table 2). Altered mental status did not show any significant association with consent rate (p-value 0.871).

Among all these parents 302(89.3%) had previous knowledge of LP and source of prior knowledge was relatives in 184(60.9%) or friends & family in 118 (39.1%). Among those who has previous knowledge of LP only 268(88.7%) had knowledge about indication for LP while knowledge of technique was found in only 57(18.8%) families. A comparison of parents' prior knowledge or experience regarding LP and its association with LP consent or refusal is presented in Table 3, which shows that LP refusal was significantly higher among parents who had previously heard about LP, its indication or complication or who knew someone who underwent LP or who developed some complication after LP.

Table 4 shows the comparison of parental decision with respect to consent process at the hospital. The data depicts that refusals were significantly lower in case when a Postgraduate doctors obtained consent as compared to a house officer (p-value <0.001). Similarly significant lower refusals were observed when no other alternative was offered or when advantages and disadvantages of LP were explained to parents. Refusal rates were significantly higher for diagnostic LP as compared to therapeutic LP (p-value 0.002)

Table 2. Comparison of clinical features of children and parental decision regarding Lumber Puncture consent

Clinical Feature		Consent Given for LP		p-value
		Given n (%)	Not given n (%)	
Provisional Diagnosis	Febrile Fits	36 (90.0)	4 (10.0)	<0.001
	Meningitis	123 (55.2)	100 (44.8)	
	Encephalitis	18 (40.0)	27 (60.0)	
	SSPE	22 (100)	0 (0.0)	
	GBS	4 (50.0)	4 (50.0)	
Total		203(60.05%)	135(39.9%)	
Fits	Yes	199 (59.6)	135 (40.4)	0.043
	No	4 (100)	0 (0.0)	
Fever	Yes	151 (52.8)	135 (47.2)	<0.001
	No	52 (100)	0 (0.0)	
Type of fits	Gen.Tonic Clonic	112 (51.9)	104 (48.1)	<0.001
	Focal	49 (80.3)	12 (19.7)	
	Tonic	18 (58.1)	13 (41.9)	
	Myoclonic	20 (76.9)	6 (23.1)	
Mental Status	Normal	150 (60.0)	100 (40.0)	0.871
	Altered	50 (61.0)	32 (39.0)	
	Coma	3 (50.0)	3 (50.0)	

Table 3. A comparison of parents' prior knowledge or experience regarding Lumber Puncture

Question	Response	Consent given for LP		p-value
		Given n (%)	Not given n (%)	
Prior information regarding LP?	Yes	173 (57.3)	129 (42.7)	0.03
	No	30 (83.3)	6 (16.7)	
Source of prior knowledge?	Doctor	24 (60.0)	16 (40.0)	0.709
	Friends/family	149 (56.9)	113 (43.1)	
Prior knowledge regarding indication of LP	Yes	148 (55.2)	120 (44.8)	<0.001
	No	55 (78.6)	15 (21.4)	
Prior knowledge about LP technique	Yes	37 (72.5)	14 (27.5)	0.048
	No	166 (57.8)	121 (42.2)	
Knowledge about complications of LP	Yes	102 (55.1)	83 (44.9)	0.042
	No	101 (66.0)	52 (34.0)	
Known someone who had LP?	Yes	106 (45.3)	128 (54.7)	<0.001
	No	97 (93.3)	7 (6.7)	
Known someone who had complication after LP	Yes	37 (25.2)	110 (74.8)	<0.001
	No	166 (86.9)	25 (13.1)	

Table 4. Factor affecting parent's decision with respect to consent process

		Consent Given for LP		p-value
		Given n (%)	Not given n (%)	
Designation of requesting doctor	House officer	50 (45.5)	60 (54.5)	<0.001
	Postgraduate training	153 (67.1)	75 (32.9)	
Any other alternative offered	Yes	1 (0.9)	107 (99.1)	<0.001
	No	202 (87.8)	28 (12.2)	
Advantages of LP explained?	Yes	201 (61.3)	127 (38.7)	0.022
	No	2 (20.0)	8 (80.0)	
Kind of advantages explained	Diagnostic	126 (54.5)	105 (45.5)	0.001
	Preventive/Therapeutic	64 (75.3)	21 (24.7)	
Disadvantages of LP explained?	Yes	113 (53.6)	98 (46.4)	0.002
	No	90 (70.9)	37 (29.1)	
Kind of disadvantages explained	Backache	41 (51.9)	38 (48.1)	<0.001
	Bleeding	19 (33.9)	37 (66.1)	
	Headache	8 (47.1)	09 (52.9)	
	Paralysis	3 (25.0)	9 (75.0)	
	Infection	41 (91.1)	4 (8.9)	

DISCUSSION

Cerebrospinal fluid examination via LP is essential for diagnosis of CNS infections and sometimes repeat LP is performed to see the response of the treatment. Although it is effective for diagnosis and management, still there are higher rates of refusal in different regions of the world (19). In our study the rate of LP refusal was 39.9%, which suggests that on average one out of three parents refused. Similar findings have been reported by Mushtaq Ahmed et al from Karachi who reported LP refusal rate of 32.6%(18) while it was 43% in the study by Haseeb Narchi et al in Al Ain Hospital UAE(15). Another study from Kuwait reported 42.5% refusals for LP (12).This was higher than in other studies who reported rate of LP refusal was 25-9% (17, 19). In our study the refusal rate was higher for male (45.1%) patients as compared to females. Which is slightly lower to the study by Nasma Naji Al-Hajjiah where it was reported to be 51.4% refusal in male children (15)

Among demographic factors, the rate of refusal was significantly higher in patients less than 1year of age as compared to other age groups in contrast to other study by Mushtaq Ahmed et al where they have reported higher consent rate for children younger than six months (18). Parents' age and education status was also significantly associated with consenting rate as parents aged less than 30 years and those without any formal education more frequently refused for LP. This is in contrast to a previously reported study where there was no significant association of age and education with the refusal (13). This may be linked with the awareness of the procedure.

In our study the signs and symptoms of the great majority of patients were suggestive of meningitis followed by encephalitis, SSPE, febrile fits and GBS . This is in contrast to the study by Acoglu, Esma Altinel et al in which majority of patient's neurological disease had in 45.25% of the patients, central

nervous system infection in 45.25%, and acute encephalopathy in 9.5%(20). In contrast to other study where they found meningitis 45-55% patients(13, 20).

Regarding provisional diagnosis the LP refusal rate was highest for encephalitis followed by GBS and meningitis, same has been observed by another study where there was higher refusal rate for meningoencephalitis followed by meningitis (13). In parents reasoning for LP refusal, 49% refused because of fear of complications, 29% refused because they thought LP is not needed while 19% preferred their family and 11% preferred some other physician's opinion. Major fear of complication for LP refusal was risk of death 13% followed by epilepsy 11% and paralysis 7%.This has been shown by other studies where the fear of paralysis and fear of death, fear of pain or trauma were reasons for refusal (2, 12).

We had higher refusal rate in parents who had prior knowledge of LP and the source of prior knowledge were most commonly observed to be friends & family. Similarly, parents with prior knowledge and experience of some complications in their friends and family had significantly higher rates of refusal. Similar results have been shown by another study where source of prior knowledge was relatives and friends while prior knowledge of known complications of epilepsy, developmental delays and other complications were significantly associated with high refusal rate (19).

We had significantly high refusal rate when consent was taken by house officer as compared to the postgraduate doctor. This is in contrast to other studies where there was no significant relation reported with respect to doctor's designation(15). However, this could be logical as more senior doctor can explain the need of LP and related complications in a better way than a junior doctor such as a house officer. Lumber Puncture refusal rate was significantly higher if parents were offered any other alternatives. Similar has been shown by other studies where LP refusal rate was significantly higher when some alternatives to LP was offered (15). Nonetheless LP is an invasive procedure with default complications and associated risks. Thus if any alternative is offered parents would choose less invasive first thus refusal rate would go up. This was a prospective questionnaire based study from a single centre. However, the sample size was smaller and the consequences of refusal of LP were not recorded. Thus it is taken as limitation of the study.

CONCLUSION

There is high frequency of LP refusal in pediatric population and most important risk factor for refusal is the fear of complications. Parent's education about LP safety and its effectiveness may improve the perception about the importance of LP and therefore the refusal rate may reduce.

ETHICAL CONSIDERATION: Study was approved from Institutional ethical review board of NICH and written informed consent was taken from parents before enrolling patients.

FUNDING SOURCE: This study required no additional funding

CONFLICT OF INTEREST: Authors declare no conflict of Interest

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Review Article

IT'S ALL ABOUT COVID-19: A REVIEW OF PATTERN OF WAVES IN PAKISTAN, DRUGS AND VACCINATION PROGRAMS

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ABSTRACT

Corona Virus (COVID)- 19 is a pathogenic viral infection that presents as a new worldwide public health crisis. This review article aims at recapitulating waves of COVID-19, vaccination programs, treatment options, and the current scenario of COVID-19 in Pakistan. All available literature on PubMed, Scopus databases and science direct and Google scholar relating to COVID-19 published between 2019 to January 2022 was reviewed. The first wave of COVID-19 presented as severe acute respiratory syndrome. The second wave had a modest intensity, affecting only Sindh's southern province, and peaked in mid-December 2020. The third wave, which primarily disturbed the regions of Khyber Pakhtunkhwa and Punjab, peaked in the late April 2021, when a new strain of SARS-CoV-2 was discovered in over 64 countries, including Pakistan, as of January 27, 2021. Fourth wave qualifying measures was forced in May, after which cases balanced out and most of the mortality was reported in Punjab, followed by Sindh. The 5th wave with a positive ratio in Karachi had the increasing trend from 2% to 6%. In clinical trials, treatment with a combination of Azithromycin and Chloroquine shown efficacy against COVID-19. The Tocilizumab was used to treat COVID-19–related symptoms. Vaccination appeared to be vital to control the COVID-19 disease outbreak with seven licensed vaccines. Currently 72% Pakistani population is either vaccinated or have been infected at least once.

Key Words: COVID -19, waves, current scenario, Pakistan, Vaccination, SARS-CoV-2

INTRODUCTION

Coronaviruses are members of the Coronaviridae family of Nidoviruses, which includes the coronaviruses and Toroviruses genera. Coronaviruses are a type of "infectious bronchitis virus." In December 2019 (1) and January 2020, the first few COVID-19 infected patients were reported, and the disease was speedily spread around the globe. Given its origin in Corona virus family and first reported in 2019 it was identified as COVID-19 (2). Since its inception in a wholesale seafood market in Wuhan, a central city in the People's Republic of China (PRC), 2019 new coronavirus (SARS-CoV-2) spread to more than 215 countries and territories (2). The emergence and re-emergence of human coronaviruses (CoVs) always caused a new global threat (3). COVID- 19) is a viral infection that emerged as a new worldwide public health crisis (4, 5). It is highly contagious causing severe acute respiratory syndrome which ca be lethal (4). On January 30, 2020, the World Health Organization (WHO) designated the COVID 19 outbreak as the Sixth public health emergency services (SPHEC) (6). This was not the first time the coronavirus had spread. The outbreak of severe acute respiratory syndrome coronavirus (SARSCoV) and

the outbreak of Middle East respiratory syndrome coronavirus (MERSCoV) are two previous outbreaks of coronavirus (7). It's an emerging worldwide menace, and the WHO has described it as a pandemic, offering multi-pronged strategies to the states around the world. The WHO had issued a warning about the pandemic's speeding up, noting that it took sixty-seven days to reach 100,000 cases from the first-reported case, eleven days to reach the second 100,000 cases, 4 days to reach the third 100,000, and only two days to reach the fourth 100,000 cases(8).

The first case of COVID-19 was certified by Pakistan's Ministry of Health on February 26, 2020 (9) and since then, the disease has been spreading across the country. It has entered through the pilgrims who were coming from Iran , Saudi Arabia (10) and from other countries where they were trapped, owing to the surging case the only panacea was lockdown and social distancing, the other measures were futile and fruitless (11). On March 24, 2020, the government instituted a countrywide lockdown that lasted more than a month(12).

In Pakistan, there were 295,849, confirmed coronavirus infections and 6,294 deaths(13). Adult male patients (age ranged 34-59 years) were most commonly infected with SARSCoV2 (14, 15). The virus was more likely to infect those with chronic comorbidities including cerebrovascular and cardiovascular diseases, as well as diabetes mellitus (16). Adults over 60, as well as those with cardiovascular and neurological diseases, and diabetes, have the highest prevalence of severe disease and it was also linked with high mortality. There have been less COVID-19 cases reportedly among children under the age of 15(17, 18). There were no cases in children under the age of 15 years in a study of 425 COVID-19 patients published on January 29 in Wuhan China (19). However, by January 2020, 28 pediatric patients have been reported. Infected children show an extensive range of clinical symptoms, albeit majority have minimal symptoms without fever and pneumonia and had a good prognosis. (20).

The purpose of this review was to highlight COVID-19 waves with drugs, immunization programs, treatment options, and Pakistan's current COVID-19 condition.

METHODS

This narrative review including studies and reports published since first report of COVID-19 (i.e. 2019) till January 2022. The study was conducted by searching websites including scientific direct, Google scholar, PubMed, and Scopus databases for published papers presenting data in first to fifth waves, medicines tried and recommended, immunization program offered for COVID-19 in Pakistan. The key words “coronavirus,” “wave”, “drugs”, “therapy”, “vaccine”, and “current scenario” were used in conjunction with the disease key words for the respective searches. The first author evaluated the titles and abstracts of 70 publications found and finally 43 were including for qualitative synthesis of this review. All authors reviewed 43 studies to confirm selection. The results were obtained from nominated studies underwent a descriptive outline of the assertion of evidence. Inappropriate literature, which included case studies of COVID-19, short communications articles studies that do not cover any of the above-mentioned items were excluded from the study. Only full-text articles were used to extract data. Because of the enormous number of submissions found in the published articles, the Google Scholar search was limited to titles only.

RESULTS

First Wave of COVID-19

In Wuhan, China, on December 29, 2019, an outbreak of pneumonia linked to the novel coronavirus of 2019 (2019-nCOV) was confirmed; affecting patients’ lower respiratory tracts, and was linked to a local human South China seafood market. The name 2019-nCOV has been changed to extreme Acute respiratory syndrome coronavirus 2 at this time (SARS-CoV-2) (21). The first wave of COVID-19 began

in late May 2020, peaked in mid-June with all-time highs in daily new confirmed cases and daily new mortality counts, and then ended in mid-July. The initial wave had a low fatality rate and passed quickly after peaking, with case and death rates rapidly declining. Following the initial wave, Pakistan's COVID-19 situation stabilized, with daily new death tolls dropped and testing positivity rates in the country stabilized at low levels. The first wave of illness was extremely unpredictable and imaginary owing to the obscurity of origin and inception, mode of infectivity, transmission, and possible treatment options. On February 26, 2020, Pakistan testified its first case of intense acute respiratory syndrome coronavirus 2 , a traveller from Iran, almost two months after the first case in Wuhan, China.

Despite having the world's 5th largest population, Pakistan had only the twenty ninth -maximum loss of life toll (round 23,087) and the 29th maximum range of cases (at approximately 1,011,708). However, the rapid response of all countries, including that of Pakistan, had helped to reduce the worrying prevalence of COVID19. Pakistan was fairly successful in directing the disease's spread during the first wave. Patients were also managed through quarantine and isolation centers, as well as COVID19 high-dependency units and critical care units. To advise and support the District COVID19 Command and Control Centers, Provincial and National COVID19 Command and Control Centers (NCOCs and PCOCs and) were established (22).

Second Wave of COVID-19

The second-wave pandemic poses an immediate threat to society, gargantuan human toll and terrible economic consequences. Traditionally, the dynamics of disease diffusion have shown using compartmental or complex network diffusion techniques (23). In early November 2020, the second wave of countries commenced. This wave stood low in strength and mostly affected areas of Pakistan were Sindh southern areas. It peaked around mid-December 2020. Nonetheless, a second wave of the disease emerged, this time with a more limited transmission and toxicity.

Third Wave of COVID-19

Testing positivity rates, as well as daily new confirmed cases and deaths, increased during the world's thirdwave, which began in mid-March 2021. The thirdwave mostly affected the provinces of Khyber Pakhtunkhwa and Punjab. In late April 2021, this wave peaked, after which positive rates, everyday new cases, and mortality dropped.

As of January 27, 2021, a new SARS-CoV-2 strain developed in the United Kingdom (UK) (24) and has been discovered in more than 64 countries, including Pakistan (25). This SARS-CoV-2 variation was shown to be related to a higher risk of death. When compared to other versions, with an average of 100 patients dying every day in Pakistan. The ten cities of Pakistan including Lahore, Faisalabad, Muzaffarabad, Bahawalpur, Hyderabad, Multan, Islamabad, Peshawar, Swat and Rawalpindi, were placed under firm lockdown until April 11, 2021.

The provincial administration was directed to strictly implement Standard Operating Procedures (SOPs). One thing that the first and third waves had in common was the time of beginning which was spring (from March till the end of April). It could lead to the creation of a theory that pollens play a crucial role in SARS-CoV-2 viral transmission (9), but surely confimed seasonal association.

FourthWave of the COVID-19

The emergence of Delta variant, a type of the virus initially found in the neighboring India, raised fear of a fourth wave of coronavirus in Pakistan (26). This number was significantly greater than it was in June (where they reported 1,200 daily new cases). Preventive measures were put in place in May, and the cases gradually started to decline as patients recovered (27). On August 20, 2021, the nationwide count of active COVID-19 cases reached 88,209, one of the highest during the fourth wave of the

pandemic, with 3,974, more persons tested positive for the fatal virus and 3,122 recovered, from the disease, in the preceding 24 hours. According to the latest information from the NCOC, 66 corona patients had died in 24 hours, 58 of them were receiving treatment in hospitals and six of whom died in their individual quarantines or homes. Punjab was the state having most deaths, followed by Sindh (28).

FifthWave of the COVID-19

The fifth wave showed most cases reported in Karachi, the positive ratio increased from 2% to 6% over a period of three days (29). The Health Ministry in the South Asian country recorded over 500 new infections during a span of 24 hours. The wave was confimed on 2nd January 2022. The country's overall caseload increased to 1,296,527, with 594 new infections, bringing the infection rate up from 0.6% to 1.3% (30). Nevertheless, five waves of COVID-19 have been witnessed in Pakistan, but the case rate and mortality remained lowered as compared to other countries.

2. Drugs and vaccination programs

There was no vaccination for COVID-19 as of June 17, 2020, but multiple medications such as Tocilizumab Ninavir lyophilized and Azithromycin, Bemsivir lyophilized and Dexamethasone were offered used to treat the condition empirically. As a result, the only way to keep the virus from spreading was to practice social distancing and self-isolation. After considering the established quality and safety requirements, the Drug Regulatory Authority of Pakistan (DRAP) approved Hydroxychloroquine as the first drug to treat COVID-19 patients. Then, in clinical trials, a combination of medicines (Chloroquine and Azithromycin) was proven to be effective against COVID-19 (31). Convalescent plasma therapy was later approved for the treatment of severely ill COVID-19 patients.

A probationary at Agha Khan Hospital in Karachi, Pakistan, found Tocilizumab, an anti–interleukin-6 receptor monoclonal antibody, to be effective (32), The Federal Government, on the other hand, did not adopt this procedure as a feasible treatment for COVID-19 patients. Supportive care, mechanical breathing, and Extracorporeal oxygenation are still the most important therapeutic option for medical experts, according to numerous sources from around the world and in Pakistan.

Anti-inflammatory drugs, Antiviral, and anti-parasitic; interferon therapy; hyper-immunoglobulin; convalescent plasma therapy; oligonucleotide-based therapies; and, occasionally, mesenchyme stem cell, and RNA interference therapy are among the therapeutic alternatives being studied and employed (33, 34). After the development of a corona vaccine, pandemic would be curbed and alleviated, Russian gave the world a ray of rope. Within 15 weeks of the corona virus outbreak, Russia released the first applicable vaccination (Sputnik- V) in this hope (35).From February to August 2020, Clinical data was collected retrospectively from 1812 confirmed COVID-19 patients admitted to four major tertiary referral hospitals in Pakistan. Monthly distributions of different medications given to COVID-19 patients (n=1562) in Pakistan during the 1st wave of the pandemic (May-July 2020) (36). Covid-19 and its related symptoms were cured by 19 drugs. Ceftriaxone and Azithromycin were mostly used ones. Anticoagulants such as heparin and Enoxaparin Sodium and steroids such as Hydrocortisone were also among the 5 most frequently used drugs (37, 38). In early February 2021, Pakistan began spreading out COVID19 vaccine across the country. China has sent vaccines to the country, including CanSino and Sinopharm vaccines, as well as vaccines as part of the COVID-19 Vaccines Global Access (COVAX) initiative (22). However, 7 vaccines approved for use in Pakistan (Table.1) (38):

Vaccine adoption and hesitation among the general public and health-care personnel are critical factors in containing the (COVID)-19 pandemic. While there is evidence of vaccine un-availability all over the world, a total of130, 958,324, vaccine doses had been provided as of August 26th 2022, and 72 percent of

Pakistanis had been infected at least once. Assuming that each individual requires two doses, this would be enough to vaccinate 48.4 percent of the country's population (39).

Table 1. Vaccines approved for use in Pakistan.

RNA	**Moderna Spikevax**	**Approved in 85 countries 60 trials in 22 countries**
RNA	Pfizer/BioNTech Comirnaty	Approved in 142 countries 73 trials in 26 countries
Non Replicating Viral Vector	CanSino Convidecia	Approved in 10 countries 13 trials in 6 countries
Non Replicating Viral Vector	Gamaleya Sputnik V	Approved in 74 countries 24 trials in 7 countries
Non Replicating Viral Vector	Oxford/AstraZeneca Vaxzevria	Approved in 138 countries 62 trials in 30 countries
Inactivated	Sinopharm (Beijing) Covilo	Approved in 91 countries 26 trials in 12 countries
Inactivated	Sinovac CoronaVac	Approved in 55 countries 37 trials in 9 countries

Pakistan's current scenario

The Omicron wave continues to subside globally. Based on vaccination, previous infection, and the current wave of Omicron, we estimate that 57% of the population is immune to Omicron (40). According to WHO, confirmed cases of COVID-19 were 1,567,893, reported in Pakistan from (3rd January 2020 to 26th August 2022), with 30,569 deaths and 1,528,145 are recovered (Fig-2) (41). Sindh province of Pakistan is more prone to COVID-19 as compared to other provinces. Province wise cases of COVID-19 in Pakistan shown in (Table 2) (42).

Table-2 Province wise cases of COVID-19 in Pakistan (42).

PROVINCE	CONFIRMED CASES	DEATHS	RECOVERIES
SINDH	593,120	8,225	567,237
PUNJAB	520,440	13,605	491,786
BALOCHISTAN	35,935	378	35,108
KPK	223,249	6,347	212,886

CONCLUSION

The current review has found that strict observation about the COVID-19 waves and a current scenario that help to build national policies reshape their strategies according to the forecasted situation for effective control of disease during this winter. A vaccine is available for everyone to halt the virus surging in the world, and despite Pakistanis' relatively good response to vaccination, issues negatively influencing their intention must be addressed quickly to control the pandemic. Measures to address public acceptability, trust, and anxiety about the safety and benefit of licensed vaccines are required. It may not be until the second or third week of January 2022 that we have enough evidence to alter our severity assessment.

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