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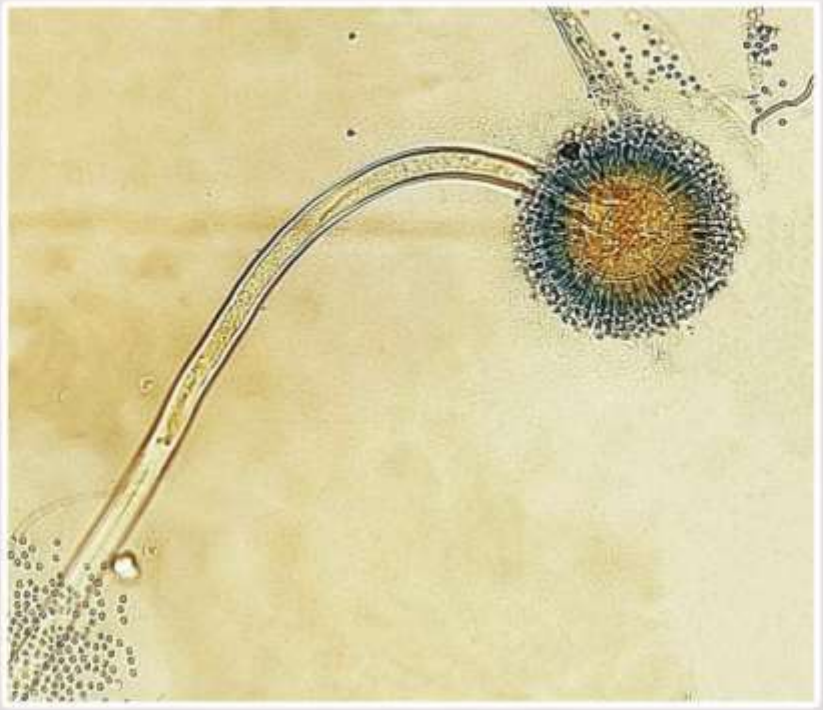

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CONTENTS

ORIGINAL ARTICLE

MOST COMMON PATHOGENIC ORGANISMS CAUSING URINARY TRACT INFECTION IN DIABETIC VERSUS NONDIABETIC PATIENTS AND THEIR ANTIBIOTIC SENSITIVITY PATTERNS

Sundas Shaukat[1], Ashfaq Hussain[2], Mazhar Shaukat[3], Laila Shaukat[4], Nayab Zamir[3], Haseeba Arif[1]

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ABSTRACT

Background: Emerging antibiotic resistance to commonly encountered urinary tract infections are the leading cause of treatment failure in our population. This study aims to identify the most common pathogen causing urinary tract infection in diabetics versus nondiabetics and their antibiotic susceptibility.

Material and Methods: This case control study was conducted Combined Military Hospital Mardan, August 2022 - January 2023. Using a stratified sampling technique 90 patients were divided into Group DM and Group NDM with 45 participants in each group. History, demographic characteristics, symptomatology and empirical treatment prescribed was recorded for each patient and midstream urine sample was collected. Urine culture and sensitivity was performed to identify the organisms and antibiotic susceptibility of the causative agent. Recorded variables included the organisms identified and their sensitivity patterns.

Results: Gender distribution revealed males were 17(37.8%) versus 12(26.7%) from Group DM and Group NDM with a mean age of the participants was 54.64 ± 9.74 years. Clinical features like history of UTI, history of catheterization, asymptomatic bacteriuria was seen more in diabetics as compared to non-diabetics with a p value of < 0.05. *Escherichia coli* was the most common causative organism found in 44 (48.5%) out of 90 patients in both diabetic and non-diabetic patients. Antibiotic susceptibility revealed increased sensitivity of *Escherichia coli* to Fosfomycin (88.6%) followed by nitrofurantoin (81.8%) and meropenem (75%).

Conclusion: *Escherichia coli* is the most common isolate causing urinary tract infection in diabetic and non-diabetic patients which shows greatest sensitivity to Fosfomycin, nitrofurantoin and meropenem.

Key Words: Antibiotic susceptibility, Culture, Pathogen, Sensitivity.

BACKGROUND

Urinary tract infection (UTI) is one of the most common infections encountered in lifetime with a greater prevalence observed in females and diabetics as compared to males and non-diabetics.[1]

Invasion and active multiplication by a variety of organisms can cause inflammatory changes in the epithelium of the urinary tract causing infection. These organisms can travel up the urinary tract into the kidneys resulting into a more complicated infection. A variety of bacteria, viral or fungal organisms can lead to infection of the urinary tract, but the most common infection is caused by bacterial pathogens of which the common invading agents include *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae* and others.[2]

Diabetes is a common metabolic disorder leading to changes in microvasculature and macrovasculature. The decrease response of the immune system, increased blood sugar levels, neuropathy, incomplete bladder emptying and damage to other organ systems leads to greater chances of urinary tract infection. The higher chances of urinary tract infection can be partially explained by impaired function, decreased chemotaxis and suppressed phagocytic activity of the white blood cells in addition to decreased activity of Killer T cells. Several environmental factors, instrumentation of the bladder, decreased hydration and urinary continence are the risk factors which can precipitate the infection.[3]

Keeping in view the hot temperatures, poverty and poor hygiene in our country, the general population are

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more prone to develop urinary tract infection. The treatment of urinary tract infection is not as simple as it was once thought. Prompt recognition of the infection, early commencement of the right empirical antibiotics and adequate hydration are required to decrease the complications.[4]

Treatment of the urinary tract infection in both diabetics and non-diabetics is challenging as the emerging pathogens colonizing the urinary tract infection cannot be eradicated by prescribing a simple antibiotic.[5] Increase in the development of resistance against antimicrobial drugs is a global issue which needs to be addressed. A number of antibiotics have been in use empirically for treatment of urinary tract infection but none to date has been considered as gold standard. Knowledge about the evolving pathogens in our population in diabetics and non-diabetics is the need of hour.[6] The aim of this cross-sectional trial was to identify the most common isolate found in urine specimen of diabetics and non-diabetic patients presenting to our institute and to assess their antimicrobial drug sensitivity patterns.[7]

MATERIAL AND METHODS

This case control study was done at Combined Military Hospital Mardan after obtaining approval from the hospital's ethical review board under ERB no 2010/Estb/EC/02/2022. Who sample size calculator was used for the calculation of sample size. Previous research shows that the presence of urinary tract infection in diabetics was 25% as compared to 2% in non-diabetic patients.[8] With a 90% power of study and a level of significance taken as 5% the calculated sample size was 90. These 90 participants were divided in two groups i.e., Group-DM (Diabetes myelitis) and Group-NDM (non-diabetes myelitis) with 45 patients assigned to each group.

Patients of either gender who were older than 18 years with or without the history of diabetes diagnosed as cases of urinary tract infection, not taking any antibiotics willing to participate in the research were included in the study. Participants who were terminally ill or having a history of genitourinary tuberculosis, females with active menstrual cycles, pelvic inflammatory disease, tubal ovarian pathology, appendicitis colitis, male patients with epididymitis and orchitis were excluded from the trial.

Patients presenting to our hospital with symptomology were interviewed and screened. On the basis of detailed history, clinical examination and laboratory investigations patients with diagnosis of urinary tract infection were recruited in the study after obtaining a written informed consent. On the basis of comorbids, 45 diabetic patients were recruited as cases in Group DM and 45 non-diabetic patients were recruited as controls in Group-NDM. Comorbidities, demographic characteristics and clinical features of each participant were recorded on a predesigned Performa.

Urine samples were collected after providing clear instructions to the patients using aseptic technique obtaining a midstream urine sample. The specimen was inoculated on Blood agar plates and Cysteine lactose electrolyte deficient media (CLED) plates and incubated for 35 degrees Celsius ± 2 under aerobic conditions in ambient air for 48 hours. Cultures yielding growth of $>10^5$ colony forming unit were identified and further processed using microbiological tests. Modified Kirby Bauer method was used for antimicrobial sensitivity. Zone sizes were measured using latest clinical and laboratory standards institute (CLSI) guidelines 2022.[8] Pathogens identified on urine culture and their antibiotic sensitivity patterns were recorded.

RESULTS

The number of patients included in the trial were 90 which were divided into two groups with equal participants in each group. 45 patients with diabetes were assigned to Group DM and 45 patients to Group NDM. Male participants were 17 (37.8%) from group DM and 12 (26.7%) from group NDM however female patients from group DM were 28(62.2%) and 33 (73.3%) from group NDM. Mean age of participants was 62.04 ± 5.59 years in group DM and 47.24 ± 6.99 years in group NDM. Clinical characteristics among both the groups revealed significant difference in regard to previous history of UTI, previous history of catheterization, asymptomatic bacteriuria and fever as shown in Table-I. *Escherichia coli* 44(48.9%) was the most common isolate found in both the groups as a causative organism of urinary tract infection followed by *Enterococcus* 18(20%) and *Klebsiella* 11(12.2%) as shown in table II. Antibiotic susceptibility revealed increased sensitivity of *Escherichia coli* to Fosfomycin

(88.6%) followed by nitrofurantoin (81.8%) and meropenem (75%). Ampicillin, amoxicillin, ceftriaxone and ciprofloxacin showed highest resistance against *E.coli* as shown in Table-III. Ceftriaxone and ciprofloxacin was the most common empirical antibiotic prescribed to patients in both groups as shown in Table-IV.

Table-I: Clinical characteristics of the two groups.

Clinical Characteristics	Group 'DM' n (%)	Group 'NDM' n (%)	p- value
Asymptomatic bacteriuria	16 (35.6%)	04 (8.9%)	0.002
Fever	25 (55.6%)	42 (93.3%)	0.000
Dysuria	08 (17.8%)	06 (13.3%)	0.561
Urinary retention	05 (11.1%)	02 (4.4%)	0.238
Increased frequency of micturition	07 (15.6%)	06 (13.3%)	0.764
Abdominal Pain	04 (8.9%)	06 (13.3%)	0.502
Vomiting	07 (15.6%)	07 (15.6%)	1.00
Pyuria	09 (20%)	11 (24.4%)	0.612
History of UTI	18 (40%)	02 (4.4%)	0.000
History of catheterization	13 (28.9%)	01 (2.2%)	0.000
Hydronephrosis	02 (4.4%)	01 (2.2%)	0.557
Pyelonephritis	03 (6.7%)	00 (0%)	0.078

Table-II: Causative organisms among groups.

Organisms	Group 'DM' n (%)	Group 'NDM' n (%)	Total n (%)
Escherichia coli	25 (55.6%)	19(42.2%)	44(48.9%)
Enterococcus spp	07(15.6%)	11 (24.4%)	18(20%)
Klebsiella pneumoniae	07(15.6%)	04 (8.9%)	11(12.2%)
Pseudomonas aeruginosa	03(6.7%)	04(8.9%)	07(7.8%)
Enterobacter cloacae	02(4.4%)	04(8.9%)	06(6.7%)
Candida	01(2.2%)	03(6.7%)	04(4.4%)

Table-III: Urine isolates and their % resistance to antimicrobials.

Antibiotics	*Escherichia coli* 44 (48.9%)	*Enterococcus spp* 18(20%)	*Klebsiella pneumoniae* 11(12.2%)	*Pseudomonas aeruginosa* 07(7.8%)	*Enterobacter cloacae* 06(6.7%)
Ampicillin	96	61.1	100	100	100
Amoxicillin clavulanate	91	61.1	82	100	100
Ceftriaxone	91	100	82	100	100
Cefepime	75	Not tested	82	71	67
Piperacillin tazobactam	71	Not tested	73	71	67
Trimethoprim sulfamethoxazole	68	Not tested	73	100	67
Ciprofloxacin	93	89	82	71	83
Levofloxacin	84	78	82	71	67
Imipenem	30	61	55	43	50
Meropenem	25	100	36	29	33
Nitrofurantoin	18	22	27	100	50
Fosfomycin	11	11	9	100	00
Vancomycin	Not tested	33	Not tested	Not tested	Not tested

Table-IV: Empirical treatment prescribed among groups.

Antibiotics prescribed empirically	Group 'DM' n(%)	Group 'NDM' n(%)	Total n (%)
Ceftriaxone	18 (40%)	17(37.8%)	35(38.9%)
Ciprofloxacin	07(15.6%)	05 (11.1%)	12(13.3%)
Meropenam	02(4.4%)	03 (6.7%)	05(5.6%)
Tazobactem piperacillin	10(22.2%)	08(17.8%)	18(20%)
Amikacin	01(2.2%)	02(4.4%)	03(3.3%)
Nitrofurantoin	04(8.9%)	05(11.1%)	09(10%)

DISCUSSION

This case control study was conducted to evaluate the most common pathogen responsible for causing UTI in diabetics and non-diabetic patients. The isolates received from all the patients in the trail revealed that *Escherichia coli* was the most common isolate identified in all the specimen collected from participants of both groups. Such patients presenting to outpatient clinics and emergency reception of our hospital were treated empirically with conventional antibiotics by attending doctors. Increase in the resistance of organisms against antimicrobials can lead to failure of treatment. Ceftriaxone was prescribed to 35(38.9%) of the patients while ciprofloxacin to 12(13.3%) of the patients however the antibiotic susceptibility patterns revealed higher resistance of 91% and 93% by *E.coli* respectively.

Similar to our study another trial performed in diabetic patients in Ethiopia revealed that the most common organism causing UTI was *Escherichia coli* (63.6%) followed by *Klebsiella pneumoniae* (13.6%).[9] History of previous urinary tract infection and duration of diabetes mellitus were the leading risk factors leading to infection in diabetics.[10]

Similar to the results achieved from our trial, Raya et al found that Diabetic patients are more prone to urinary tract infections as compared to non-diabetic patients. The most common pathogen responsible for UTI in both diabetics and non-diabetics was *Escherichia coli*. Similarly, the antibiotic susceptibility revealed that the pathogen carry a high resistance against quinolones, penicillin and cephalosporin as found in our trial.[11]

A local trial performed at a tertiary care hospital in Northwest General Hospital and Research Centre, Pakistan also revealed that *Escherichia coli* was the most common pathogen responsible for causing UTI in 71% of diabetic patients and drugs similar to our study including Fosfomycin, nitrofurantoin and imipenem were the most sensitive drugs against the pathogen.[12]

A comparative study conducted in diabetics versus nondiabetics in Nepal revealed that gram negative rods were the most common isolates causing urinary tract infection including *E.coli* and *Klebsiella pneumoniae*.[13] Female gender and diabetes were the predominant risk factors which can cause UTI.[14]

The emergence of resistant strains has led to treatment failure leading to increased morbidity. The usual empirical treatment prescribed to patients presenting with UTI is usually not effective as proved by a study done by et al. This trial suggested to modify the empirical antibiotics for the treatment of UTI for better outcomes.[15]

Diabetics were found to be twice as prone to UTI as in comparison with a nondiabetic patient. Female gender increased the risk up to 5 folds. Asymptomatic bacteriuria was found in 31.4%[16] of the patients as compared to 35.6% in our trial. *Pseudomonas* was identified in 16% of the urine cultures as compared to a lower percentage of 6.7% in our patients.[17]

The increase in prevalence of diabetes among our population leads to increased chances of urinary tract infection.[18] Empirical treatment of antibiotics cannot eradicate the organisms which can result in multiple complications in patients presenting with urinary tract infections.[19] Similar to several trials our study proves that diabetic patients are more prone to develop urinary tract infection and the most common organism responsible for UTI in both diabetics and nondiabetic patients remains *Escherichia coli* which shows greater sensitivity to Fosfomycin followed by nitrofurantoin and meropenem. Empirical treatment given to such patients requires modification and addition of more sensitive drugs for better outcome.

CONCLUSION

Escherichia coli is the most common isolate causing urinary tract infection in diabetic and non-diabetic patients which shows greatest sensitivity to Fosfomycin, nitrofurantoin and meropenem.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Sundas Shaukat: Conception, Research Analysis and Manuscript Drafting.

Ashfaq Hussain: Data collection, Research Supervision.

Mazhar Shaukat: Research analysis and final approval of the version to be published.

Laila Shaukat: Research supervision and analysis

Nayab Zamir: Data interpretation and drafting.

Haseeba Arif: Research analysis and final approval of the draft.

REFERENCES

1. Jameel AY, Artoshi DM. Prevalence of urinary tract infections and their antimicrobial sensitivity among diabetic and non-diabetic patients in Zakho. Sci J Univ Zakho. 2019;7(4):125-31. DOI: 10.25271/sjuoz.2019.7.4.629
2. Bhagat ZP, Sahu MC. Pervasiveness of urinary tract infection in diabetic patients and their causative organisms with antibiotic sensitivity pattern. Apollo Medicine. 2020; 17(1):26. DOI: 10.4103/am.am_2_20
3. Shill MC, Mohsin MN, Showdagor U, Hasan SN, Zahid MZ, Khan SI, et al. Microbial sensitivity of the common pathogens for UTIs are declining in diabetic patients compared to non-diabetic patients in Bangladesh: An institution-based retrospective study. Heliyon. 2023: e12897. DOI: 10.1016/j.heliyon.2023.e12897
4. Shohid S, Ali MT, Saha S, Jahan M, Hossain MB, Roy PK. Current Antibiotic resistance pattern of prevalent uropathogens in urinary tract infection of diabetic patients in comparison with non-diabetics. Sch J App Med Sci. 2022; 5: 816-22. DOI: 10.36347/sjams.2022.v10i05.024
5. Rafeeq M. Isolation and drug susceptibility pattern of uropathogens in Saudi diabetic and non-diabetic patients with urinary tract infection. Bioinformation. 2022; 18(8): 710-7. DOI: 10.6026/97320630018710
6. Owusu E, Adjei H, Afutu E. Similarities in bacterial uropathogens and their antimicrobial susceptibility profile in diabetics and their non-diabetic caregivers at a National Diabetes Management and Research Centre, Accra-Ghana. Diseases. 2022; 10(4): 124. DOI: 10.3390/diseases10040124
7. Zubair KU, Shah AH, Fawwad A, Sabir R, Butt A. Frequency of urinary tract infection and antibiotic sensitivity of uropathogens in patients with diabetes. Pak J Med Sci. 2019; 35(6): 1664. DOI: 10.12669/pjms.35.6.115
8. Kiranmala K, Johnson R, Savio J, Idiculla J. Microbiologic profile and clinical practices in urinary tract infections in a tertiary care center in Southern India. Fam. Med. Prim. Care Rev. 2019; 8(9): 2888. DOI: 10.4103/jfmpc.jfmpc_346_19
9. Worku GY, Alamneh YB, Abegaz WE. Prevalence of bacterial urinary tract infection and antimicrobial susceptibility patterns among diabetes mellitus patients attending Zewditu memorial hospital, Addis Ababa, Ethiopia. Infect Drug Resist. 2021: 1441-54. DOI: 10.2147/IDR.S298176
10. Clare EC, Juliet AO, Maureen OC, Romeo AJ, Ada KO. Urinary tract infections and antimicrobial sensitivity patterns of uropathogens isolated from diabetic and non-diabetic patients attending some hospitals in Awka. Am J Microbiol Res. 2021; 9(3): 83-91. DOI:10.12691/ajmr-9-3-3
11. Raya S, Belbase A, Dhakal L, Prajapati KG, Baidya R. In-vitro biofilm formation and antimicrobial resistance of Escherichia coli in diabetic and nondiabetic patients. Biomed Res. Int. 2019; 2019. DOI: 10.1155/2019/1474578
12. Ahmad S, Hussain A, Khan MS, Shakireen N, Ali I. Diabetes mellitus and urinary tract infection: Causative uropathogens, their antibiotic susceptibility pattern and the effects of glycemic status. Pak J Med Sci. 2020; 36(7): 1550. DOI: 10.12669/pjms.36.7.2881
13. Gautam S, Sapkota R. Comparative study of isolates associated with urinary tract infection among diabetic and non-diabetic patients attending tertiary care hospital, Chitwan, Nepa. Int J Innov Sci Res. 2020; 5: 531.
14. Andaleeb H, Zia W, Shahid A, Tarar ZI, Shams N, Haq K, Hamid MF. Spectrum of antimicrobial susceptibility pattern of urinary tract infection in in adults: Antimicrobial susceptibility pattern of urinary tract infection. Pak Bio Medical J. 2022 Aug 31:60-6. DOI: 10.54393/pbmj.v5i8.751
15. Elsaeed AM, Radwan AA, Mohamed MK. Prevalence of bacteriuria, candiduria and antibiotics susceptibility patterns among diabetic verses nondiabetic patients. Al-Azhar Int Med J. 2020 Sep 1;1(9):189-95. DOI: 10.21608/AIMJ.2020.38649.1297
16. Kumar R, Kumar R, Perswani P, Taimur M, Shah A, Shaukat F. Clinical and microbiological profile of urinary tract infections in diabetic versus non-diabetic individuals. Cureus. 2019; 11(8). DOI: 10.7759/cureus.5464
17. Akash MS, Rehman K, Fiayyaz F, Sabir S, Khurshid M. Diabetes-associated infections: Development of antimicrobial resistance and possible treatment strategies. Arch. Microbiol. 2020; 202: 953-65. DOI: 10.1007/s00203-020-01818-x
18. Ajith R, Kumar RN, Ozioma NC, Pratthyusha VT, Pranavi Y, BK, Karuppusamy UB, et al A Clinical audit study on the management of urinary tract infection in a Tertiary Care Hospital. J Pharm Negat Results. 2022: 1765-72. DOI: 10.47750/pnr.2022.13.S08.213
19. Nath T, Das SK, Hazra S. Pattern of uropathogens and antibiotic sensitivity in diabetes patients attending to out–patient department and diabetes clinic of a teaching hospital: A cross-sectional study. J Family Med Prim Care. 2021; 10(10): 3638. DOI: 10.4103/jfmpc.jfmpc_71_21

ORIGINAL ARTICLE

COMPLICATIONS OF PLASMAPHERESIS IN COVID-19 PATIENTS AT PAKISTAN AIR FORCE HOSPITAL MUSHAF

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ABSTRACT

Background: Plasmapheresis is a method of blood purification in which blood plasma and components are removed and treated from blood circulation. With advances in treatment of COVID-19, plasma exchange was found effective against disease complicated by cytokine storm syndrome. Plasmapheresis was started in our setup in January 2021. Study was conducted to observe adverse events related to procedure.

Material and Methods: A hospital based descriptive case series was conducted from January 2021 to June 2021, in Pakistan Air Force Hospital Mushaf, Sargodha, Punjab, Pakistan. Adverse events like hypocalcemia, hypotension and cardiac arrest were observed in 44 plasmapheresis sessions. Data was collected by generating a proforma and was analyzed by using IBM SPSS. v.24.

Results: Data from our study revealed that complications were seen in 25% of plasmapheresis sessions with most of the complications being minor and easy to manage and fatal events in 0.04% cases only. Complication during maintenance of Intra Venous line was seen in only one session, during procedure in 2 sessions and after procedure in 8 sessions. The only complication related to maintenance of Intra Venous access was atrial fibrillation in a single case [1]. Complications seen during procedure included hypotension in 1 session and cardiac arrest in one. Adverse events seen after plasmapheresis were apprehension & palpitation in one case, drowsiness in one, drowsiness and Surgical Emphysema at Intra Venous access site in one, Hypocalcemia in three, Hypocalcemia with drowsiness in one and Hypomagnesemia in one case.

Conclusion: After interpretation of analysis and reviewing literature, it was seen that plasmapheresis is a safe procedure if it is used with caution and professional expertise.

Key Words: Plasmapheresis, Cytokine Storm, Adverse Effects, Complications, COVID-19

BACKGROUND

Plasmapheresis is a method of blood purification in which blood plasma and components with large molecular weight are removed and treated from blood circulation and then returned to it.[2] This method is being used as a treatment modality in different disease states for many decades. Plasmapheresis was well established in late 70's. Dr. Emil Freireich was the pioneer in using this procedure in different types of leukemias and other malignancies.[3]

With advances in treatment of Corona Virus Disease of 2019 (COVID-19), plasmapheresis was found effective against disease complicated by cytokine storm syndrome.[4,5,6]

Cytokine Storm, being predictor of mortality and disease severity, is frequently seen in severe infections with *SARS*, *MERS* and *Influenza* as well as in *COVID-19*.[7,8] Cytokine storm is the release of excessive amounts of cytokines (tumor necrosis factors, chemokines, interleukins, interferons, colony stimulating factors etc.) from cells of immune system into blood.

After receipt of data regarding its efficacy, plasmapheresis is being used in critically ill COVID-19 patients, complicated by cytokine storm and respiratory failure in PAF Hospital Mushaf after taking informed consent from patient's attendants. This treatment modality was started in this setup in January, 2021 by using Spectra Optia Apheresis system (an alternate to COBE spectra device).[9] Fresh frozen plasmas are being used as an exchange fluid.

A case study of 31 patients was conducted in Oman which revealed beneficial role of therapeutic plasma exchange in COVID-19 patients with higher extubation rate and decreased mortality.[5] A study was published in Dec 2020 which reviewed the beneficial role of therapeutic plasma exchange in COVID-19 patients and its complications.[10]

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A hospital based descriptive case study was conducted at PAF Hospital Mushaf to assess the adverse events and complications in patients while preparing for plasmapheresis, during the procedure and after completion of the process. So that, the effective treatment modality against the novel corona virus disease can be used in a safer way. A separate study was conducted in same setup to assess beneficial role of plasmapheresis in COVID-19 patients.

MATERIAL AND METHODS

A hospital based descriptive case series was conducted for six months duration i.e. from January 2021 to June 2021 in COVID ITC of PAF Hospital Mushaf to observe adverse effects of plasmapheresis in COVID-19 patients. Study included all plasmapheresis sessions performed during the study interval i.e. 44 sessions performed in 13 COVID-19 patients complicated by cytokine storm and having respiratory failure. Procedure was performed after taking informed written consent from attendants of all patients.

Patients who received plasmapheresis therapy were observed for complications along with review of their medical records. For plasmapheresis, Spectra Optia apheresis system was used. Fresh Frozen Plasma from healthy donors was used as an exchange fluid with anticoagulant and normal saline. Data was collected by using a self-generated Performa filled while directly observing patients and with review of their medical records. Data was analyzed by using IBM SPSS.V.24.

Study included critically ill patients with COVID-19 pneumonia having

- Positive/negative PCR for SARS COV-2
- >50% lung involvement on CT Chest and
- Raised inflammatory markers including IL-6, serum ferritin, LDH and D-Dimers

Operational Definitions:

Adverse events: Any unexpected medical problem related to technique, instrument or procedure of plasmapheresis.

Serious Adverse event: Any adverse event leading to death or directly causing death of patient.

Causality of adverse event with procedure: Causes of adverse events related to procedure.

RESULTS

In our study 44 plasmapheresis sessions were performed in 13 critically ill COVID-19 patients. In 27 sessions, patients had positive PCR for SARS COV 2. In other cases patients had negative PCR but were diagnosed as case of COVID-19 pneumonia clinically. The positive percentage was 61.4% while the negative percentage was 38.6%. All sessions were performed in patients with greater than 50% lung involvement on CT scan chest. For twenty-two Plasmapheresis sessions IV access was secured in Right Femoral Vein, for twenty-one sessions in Internal Jugular Vein, and for only a single session IV line was maintained in Right Subclavian vein. Complication during maintenance of IV access, developed in only one case, i.e. atrial fibrillation [1], in which IV line was placed in Right Subclavian vein i.e. 2.3% (Figure-I).

No anaphylactic reaction was observed. Complications were found during two out of 44 sessions (Table-I), i.e 4.5%. One of the complications was hypotension, which was managed with IV Phenylephrine. The other was sudden cardiac arrest just after starting plasmapheresis leading to death of patient. Cause of death could not be confirmed as consent for autopsy was not granted by attendants.

Prophylactic Calcium Gluconate was given before 7 plasmapheresis sessions only.[14,15] Adverse events seen after plasmapheresis were, apprehension & palpitation in one case, drowsiness in one, drowsiness and Surgical Emphysema at IV-line site in one, Hypocalcemia in three, Hypocalcemia with drowsiness in one case and Hypomagnesemia in one case (Figure-II).

Data compilation of all 44 sessions revealed that adverse events were observed in 11 plasmapheresis sessions with a cumulative percentage of 25%. Most of them are minor and can easily be dealt with. Fatal events were seen in 2 sessions only i.e 0.04%.

Table-I: Complications during Plasmapheresis (cross tabulation)

		What was the complication			Total
			Hypotension	Cardiac Arrest	
Complications during procedure	Yes	2	1	1	2
	No	42	0	0	42
Total		**44**	**1**	**1**	**44**

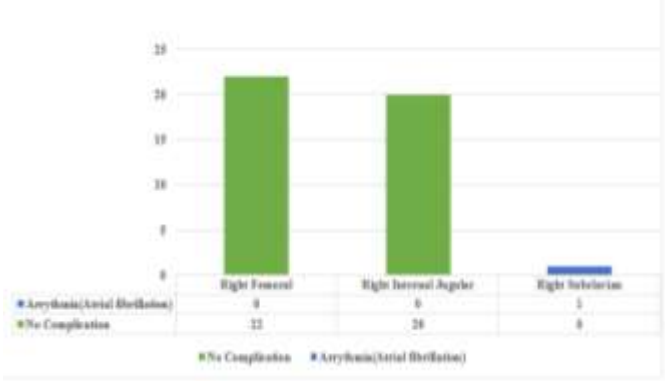

Figure-I: IV access and complications related to the site of insertion.

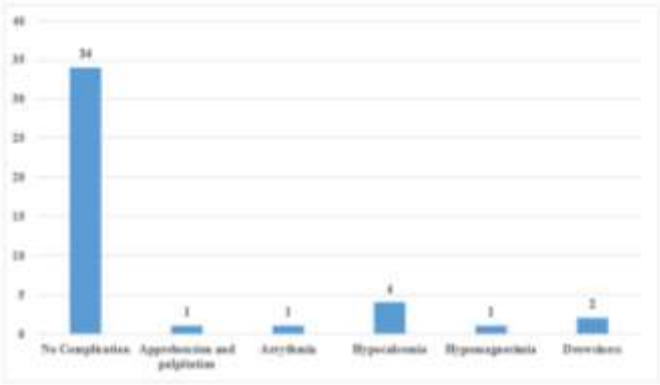

Figure-II: Side effects of plasmapheresis.

DISCUSSION

Plasmapheresis and other modalities to overcome cytokine storm were found effective in life threatening COVID-19 infections[11,4,6,12] but there are few complications which are related to procedure and can cause morbidity and mortality. Different studies have been conducted across the globe for decades to assess the complications of plasmapheresis in different settings and diseases. Our study and literature revealed that different factors can have impact on adverse effects related to plasmapheresis like underlying disease, type of vascular access, type of replacement fluid and type of anticoagulation. Vascular access complications can be infections and others as for vascaths. Procedural problems can be hypocalcemia (due to citrate anticoagulants), hypotension due to vasovagal response, hemolysis and air embolism. Replacement fluid effects can be transfusion related reaction, coagulopathy, removal or dilution of drugs, hypothermia, infections, anemia, hepatitis, electrolyte imbalance, sepsis and hypoproteinemia.

A study in Iran showed role of plasmapheresis in COVID-19 and concluded that main factor in success of therapy is in starting therapy at early stages of inflammation. Study also revealed, safety of technique in COVID-19 patients. In this study use of albumin as an exchange fluid was considered to be associated with complications like reduction of immunoglobulins and coagulopathy as COVID-19 patients are already at risk of coagulopathy and use of albumin instead of FFPs may increase the risk of bleeding and depletion of procoagulant factors. Study suggested that a potential complication with plasmapheresis was removal or dilution of antivirals or steroids. So these drugs must be administered after procedure. [10]

Keeping in view of all available data plasmapheresis was started in our setup in patients of Novel Corona Virus, complicated with cytokine storm and respiratory distress syndrome.

We used Calcium Gluconate prophylactically in 7 plasmapheresis sessions as it was supported by previous studies. A study published in 2018 compared two methods of Calcium gluconate administration during therapeutic plasma exchange, one method was use of 5ml of 10% Calcium Gluconate in 500ml of replacement fluid and other was IV infusion of 2g Calcium Gluconate in 50mL normal saline at 25ml/hr. First method was found better with lesser hypocalcemic events but more use of Calcium Gluconate.[14] In other study, 2g of Calcium Gluconate was given in 50ml Normal saline either at rate of 1.0 or 1.6 g/hr. Hypocalcemic events were seen in 5 of 17 sessions with 1.0g/hr but in 0 of 17 with 1.6 g/hour.[15] In our setup we used 10ml of 10% calcium gluconate in 100ml of Normal Saline.

Complications related to plasmapheresis were studied in past for different diseases but no such studies are conducted in patients of COVID-19, so complications are compared with procedure done in other diseases.

A study was published in Journal of Clinical apheresis in which retrospective review of 3624 sessions in 401 patients was done. In 67.7% of those procedures 80% albumin and 20% saline was used, while in remaining procedures albumin alone was used as replacement fluid. It was concluded that partial use of saline was associated with greater risk of hypotension . [13]

A prospective observational study was conducted in India regarding effect of Double filtration plasmapheresis on various plasma components. Study revealed that bleeding complications were seen in only

those cases in which fibrinogen level was lesser than 50% and hypocalcemic events were lesser with use of effluent albumin concentration as replacement fluid [16].

A paper was published in 2014, which revealed complications of plasma exchange in patients with Thrombotic Thrombocytopenic purpura and related microangiopathic anemias. Results showed that overall percentage of complications was low with 6.45% venous thrombotic events, 6.45% allergic plasma reactions with only 1 acute anaphylaxis, 9.6% citrate reactions and 8.3% line associated infections.[17]

A study was conducted to analyze incidence of plex related complications in ICU patients. Study documented episodes of hypotension in 8.4%, arrythmia in 3.5%, fever with chills in 1.1% and paresthesias in 1.1% procedures.[18]

A study was published in 2012, which was based on concepts, mechanisms and overview of apheresis guidelines. Study revealed that frequency of complications with plasma exchange is different in literature and depends on whether an effect is considered complication or not. Study reported that in an older study, rate of complications was 17% with 6.14% severe reactions and newer studies had more divergent data ranging between 4.75% and 36%.[19]

An article was published in Journal of Clinical Apheresis in which 883 plasma exchange sessions were analyzed retrospectively for complications and their causes. Study concluded that adverse events were present in 25.6% procedures, with 13.7 % being mild, 11.0% moderate and only 0.7% were severe adverse effects including 0.4% anaphylaxis and 0.3% sepsis. Complications were more common in patients with neurological disorders.[20]

In an article data was compiled of prospective examination of 1727 plasma exchange sessions. Total 889 complications were observed in 614 or 36% of treatments. Most of the complications were minor with fever in 7.7%, urticaria in 7.4% and hypocalcemia in 7.3% treatments. Complications were seen in 42% of sessions with Fresh Frozen Plasma and in 30% with Albumin Saline as an exchange fluid. Hypotension was more common in sessions with Albumin saline as an exchange fluid.[21]

A study analyzed data of 4857 plasma exchange sessions retrospectively and reported that total 231(4.75%) adverse events were seen. Paresthesias being the most common was seen in 2.7%, hematoma at site of IV access in 2.4%, clotting in 1.7%, urticaria in 1.6% and bleeding in 0.06% cases. The incidence of life threatening adverse effects was 0.12% and true anaphylactoid reactions were seen in only 5 cases. Use of prophylactic Calcium and potassium lead to lesser electrolyte disturbances.[22]

It was revealed in a research that hemolysis was the most common complication of Double Filtration Plasmapheresis and occurred in 20% of patients. Hypotension was reported in 3.3% sessions and in 17% sessions Vascular-access-related complications were seen. One episode of bleeding was reported due to overt coagulopathy.[23]

CONCLUSION

Based on literature and results of this study, it is concluded that plasma exchange is associated with adverse events which are related to technique, exchange fluid used and coagulopathy. Out of complications and adverse events most of the adverse events observed are of minor significance only. Patients can get more benefits and lesser side effects if plasma exchange is started earlier in COVID-19. Further benefits can be achieved with administration of electrolytes like calcium gluconate prophylactically.

CONFLICT OF INTEREST

Authors declare no conflict of interest

CONTRIBUTION

Hania Afzal: Conception, the acquisition, analysis, interpretation of data and manuscript writing.

Muhammad Asif Naseer: Conception, the acquisition, analysis, interpretation of data and manuscript writing.

Muzammil Khalid: Discussed the results and contributed to final manuscript.

REFERENCES

1. Deere M, Singh A, Burns B. Central Venous Access of The Subclavian Vein. [Updated 2022 Jan 25]. In: StatPearls [Internet]. Treasure Island (FL)
2. Kaplan AA. Therapeutic plasma exchange: A technical and operational review. J Clin Apher. 2013; 28(1):3-10. DOI: 10.1002/jca.21257
3. Rock G. Apheresis then and now. Transfus Apher Sci. 2019; 58(3): 230-6. DOI: 10.1016/j.transci.2019.04.005
4. Khamis F, Al-Zakwani I, Al Hashmi S, Al Dowaiki S, Al Bahrani M, Pandak N, *et al*. Therapeutic plasma exchange in adults with severe COVID-19 infection. Int J Infect Dis. 2020; 99: 214-8. DOI: 10.1016/j.ijid.2020.06.064
5. Tabibi S, Tabibi T, Conic RRZ, Banisaeed N, Streiff MB. Therapeutic plasma exchange: A potential management

strategy for critically Ill COVID-19 patients. J Intensive Care Med. 2020; 35(9): 827-35. DOI: 10.1177/0885066620940259

6. Shi H, Zhou C, He P, Huang S, Duan Y, Wang X. Successful treatment with plasma exchange followed by intravenous immunoglobulin in a critically ill patient with COVID-19. Int J Antimicrob Agents. 2020; 56(2): 105974. DOI: 10.1016/j.ijantimicag.2020.105974
7. Zhang Y, Yu L, Tang LL, Zhu Mengfei, Jin Yanqi, Wang Z, *et al*. A promising anti-cytokine-storm targeted therapy for COVID-19: The artificial-liver blood-purification system. Engineering. 2021; 7(1): 11-3. DOI: 10.1016/j.eng.2020.03.006
8. Huang C, Wang Y, Li XW, Ren L, Zhao J, Hu Y, *et al*. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020; 15; 395(10223): 497-506. DOI: doi.org/10.1016/S0140-6736(20)30183-5
9. Hequet O, Stocco V, Assari S, Drillat P, Le QH, Kassir A, *et al*. Comparison of plasma exchange performances between Spectra Optia and COBE Spectra apheresis systems in repeated procedures considering variability and using specific statistical models. Transfus Apher Sci. 2014; 51(1): 47-53. DOI: 10.1016/j.transci.2014.07.005
10. Balagholi S, Dabbaghi R, Eshgi P, Mousavi SA, Heshmati F, Mohammadi S. Potential of therapeutic plasmapheresis in treatment of COVID-19 patients: Immunopathogenesis and coagulopathy. Transfus Apher Sci. 2020; 59(6): 102993. DOI: 10.1016/j.transci.2020.102993
11. Turgutkaya A, Yavaşoğlu İ, Bolaman Z. Application of plasmapheresis for Covid-19 patients. Ther Apher Dial. 2021; 25(2): 248-9. DOI: 10.1111/1744-9987.13536
12. Dogan L, Kaya D, Sarikaya T, Zengin R, Dincer A, Akinci IO, *et al*. Plasmapheresis treatment in COVID-19–related autoimmune meningoencephalitis: Case series. Brain Behav Immun. 2020; 87: 155-8. DOI: 10.1016/j.bbi.2020.05.022
13. Irani MS, Toushan M, Zhang L, Simpson PM, Karafin MS. Risk of hypotensive reactions is increased when using partial saline replacement for therapeutic plasma exchange. J Clin Apher. 2019; 34(5): 524-7. DOI:10.1002/jca.21703
14. Zhao Y, Linden J, Welch L, St Pierre P, Graves M, Garrity D, *et al*. Prophylactic infusion of calcium gluconate to prevent a symptomatic fall in plasma ionized calcium during therapeutic plasma exchange: A comparison of two methods. J Clin Apher. 2018; 33(5): 600-3. DOI:10.1002/jca.21648
15. Zhao Y, Garrity D, Graves M, Linden J, St Pierre P, Ducharme P, *et al*. Optimization of infusional calcium gluconate for prevention of hypocalcemic reactions during therapeutic plasma exchange. J Clin Apher. 2019; 34(6):656-60. DOI:10.1002/jca.21742
16. Jagdish K, Jacob S, Varughese S, David VG, Mohapatra A, Valson A, *et al*. Effect of double filtration plasmapheresis on various plasma components and patient safety: A prospective observational cohort study. Indian J Nephrol. 2017; 27:377-83. DOI: 10.4103/ijn.IJN_64_17
17. McGuckin S, Westwood JP, Webster H, Collier D, Leverett D, Scully M. Characterization of the complications associated with plasma exchange for thrombotic thrombocytopaenic purpura and related thrombotic microangiopathic anaemias: A single institution experience. Vox Sanguinis. 2014; 106(2):161-6. DOI: 10.1111/vox.12090
18. Wojciech S, Katarzyna W-A, Lek WA, Wludarczyk A. Complications in patients treated with plasmapheresis in the intensive care unit. Anaesthesiol Intensive Ther. 2013; 45(1):7-13. DOI:10.5603/AIT.2013.0002
19. Winters JL. Plasma exchange: concepts, mechanisms, and an overview of the American Society for Apheresis guidelines. Am Soc Hematol Educ Program. 2012; 2012(1):7-12. DOI: 10.1111/vox.12090
20. Bramlage CP, Schröder K, Bramlage P, Ahrens K, Zapf A, Muller GA, *et al*. Predictors of complications in therapeutic plasma exchange. J Clin Apher. 2009; 24(6): 225-31. DOI: 10.1002/jca.20217
21. Shemin D, Briggs D, Greenan M. Complications of therapeutic plasma exchange: A prospective study of 1,727 procedures. J Clin Apher. 2007; 22(5): 270-6. DOI: 10.1002/jca.20143
22. Basic-Jukic N, Kes P, Glavas-Boras S, Brunetta B, Bubic-Filipi Ljubica, Puretic Z. Complications of therapeutic plasma exchange: Experience with 4857 treatments. Ther Apher Dial. 2005; 9(5): 391-5. DOI: 10.1111/j.1744-9987.2005.00319.x
23. Yeh JH, Chen WH, Chiu HC. Complications of double-filtration plasmapheresis. Transfusion. 2004; 44(11): 1621-5. DOI: 10.1111/j.1537-2995.2004.04154.x

ORIGINAL ARTICLE

PERFORMANCE OF THE VITEK MS MATRIX-ASSISTED LASER DESORPTION IONIZATION-TIME OF FLIGHT MASS SPECTROMETRY SYSTEM FOR RAPID IDENTIFICATION OF CLINICAL MICROBIOLOGY ISOLATES

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ABSTRACT

Background: Rapid and precise microbial identification is essential for timely patient management and control of infection. Conventional microbiological procedures are time-consuming, laborious, and require expertise. Recent coalition of MALDI-TOF in the microbiology workflow has yielded excellent results so far. Here we have assessed this quick proteomic based technique for clinical bacterial isolates identification.

Material and Methods: All fresh bacterial isolates were selected for VITEK MS (bioMérieux, France*)* analysis, the bacteria were identified by using MALDI-TOF System (IVD 3.2) software. For same isolates, API (bioMérieux, France*)* was set as per kit instructions and conventional/biochemical tests were performed according to ASM Guidelines.

Results: Among 200 isolates 99% were accurately identified by the VITEK MS system to the species level, one of two isolates was mis-identified (*Shigella* as *E. coli*) while the other was identified later by re-spotting. On testing these samples in parallel by APIs, 91.50% were correctly identified, while 8.50% (17 samples) showed discrepant results. These were re-analyzed by VITEK-2 (bioMérieux, France*)* semi-automated system which showed the same results as those of VITEK MS. Our findings revealed diagnostic accuracy of VITEK MS in comparison with APIs in terms of time, cost and patient management.

Conclusion: For bacterial identification MALDI-TOF MS is an expeditious, authentic and comparatively inexpensive system. Our results emphasize that it is speedy technique which can replace the traditional identification methods for most of bacterial strains on their routine isolation. This ingenious approach complies with advanced patient management and therapeutic intervention.

Key Words: MALDI-TOF MS, bacterial identification, VITEK-2, Analytical profile index (API), Clinical microbial isolates

BACKGROUND

Due to substantial increase in drug resistance, prompt bacterial identification is imperative for the initiation of antimicrobial treatment and infection prevention.[1] Conventional identification methods are laborious, and have a long turnaround time as these involve observational procedures like growth on appropriate medias, colony appearance, Gram stain results, microscopy, and biochemical reactions. However molecular methods are not commendable for extensive routine identification. Accurate and quick microbial identification provides exact knowledge about the infectious cause. Un-identified organisms could also be identified using DNA sequencing by reference laboratories. Currently semi-automated biochemical test programs like VITEK-2 (bioMérieux, France*)* are commonly used in analytical microbiology laboratories alongside regular culture methods for day-to-day microbial isolation thus decreasing mean processing time up to a few hours. Although, cost of the reagents is a limiting factor in the laboratory setting of a developing country, like Pakistan.[2,3]

Genomics and proteomics are among various methods used to study bacterial macromolecules (genome, protein, polysaccharides, phospholipids). A genomic method gives us extensive information about bacterial genome while proteomics helps us to recognize multiple bacterial proteins.[4] VITEK MS Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry uses proteomics, to study bacterial protein content and provides detailed information about the cell and helps in identifying microorganism including mycobacterium, viruses,

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parasites, molds and yeast. This instrument along with antimicrobial susceptibility testing results could prove helpful in early patient management, monitoring and epidemiological surveillance.[5]

MALDI-TOF MS instrument was officially launched for diagnostic use in clinical microbiology department in Chughtai Laboratory, Lahore in January 2021. The aim of our study is to assess the performance and diagnostic accuracy of the VITEK MS (bioMérieux, France) on clinical microbiology aerobic and anaerobic isolates by processing 200 samples during a 6-months period (February -July 2022).

MATERIAL AND METHODS

After approval from institutional review board (Letter No CIP/IRB/1101) this cross-sectional study was conducted over a six-month period February 2022 to July 2022 at the Chughtai Laboratory Lahore, Pakistan. Approximately 200 samples were processed using non-probability convenience sampling technique (aerobic/anaerobic), clinical isolates were recovered from different clinical samples including blood, urine, CSF, wound, and sputum.

Before subjecting isolates to MALDI-TOF MS, they were initially isolated on selective/non-selective media as 5% Blood agar plate (BAP), Chocolate agar (CHOC), and MacConkey agar (MAC) for aerobes and Sheep blood agar (SBA) for anaerobes. Respective plates were incubated aerobically for 18-24 hours and 48-72 hours for anaerobes under 35C incubation shown in Figure-I.

All isolates were identified with conventional/biochemical tests as per ASM guidelines. The APIs or Analytic Profile Index classifies bacteria on the basis of biochemical reactions, allowing rapid identification of known bacteria (Figure-II). Results of APIs were compared with VITEK MS. Any discrepancies when occurred were subsequently resolved by semi-automated biochemical test platform, VITEK-2.

Clinical isolates identification done by VITEK-MS system utilizing direct single deposit from bacterial colonies in absence of prior extraction according to manufacturer's guidelines as shown in Figure-III.

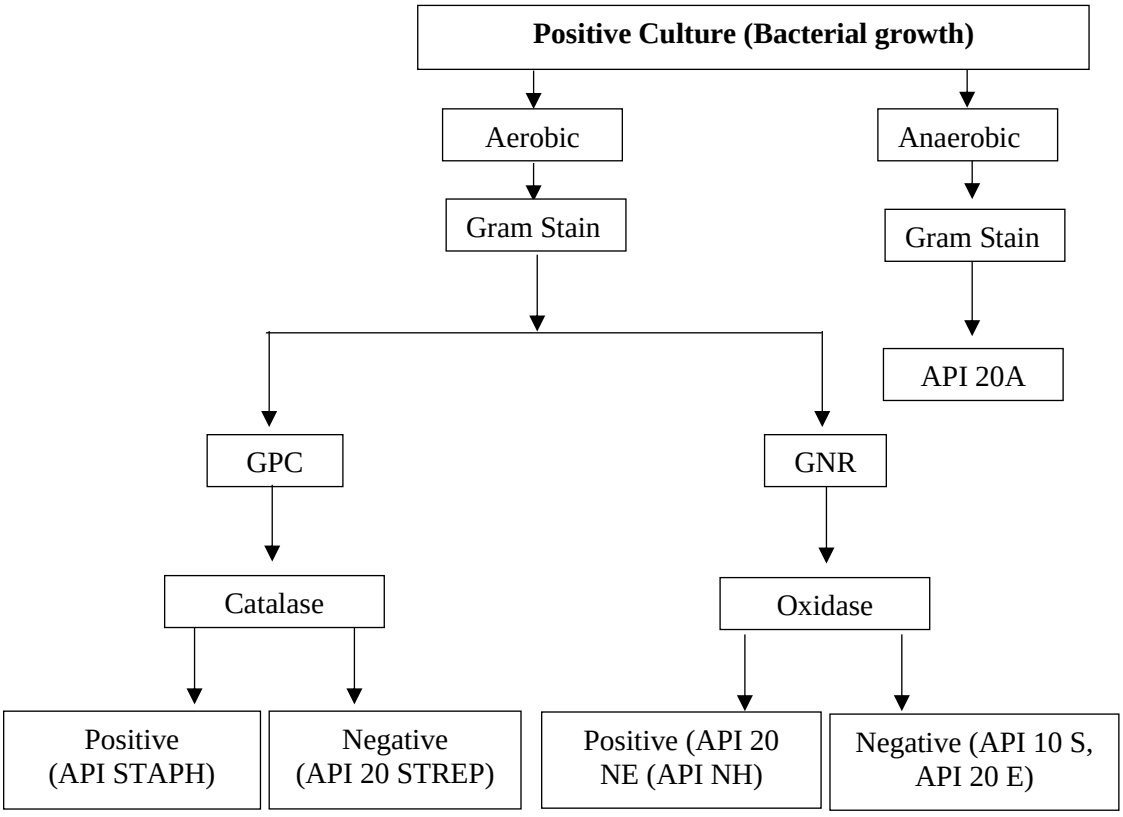

Figure -I: Workflow chart for conventional identification of isolates.

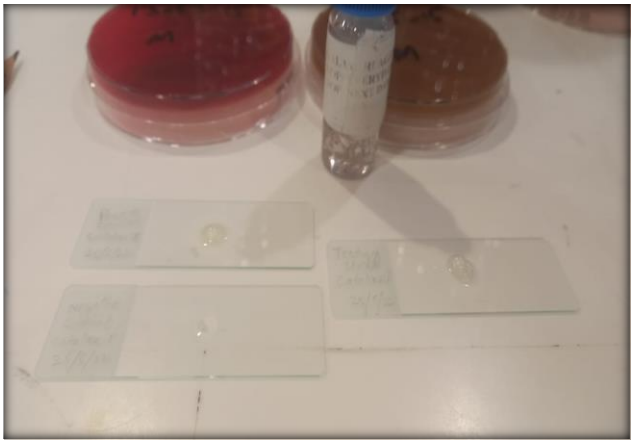

Figure-II: API System for identification of clinical bacterial isolates.

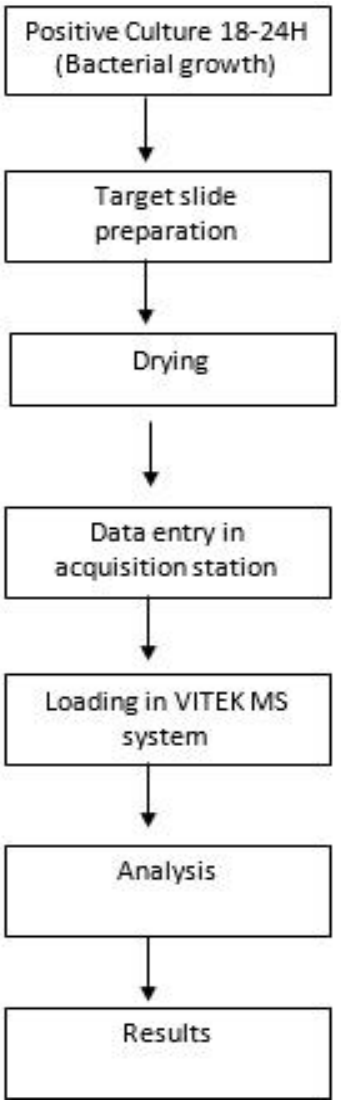

Figure-III: VITEK MS (bioMérieux, France) system.

RESULTS

200 isolates were analyzed by the VITEK MS (IVD 3.2, >15000 bacterial spp. in database) during six months study period and in parallel by the conventional/biochemical tests, APIs. Among all the bacterial isolates (*Enterobacterales*, Non-fermentor, Gram-negative rods, *Staphylococci*, *Streptococci*,

Enterococcus and other GPCs, Anaerobes) 198/200 (99%) were correctly identified by the VITEK MS system with confidence value 99.9% as shown in Table-I. On account of remaining two isolates, one was misidentified (*Shigella* as *E.coli*) while another was unidentified first due to insufficient quantity of colony while spot preparation however identified later by re-spotting which emphasizes the fact that improper spot can result in poor identification.

APIs with regular biochemical tests were also performed simultaneously on the same 200 isolates along with controls which showed correct identification for 183/200 (91.50%) isolates. While analyzing the results of VITEK MS and conventional system, 17 samples (8.50%) showed discrepant result. The discrepant results were analyzed by VITEK-2 Semi automation and the obtained results found in agreement with VITEK MS results as shown in Table-II. Semi-automated VITEK 2 system was used to cope discrepancies and to provide correct identification up to specie/subspecies level. Comparison of APIs and VITEK MS identification is shown in Figure-IV.

Table-I: Identification comparison between VITEK MS and API systems.

Clinical bacterial isolate	Number of isolates	Correct identification		Misidentification		No identification	
		VITEK MS	API	VITEK MS	API	VITEK MS	API
		Enterobacterales					
Citrobacter amalonaticus	1	1	1				
Citrobacter freundii	1	1	1				
Enterobacter aerogenes	1	1	1				
Enterobacter cloacae	6	6	6				
Enterobacter fergusoni	1	1					1
Enterobacter hormaechei	3	3					3
Escherichia coli	36	36	36				
Klebsiella pneumoniae	18	18	18				
Morganella morganii	5	5	5				
Pantoea dispersa	1	1					1
Proteus mirabilis	2	2	2				
Proteus vulgaris	1		1			1	
Providencia rettgeri	2	2	2				
Providencia stuarti	1	1	1				
*Salmonella enterica**	11	11	11				
Serratia marcescens	19	19	19				
Shigella sonnei	1		1	1			
Non-fermenters							
Acinetobacter baumannii	13	13	13				
Acinetobacter calcoaceticus	1	1	1				
Acinetobacter haemolyticus	1	1	1				
Acinetobacter junii	1	1	1				
Acinetobacter pitti	2	2					2
Achromobacter denitrificans	1	1					1
Aeromonas jandaei	1	1					1
Aeromonas punctata	1	1					1
Aeromonas sobria	1	1	1				
Burkholderia cenocepacia	1	1					1
Burkholderia cepacia	1	1	1				
Burkholderia contaminans	6	6					6
Chryseobacterium indologenes	1	1	1				
Pseudomonas aeruginosa	8	8	8				
Pseudomonas mendocina	2	2	2				
Pseudomonas putida	1	1	1				
Pseudomonas stutzeri	4	4	4				
Rhizobium radiobacter	1	1	1				
Stenotrophomonas maltophilia	13	13	13				
		Staphylococci					
Staphylococcus aureus	8	8	8				

Staphylococcus capitis	1	1	1
Staphylococcus cohnii	1	1	1
Staphylococcus haemolyticus	3	3	3
Staphylococcus hominis	1	1	1
Staphylococcus saprophyticus	1	1	1
Staphylococcus sciuri	1	1	1
Streptococci			
Streptococcus agalactiae	2	2	2
Streptococcus anginosus	1	1	1
Streptococcus dysgalactiae	1	1	1
Streptococcus mitis	2	2	2
Streptococcus pneumoniae	2	2	2
Streptococcus pyogenes	1	1	1
Enterococcus* & other *GPC			
Enterococcus faecalis	1	1	1
Micrococcus luteus	2	2	2
Anaerobe			
Bacteroides thetaiotaomicron	1	1	1
HACEK			
H. influenzae	1	1	1

Table-II: Conformation of discrepant results by VITEK-2 semi-automated system.

API result	VITEK-MS result	VITEK-2 result	Total (n=17)
Enterobacter spp.	*Enterobacter fergusoni*	*Enterobacter fergusoni*	1
Enterobacter spp.	*Enterobacter hormaechei*	*Enterobacter cloacae complex**	3
Pantoea spp.	*Pantoea dispersa*	*Pantoea spp.**	1
Acinetobacter spp.	*Acinetobacter pitti*	*Acinetobacter baumannii complex**	2
Achromobacter spp.	*Achromobacter denitrificans*	*Achromobacter denitrificans*	1
Aeromonas spp.	*Aeromonas jandaei*	*Aeromonas jandaei*	1
Aeromonas spp.	*Aeromonas punctata*	*Aeromonas punctata*	1
Burkholderia spp.	*Burkholderia cenocepacia*	*Burkholderia cenocepacia*	1
Burkholderia spp.	*Burkholderia contaminans*	*Burkholderia contaminans*	6
Shigella sonnei	*Escherichia coli*	*Shigella sonnei*	1

Table-III: Time dynamics for VITEK-MS.

Workflow	Time (seconds)
Slide spot preparation	20
Drying	120
Matrix application	10
Drying	120
Loading into system	60
Result time	40
Total time	**370 (6minutes,10seconds)**

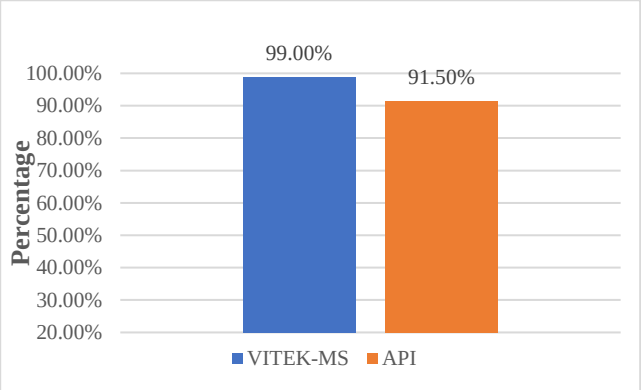

Figure-IV: Comparison of API and VITEK-MS identification percentage.

DISCUSSION

MALDI-TOF MS, is a rapid and sensitive technique for identifying clinical microbiological isolates. This study proposes bacterial identification comparison between VITEK MS and APIs, performed at Chughtai Laboratory Lahore, Pakistan. The VITEK MS (bioMérieux, France) is an advance automated system, without need for intensive background training for its operator.

In our study on comparing the identification of clinical samples, VITEK MS correctly identified 99%, while APIs up to 91.50%, whereas 8.50% results were

discordant. Some of the bacterial species such as *Citrobacter amalonaticus, Acinetobacter junni, Rhizobacterium radiobacter, Acinetobacter calcoaceticus* and *Micrococcus*, identified by APIs, were only possible with the help of certain additional tests. The results of APIs were the same as that of MALDI-TOF in terms of genus identification. However, APIs could not speciate the isolates to the level of accuracy of MALDI-TOF. Although accuracy of VITEK MS was 99%, but in case of certain bacteria, such as *Salmonella spp*. further serological testing is warranted. Literature review has revealed that MALDI-TOF is a safe and accurate method of bacterial identification with markedly reduced biowaste production. In comparison to the current study previous studies have documented non-clinical isolates .[6-10,12-14] Furthermore, additional benefit of using MALDI-TOF is that it's a safe option to identify hazardous pathogens (*Brucella spp, Bacillus anthracis, Francisella tularensis*) due to shorter handling time, absence of aerosol generating steps.[15,16]

In present study primary focus was centered on clinical bacterial isolates identification. In comparison with MALDI-TOF, API is a conventional method but it has the advantage of identifying *salmonella enterica subspecies enterica* serotypes without need for an additional serogrouping, however it requires 24-48 hours for analysis, and late identification results in delay of both empiric and targeted treatment. This means that rapid, reliable, cost-effective procedures are important prerequisites of a clinical microbiology.[11] In our study VITEK MS has analyzed the samples within few minutes (6min 10 seconds). The time dynamics for MALDI-TOF were calculated and shown in the Table-III. In addition to this reduced running cost, technical expertise, provision of the authentic results gives an edge to the VITEK MS over other identification procedures. However, there are certain precautionary measures which include careful application, avoiding spillage of matrix between spots and avoiding marking on slide.[6,11]

There were a few limitations of this study. Firstly, the current study was single centered, further validation with a similar study containing large number of samples(multicentric) should be done. Secondly, discrepant results could have been more precisely confirmed by 16s rRNA sequencing.

CONCLUSION

Addition of the VITEK MS to our laboratory facilitates turnaround time and adds in diagnostic efficiency for bacterial identification. It was observed that rapid bacterial identification can affect patient's management. Number of database entries are crucially important for reliable identification of microorganisms thus continuous update of database software is mandatory for excellent performance. MALDI-TOF is a game changer. It seems to play a vital role in future clinical microbiological laboratories in Pakistan. More research is required to achieve efficient identification directly from samples like blood, CSF, Pus and urine cultures along with antibiotic susceptibilities.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

CONTRIBUTION

Alina Mehwish: Original concept and design of work; the acquisition, drafting, revision.

Irim Iftikhar: Critical revision, final approval of version, questions related to accuracy/integrity of different parts of work resolution.

Karam Rasool: Analysis, interpretation of data for the work.

REFERENCES

1. Dubois D, Grare M, Prere MF, Segonds C, Marty N, Oswald E. Performances of the Vitek MS matrix-assisted laser desorption ionization–time of flight mass spectrometry system for rapid identification of bacteria in routine clinical microbiology. J Clin Microbiol. 2012; 50(8): 2568-76. DOI: 10.1128/JCM.00343-12
2. Luo Y, Siu GK, Yeung AS, Chen JH, Ho PL, Leung KW, *et al*. Performance of the VITEK MS matrix-assisted laser desorption ionization-time of flight mass spectrometry system for rapid bacterial identification in two diagnostic centres in China. J Med Microbiol. 2015; (64): 18-24. DOI: 10.1099/jmm.0.080317-0
3. Mitchell SL, Alby K. Performance of microbial identification by MALDI-TOF MS and susceptibility testing by VITEK 2 from positive blood cultures after minimal incubation on solid media. Eur J Clin Microbiol Infect Dis. 2017; 36 (11): 2201-6. DOI: 10.1007/s10096-017-3046-0
4. Alizadeh M, Yousefi L, Pakdel F, Ghotaslou R, Rezaee M. A, Khodadadi E, Oskouei M. A., Soroush Barhaghi M. H., & Kafil H. S. MALDI-TOF Mass Spectroscopy Applications in Clinical Microbiology. Advances in pharmacological and pharmaceutical sciences, 2021, 9928238. DOI://doi.org/10.1155/2021/9928238
5. Bilecen K, Yaman G, Ciftci U, Laleli YR. Performances and reliability of Bruker Microflex LT and VITEK MS MALDI-TOF mass spectrometry systems for the identification of clinical microorganisms. BioMed Res Int. 2015 Oct; :516410. DOI: 10.1155/2015/5164102015

1. Panda A, Ghosh AK, Mirdha BR, Xess I, Paul S, Samantaray JC, *et al*. MALDI-TOF mass spectrometry for rapid identification of clinical fungal isolates based on ribosomal protein biomarkers. J Microbiol Methods. 2015; 109: 93-105. DOI: 10.1016/j.mimet.2014.12.014
2. Bizzini A, Greub G. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry, a revolution in clinical microbial identification. Clinical Microbiology and infection. 2010; 16(11): 1614-9.
3. Barbuddhe SB, Maier T, Schwarz G, Kostrzewa M, Hof H, Domann E, *et al*. Rapid identification and typing of listeria species by matrix-assisted laser desorption ionization-time of flight mass spectrometry. Appl Environ Microbiol. 2008;74(17):5402–7. DOI: 10.1128/AEM.02689-07
4. Schulthess B, Brodner K, Bloemberg GV, Zbinden R, Böttger EC, Hombach M. Identification of gram-positive cocci by use of matrix-assisted laser desorption ionization–time of flight mass spectrometry: Comparison of different preparation methods and implementation of a practical algorithm for routine diagnostics. J Clin Microbiol. 2013;51(6):1834–40. DOI: 10.1128/JCM.02654-12.
5. Shitikov E, Ilina E, Chernousova L, Borovskaya A, Rukin I, Afanas'ev M, *et al*. Mass spectrometry-based methods for the discrimination and typing of Mycobacteria. Infect, Genet Evol. 2012; 12(4): 838–45. DOI: 10.1016/j.meegid.2011.12.013
6. Singhal N, Kumar M, Kanaujia PK, Virdi JS. MALDI-Tof Mass Spectrometry: An emerging technology for microbial identification and diagnosis. Front Microbiol. 2015;6. DOI: doi.org/10.3389/fmicb.2015.00791
7. Saleeb PG, Drake SK, Murray PR, Zelazny AM. Identification of mycobacteria in solid-culture media by matrix-assisted laser desorption ionization–time of flight mass spectrometry. J Clin Microbiol. 2011; 49(5): 1790–4. DOI: 10.1128/JCM.02135-10
8. Lotz Aurélie, Ferroni Agnès, Beretti J-L, Dauphin B, Carbonnelle E, Guet-Revillet Hélène, *et al*. Rapid identification of mycobacterial whole cells in solid and liquid culture media by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol. 2010;48(12):4481–6. DOI: 10.1128/JCM.01397-10
9. Fedorko DP, Drake SK, Stock F, Murray PR. Identification of clinical isolates of anaerobic bacteria using matrix-assisted laser desorption ionization-time of flight mass spectrometry. Eur J Clin Microbiol Infect Dis. 2012;31(9):2257–62. DOI: 10.1007/S10096-012-1563-4
10. Vranakis I, Papadioti A, Tselentis Y, Psaroulaki A, Tsiotis G. The contribution of proteomics towards deciphering the enigma of coxiella burnetii. Proteomics Clin Appl. 2013;7(1-2):193–204. DOI: 10.1002/prca.201200096
11. Lista F, Reubsaet FAG, De Santis R, Parchen RR, de Jong AL, Kieboom J, *et al*. Reliable identification at the species level of Brucella isolates with MALDI-TOF-MS. BMC Microbiol. 2011;11(1). DOI: doi.org/10.1186/1471-2180-11-267

ORIGINAL ARTICLE

FREQUENCY OF HUMAN PAPILLOMAVIRUS (HPV) WITH HEAD AND NECK CANCER: A SINGLE ONCOLOGY CENTER EXPERIENCE FROM KARACHI, PAKISTAN

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ABSTRACT

Background: The global incidence of head and neck cancer (HNC) is escalating. This research aimed to analyze the prevalence of human papillomavirus (HPV) in patients suffering from HNC.

Material and Methods: We conducted a forward-looking observational study at the Department of Medical Oncology, Jinnah Postgraduate Medical Center, Karachi, Pakistan, from January through September 2021. The study included patients above 15 years old, of any gender, with a confirmed diagnosis of HNC. HPV presence was determined via Polymerase Chain Reaction (PCR) for each patient. Socio-demographic data was collected using a pre-established proforma, and data analysis was executed using SPSS version 23.

Results: A total of 305 patients with HCNs were included. The mean age of patients was 46.46±12.58 years. The most common site of HNCs was found to be oral cavity (82%) and squamous cell carcinoma was the most common histological type (94.1%). Of 305 HNCs, 134 had tumor size of T3, 200 had nodal status of N1, 263 had grade II and only had metastasis (2.3%). Out of 305 patients with HNCs, 39 cases were found to be HPV positive. Statistically significant differences were observed between HPV and gender (p=0.048), naswar (p=0.018), betel nut consumption (p=0.020) and site of tumor (p=0.038).

Conclusion: The presence of HPV presents distinctive HNC characteristics and may serve as a survival indicator for these types of malignancies. Given the inherent genetic and socio-demographic disparities, along with differences in sexual behaviors and harmful lifestyle habits, the need for sex and race-specific clinical trials for HNC treatment is becoming increasingly evident.

Key Words: Cancer, Head and neck cancer, Human papillomavirus, Oral cavity cancer

BACKGROUND

Head and neck cancer (HNC) encapsulates a diverse array of neoplasms located in the head and neck region. As per the World Health Organization (WHO), HNCs can be classified based on site, etiology, and histology.[1] Squamous cell carcinoma, the most common type of HNC, ranks as the sixth most prevalent cancer in Pakistan.[2] Etiologically, HNCs can be categorized into two groups: Human papillomavirus (HPV) positive and HPV negative cancers.[3]

Existing data concerning HPV infection prevalence in Pakistan is not substantial or conclusive. Previous research provides conflicting perspectives on the extent of HPV infection among HNC patients. In the context of cervical HPV detection, studies report a prevalence rate ranging from 18% to 98%.[4-6] Another study specifically examined HPV prevalence in head and neck squamous cell cancers (HNSCC) across variables such as sex, race, tumor site, and HPV detection method. The findings showed that overall HPV prevalence in HNSCC was 47.4%, with higher rates observed in oropharyngeal tumors compared to non-oropharyngeal tumors. HPV positivity was also found to be more prevalent among men than women and white individuals compared to non-white individuals. Additionally, HPV detection rates varied depending on the method used, with p16 immunohistochemistry demonstrating the highest sensitivity and specificity. This study shed crucial light on the epidemiology of HPV-related HNSCC, underscoring the necessity for prevention and treatment strategies tailored to sex, race, and tumor site.[7]

HPV, a member of the Papillomaviradae family, is a non-enveloped, double-stranded DNA virus. Its size ranges from 52 to 55 nm, composed of seventy-two pentameric capsomers.[8] Although its role in causing cervical cancer is well-established, accounting for 5% of all cancers and nearly 113,400 cases annually, the connection between HPV and other cancers like HNC

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remains understudied.9 HPV-driven cancer stems from persistent HPV infections known to induce dysplastic and neoplastic changes.[10]

With limited research focused on HPV infection in HNCs, the need for further investigation is clear. This study, therefore, aims to evaluate the frequency of HPV-driven cancers, characterize the tumors associated with HPV infection, and understand the correlation between socio-demographic factors and HPV infection

MATERIAL AND METHODS

It was a prospective observational study conducted at the department of medical oncology, Jinnah postgraduate medical center, Karachi, Pakistan from January to September 2021. Sample size of 305 was estimated using proportion of HPV as 31.1% [11,12], taking margin of error as 5.2% and 95% confidence level. Patients of age more than 15 years of either gender with confirmed diagnosis of HNCs were included. Patients with non-HPV driven origins, patients with inoperable disease and palliative care cases were excluded from the study. Non-random probability consecutive sampling technique was applied for sample selection.

Ethical approval (ERC NO.F.2-81/2021-GENL/5845/JPMC) was taken from ERC of Jinnah postgraduate medical center, Karachi, Pakistan. Informed consent was obtained from all the eligible participants before collecting data. In this study, we used the p16 immunohistochemistry test, a surrogate marker for HPV infection, on tissue biopsies for HPV detection. This involved staining the tissues for the p16 protein, which is often overexpressed in HPV-related cancers. The test results were interpreted by experienced pathologists, who determined the presence of HPV based on the degree and pattern of staining. p16 positivity or negativity was determined by the intensity and extent of staining observed under the microscope. A sample was considered p16 positive when there was a strong and diffuse staining in the nucleus and/or cytoplasm of the cells in the tissue section. In the case of HNCs, p16 positivity was defined as staining in 70% or more of the tumor cells. Data on socio-demographics was collected on pre-designed proforma including sociodemographic, addiction, family history of cancer, smoking, tobacco, naswar, betel nut, betel leaf, alcohol, and tumor characteristics.

SPSS version 23 was used to analyze data. Mean and standard deviation (SD) were reported for quantitative data. Frequency and percentage were reported for categorical data. Association between HPV and socio-demographics was assessed using Chi-square test. A p-value<=0.05 was considered as statistically significant.

RESULTS

The study included 305 patients with HNCs from tertiary care hospitals of Karachi, Pakistan over a period of six months. The mean of the study participants was 46.46±12.58 years (range: 16-76 years). There were 227 males and 78 females diagnosed with HNCs. Most of the study participants were Urdu speaking (47.9%), urban area residents (72.1%), and illiterate (43.6%). The majority of participants (61.3%) in the study have a monthly family income less than 15,000 PKR, while 35.4% earn between 15,000-30,000 PKR and only a small fraction (3.3%) have income exceeding 30,000 PKR. In the study, 47 (15.4%) of participants reported a family history of any type of cancer, while the majority, 258 (83.6%), indicated no such history. A small proportion of the participants, 14 (4.6%), reported a family history of head and neck cancers (HNCs), while the vast majority, 291 (95.4%), had no family history of HNCs. There were 85 smokers and 106 were betel nut consumers. Almost 15.4% had a positive family history of any cancer and 4.6% had a positive family history of HNCs. The most common site of HNCs was oral cavity (82%), followed by larynx (7.9%). SSC was present in the majority of the cases (94.1%) and 5.9% had adenocarcinoma. Of 305 HNCs, 134 had tumor size as T3, 200 had nodal status as N1, 263 had grade II of tumor and only had metastasis (2.3%) (Table-I).

Out of 305 patients with HNCs, 39 (12.8%) cases were HPV positive and 266 cases were HPV negative. HPV was positive in 24 males (61.5%) and 15 females (38.5%). Statistically significant difference was observed between HPV and gender (p=0.048). We found HPV was positive in 2 naswar users (5.1%) and 37 non-naswar users. There was a statistically significant difference observed between HPV positivity and naswar consumption (p=0.018). Further, we found 20 HPV positive patients were betel nut consumers and 19 were non-betel nut consumers. We found a

statistically significant difference between HPV positivity and betel nut consumption (p=0.020). We observed HPV was positive in 36 patients with oral cavity tumors (92.3%), 1 patient with larynx (2.6%) and 2 patients with oropharynx (5.1%). There was a statistically significant difference observed between HPV positivity and site of tumor (p=0.038) (Table-II).

Table-I: Baseline characteristics of patients with HNCs (n=305).

Characteristics	Mean ± SD
Age in years	46.46±12.58
Gender	
Male	227 (74.4%)
Female	78 (25.6%)
Ethnicity	
Urdu	146 (47.9%)
Sindhi	66 (21.6%)
Punjabi	30 (9.8%)
Pashto	20 (6.6%)
Balochi	24 (7.9%)
Others	19 (6.2%)
Locality	
Urban	220 (72.1%)
Rural	85 (27.9%)
Education	
Illiterate	133 (43.6%)
Primary	89 (29.2%)
Matric	35 (11.5%)
Intermediate	32 (10.5%)
Graduate	14 (4.6%)
Post graduate	2 (0.7%)
Site of tumor	
Oral cavity	250 (82%)
Larynx	24 (7.9%)
Hypopharynx	8 (2.6%)
Oropharynx	4 (1.35)
Nasopharynx	19 6.2%)
Subtype of tumor	
Adenocarcinoma	18 (5.9%)
SSC	287 (94.1%)
Tumor size	
T1	5 (1.6%)
T2	64 (21%)
T3	134 43.9%)
T4	102 (33.45)
Nodal status	
No	43 (14.1%)
N1	200 (65.6%)
N2	61 (20%)
N3	1 (0.3%)
Grade	
I	26 (8.5%)
II	263 (86.2%)
III	16 (5.2%)
Metastasis	
Mo	298 (97.7%)
M1	7 (2.3%)

Table-II: Stratification of HPV with respect to socio-demographics and tumor characteristics.

Characteristics	HPV		P-value
	Yes (n=39)	No (n=266)	
Age in years	43.82±10.77	46.85±12.79	0.161
Gender			
Male	24 (61.5%)	203 (76.3%)	0.048*
Female	15 (38.5%)	63 (23.7%)	
Ethnicity			
Urdu	19 (48.7%)	127 (47.7%)	0.981
Sindhi	8 (20.5%)	58 (21.8%)	
Punjabi	3 (7.7%)	27 (10.2%)	
Pashto	3 (7.7%)	17 (6.4%)	
Balochi	4 (10.3%)	20 (7.5%)	
Others	2 (5.1%)	17 (6.4%)	
Locality			
Urban	24 (61.5%)	196 (73.7%)	0.114
Rural	15 (38.5%)	70 (26.3%)	
Education			
Illiterate	18 (46.2%)	115 (43.2%)	0.435
Primary	9 (23.15%)	80 (30.1%)	
Matric	7 (17.9%)	28 (10.5%)	
Intermediate	5 (12.8%)	27 (10.2%)	
Graduate	0	14 (5.3%)	
Post graduate	0	2 (0.8%)	
Monthly Family Income			
<15,000 PKR	29 (74.45)	158 (59.45)	0.199
15,000-30,000 PKR	9 (23.1%)	99 (37.2%)	
>30,000 PKR	1 (2.6%)	9 (3.4%)	
Addiction			
Smoking	7 (17.9%)	78 (29.3%)	0.139
Tobacco	5 (12.8%)	60 (22.6%)	0.166
Naswar	2 (5.1%)	56 (21.1%)	0.018*
Betel nut	20 (51.3%)	86 (32.3%)	0.020*
Betel leaf	7 (17.9%)	62 (23.3%)	0.455
Alcohol	0	9 (3.4%)	0.243
Family history of any cancer			
Yes	8 (20.5%)	39 (14.7%)	0.526
No	31 (79.5%)	227 (85.3%)	
Family history of HNCs			
Yes	3 (7.7%)	11 (4.1%)	0.322
No	36 (92.3)	255 (95.9%)	
Site of tumor			
Oral cavity	36 (92.3%)	214 (80.5%)	0.038*
Larynx	1 (2.6%)	23 (8.6%)	
Hypopharynx	0	8 (3%)	
Oropharynx	2 (5.1%)	2 (0.8%)	
Nasopharynx	0	19 (7.1%)	
Subtype of tumor			
Adenocarcinoma	2 (5.1%)	16 (6%)	0.589
SSC	37 (94.9%)	250 (94%)	
Tumor size			
T1	0	5 (1.9%)	0.454
T2	5 (12.8%)	59 (22.2%)	
T3	21 (53.8%)	113 (42.5%)	
T4	13 (33.3%)	89 (33.5%)	

Nodal status			
No	6 (15.4%)	37 (13.9%)	0.818
N1	27 (69.2%)	173 (65%)	
N2	6 (15.4%)	55 (20.7%)	
N3	0	1 (0.4%)	
Grade			
I	3 (7.7%)	23 (8.6%)	0.7
II	35 (89.7%)	228 (85.7%)	
III	1 (2.6%)	15 (5.6%)	
Metastasis			
Mo	39 (100%)	259 (97.4%)	0.601
M1	0	7 (2.6%)	

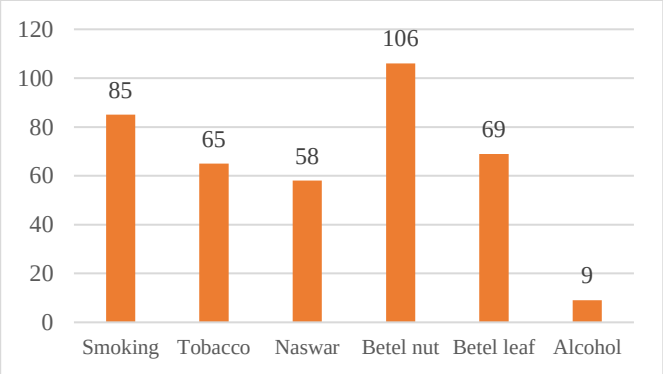

Figure-I: Frequency of substance use among participants (N=305).

DISCUSSION

The role of high-risk HPV in cervical cancer is well-established, yet recent decades have also seen emerging evidence of its involvement in other malignancies, including a subset of head and neck cancers.[13, 14] This latter group exhibits distinct clinical, epidemiological, and molecular characteristics compared to non-HPV head and neck carcinoma.[15]

While oropharyngeal squamous cell carcinoma (OPSCC), which represents approximately a quarter of all head and neck squamous cell carcinoma (HNSCC) cases, has been robustly linked to HPV, its association with other HNSCC types is less certain.[16, 17] High HPV prevalence is observed in oral malignancies in Western countries, with rates ranging from 70-90%.[18] However, our study reports a comparatively lower incidence of HPV-associated HNC at around 13%, a finding consistent with data from other regions.[19, 20]

Despite HPV's frequent detection in oral cavities, its contribution to oral cavity squamous cell carcinoma (OCSCC) remains limited (3.9% of cases), contrasting starkly with the 47% case contribution to OPSCC.[21] Interestingly, our findings revealed a high HPV prevalence of 92.3% in oral cavity cancer, compared to 5.1% in oropharyngeal and 2.6% in larynx cancer. Tobacco use, betel quid chewing, and alcohol consumption all pose potential risks for oral cavity malignancies.[22] Moreover, our study corroborates previous research indicating a higher HPV prevalence in men than women.[23,24] This may suggest gender-based transmission patterns, though the data remains inconclusive. Evidence points to potential protective roles of female hormones against HNSCC development.[25] Further, a study by Ochoa *et al*. underscored the survival advantage conferred by HPV positivity in HNSCC patients, with HPV-negative individuals exhibiting significantly lower survival rates.[26]

Our analysis also highlights the link between HPV infection and hazardous habits such as tobacco and betel nut chewing, both independent risk factors for oral cancer. The increasing prevalence in recent years reflects not just the addictive and stimulatory properties of tobacco, but also the lack of social stigma associated with betel nut use among Pakistan's lower and middle socioeconomic classes.[19] The results of this study lay a foundation for devising improved diagnostic strategies, effective treatment modalities, and advanced screening programs to mitigate the future burden of HPV-driven HNC in Pakistan.

CONCLUSION

This study underscores the significant, yet complex, relationship between HPV and head and neck cancer, particularly highlighting the demographic, behavioral, and gender differences. The findings provide valuable insights for healthcare providers to tailor preventive strategies, diagnostic procedures, and treatment modalities. They also stress the importance of developing comprehensive HPV screening programs and targeted vaccinations, particularly in regions with lower socioeconomic status. Further research is warranted to delve deeper into the multifaceted role of HPV in head and neck cancers and to further elucidate the factors impacting its prevalence and progression.

CONFLICT OF INTEREST

Authors declare no conflict of interest

AUTHOR CONTRIBUTIONS

Nargis Aalam Abro: Study design and concept and data acquisition.

Ghulam Haider: Supervision and critical appraisal

Aamera Shah and Tooba Sarim
: Literature review
Reeta Kumari: Result interpretation
Sorath Bhutto and Sana Hashmat: Data acquisition and draft writing

REFERENCES

1. Huang SH, O'Sullivan B. Overview of the 8th edition TNM classification for head and neck cancer. Curr Treat Options Oncol. 2017 Jul;18(7):40. DOI: 10.1007/s11864-017-0484-y
2. Manzoor H, Ahmad N, Shuja J, Rehman A, Naheed H, Iftikhar S. Epidemiology characteristic of head & neck cancers (HNCs) in Southwestern Pakistan: 21 years experience. Pak J Med Health Sci. 2020; 14(4): 1955-7.
3. Kobayashi K, Hisamatsu K, Suzui N, Hara A, Tomita H, Miyazaki T. A review of HPV-related head and neck cancer. J Clin Med. 2018; 7(9): 241. DOI: 10.3390/jcm7090241
4. Manzoor H, Naheed H, Ahmad K, Iftikhar S, Asif M, Shuja J, Sultan N, Ali I, Inayatullah S, Khan YH. Pattern of gynaecological malignancies in south western region of Pakistan: An overview of 12 years. Biomed Rep. 2017; 7(5): 487-91. DOI: 10.3892/br.2017.993
5. Raza SA, Franceschi S, Pallardy S, Malik FR, Avan BI, Zafar A, *et al*. Human papillomavirus infection in women with and without cervical cancer in Karachi, Pakistan. Br J Cancer. 2010; 102(11): 1657-60. DOI: 10.1038/sj.bjc.6605664
6. Khan S, Jaffer NN, Khan MN, Rai MA, Shafiq M, Ali A, *et al*. Human papillomavirus subtype 16 is common in Pakistani women with cervical carcinoma. Int J Infect Dis. 2007; 11(4): 313-7. DOI: 10.1016/j.ijid.2006.06.007
7. Alsbeih G, Al-Harbi N, Bin Judia S, Al-Qahtani W, Khoja H, El-Sebaie M, *et al*. Prevalence of human papillomavirus (HPV) infection and the association with survival in Saudi patients with head and neck squamous cell carcinoma. Cancers (Basel). 2019; 11(6):820. DOI: 10.3390/cancers11060820
8. Boda D, Docea AO, Calina D, Ilie MA, Caruntu C, Zurac S, *et al*. Human papilloma virus: Apprehending the link with carcinogenesis and unveiling new research avenues (Review). Int J Oncol. 2018; 52(3): 637-55. DOI: 10.3892/ijo.2018.4256
9. Roden RBS, Stern PL. Opportunities and challenges for human papillomavirus vaccination in cancer. Nat Rev Cancer. 2018; 18(4): 240-54. DOI: 10.1038/nrc.2018.13
10. Kjær SK, Frederiksen K, Munk C, Iftner T. Long-term absolute risk of cervical intraepithelial neoplasia grade 3 or worse following human papillomavirus infection: Role of persistence. J Natl Cancer Inst. 2010; 102(19): 1478-88. DOI: 10.1093/jnci/djq356
11. Okunade KS. Human papillomavirus and cervical cancer. J Obstet Gynaecol. 2020; 40(5): 602-8. DOI: 10.1080/01443615.2019.1634030
12. Khasawneh A, Himsawi N, Abu-Raideh J, Salameh M, Abdullah N, Khasawneh R, *et al*. Prevalence of human papillomavirus associated with head and neck squamous cell carcinoma in Jordanian patients. Open Microbiol J. 2020; 14: 57-64. DOI: 10.2174/1874285802014010057
13. Saira, Ahmed R, Malik S, Khan MF, Khattak MR. Epidemiological and clinical correlates of oral squamous cell carcinoma in patients from north-west Pakistan. J Pak Med Assoc. 2019; 69(8): 1074-8.
14. Götz C, Bischof C, Wolff KD, Kolk A. Detection of HPV infection in head and neck cancers: Promise and pitfalls in the last ten years: A meta-analysis. Mol Clin Oncol. 2019; 10(1): 17-28. DOI: 10.3892/mco.2018.1749
15. Sabatini ME, Chiocca S. Human papillomavirus as a driver of head and neck cancers. Br J Cancer. 2020; 122(3): 306-14. DOI: 10.1038/s41416-019-0602-7
16. Faraji F, Eisele DW, Fakhry C. Emerging insights into recurrent and metastatic human papillomavirus-related oropharyngeal squamous cell carcinoma. Laryngoscope Investig Otolaryngol. 2017; 2(1): 10-8. DOI: 10.1002/lio2.37
17. Hay A, Nixon IJ. Recent advances in the understanding and management of oropharyngeal cancer. F1000Res. 2018; 7. DOI: 10.12688/f1000research.14416.1
18. Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. Cancer Epidemiol Biomarkers Prev. 2005; 14(2): 467-75. DOI: 10.1158/1055-9965.EPI-04-0551
19. Ali SMA, Awan MS, Atif S, Ali N, Mirza Y. Correlation of human papillomavirus infection and clinical parameters with five-year survival in oral squamous cell carcinoma. J Laryngol Otol. 2018; 132(7): 628-35. DOI: 10.1017/S0022215118000361
20. Abu Qatouseh L, Sabri I, Alkhatib I, Atwa E, Arafat T. Detection of high-risk human papillomavirus genotypes 16 and 18 in head and neck squamous cell carcinomas in Jordan. Asian Pac J Cancer Prev. 2017; 18(5): 1337-41. DOI: 10.22034/APJCP.2017.18.5.1337
21. Leemans CR, Snijders PJF, Brakenhoff RH. The molecular landscape of head and neck cancer. Nat Rev Cancer. 2018; 18(5): 269-82. DOI: 10.1038/nrc.2018.11
22. Hübbers CU, Akgül B. HPV and cancer of the oral cavity. Virulence. 2015; 6(3): 244-8. DOI: 10.1080/21505594.2014.999570
23. Saunders CL, Meads C, Abel GA, Lyratzopoulos G. Associations Between sexual orientation and overall and site-specific diagnosis of cancer: Evidence from two national patient surveys in england. J Clin Oncol. 2017; 35(32): 3654-61. DOI: 10.1200/JCO.2017.72.5465
24. Widdice L, Ma Y, Jonte J, Farhat S, Breland D, Shiboski S, *et al*. Concordance and transmission of human papillomavirus within heterosexual couples observed over short intervals. J Infect Dis. 2013; 207(8): 1286-94. DOI: 10.1093/infdis/jit018
25. Hashim D, Sartori S, La Vecchia C, Serraino D, Maso LD, Negri E, *et al*. Hormone factors play a favorable role in female head and neck cancer risk. Cancer Med. 2017; 6(8): 1998-2007. DOI: 10.1002/cam4.1136
26. Sharkey Ochoa I, O'Regan E, Toner M, Kay E, Faul P, O'Keane C, *et al*. The role of HPV in determining treatment, survival, and prognosis of head and neck squamous cell carcinoma. Cancers. 2022; 14(17): 4321. DOI: 10.3390/cancers14174321

ORIGINAL ARTICLE

COMPARISON BETWEEN MINI-BAL AND BAL RESULTS AMONG ICU PATIENTS WITH PREDOMINANT RIGHT LOWER LOBE PNEUMONIA

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ABSTRACT

Background: Bacterial pneumonia is one of the public health concerns with high ICU admissions, morbidity and mortality in individuals of all ages worldwide. The diagnosis in intensive care unit (ICU) settings poses challenges, primarily obtaining rapid and reliable microbiological confirmation. Objective of the present study is to compare the results of mini-BAL and BAL among ICU patients with predominant right lower lobe pneumonia.

Material and Methods: This Prospective randomized comparative cross-sectional study as done Medical ICU, Services Hospital, Lahore – 1 year. The study was carried out after approval from the institute's ethics committee. Our study had 66 participants, 33 in each group. Each group of patients was assigned at random. The first group had the BAL procedure, whereas the second group received the mini-BAL procedure. Both groups' demographic, clinical, laboratory, bacteriological, and mycological data were obtained. SPSS version 23 was used to analyse the data collected. Mean, standard deviation, frequency, and percentages were utilised in descriptive statistics. The Chi square test was also used to compare groups. Cut off set to $p<0.05$.

Results: The BAL technique isolated bacteriological pathogens in 13(39.4%) patients and mini-BAL technique isolated bacteriological pathogens in 15(45.5%) patients. The BAL technique isolated mycological pathogens in 12(36.4%) patients and mini-BAL technique isolated mycological pathogens in 14(42.4%) patients. There was no statistically significant difference among the two procedures. ($p>0.05$)

Conclusion: In the mini-BAL group, the frequency of isolation of bacteria and mycological pathogens is slightly higher. Although there was no statistically significant difference, we propose that mini-BAL be used instead BAL because it is less invasive.

Key Words: Comparison, Mini-BAL, BAL ICU patients, Right lower lobe, Pneumonia

BACKGROUND

Bacterial pneumonia is one of the most important public health challenges due to its high medical and economic expenses, which result in increased morbidity and mortality in individuals of all ages worldwide.[1-3] Pneumonia is an acute inflammation and consolidation of lung tissue caused by infectious organisms such as bacteria, viruses, fungi, and parasites.[1] Bacterial pneumonia is an inflammation of one or two lobes of the lung caused by bacterial infection.[4]

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The most prevalent ICU-acquired infection is ventilator-associated pneumonia (VAP). Five to forty percent of incidents are reported. It is strongly recommended that infections be confirmed microbiologically. Which sampling technique to employ is still a matter of debate. Emerging microbiological tools will likely alter our standard diagnostic and treatment procedures for VAP in the near future. [5]

The diagnosis in intensive care unit (ICU) settings poses challenges, primarily obtaining rapid and reliable microbiological confirmation. BAL is a diagnostic tool for bacteriological and fungal infections. [6] The BAL bronchoscopic technique is costly and requires experienced personnel. Complications associated with invasive procedures, such as BAL, are more likely to occur in critically ill patients. Therefore, the hazards and advantages of BAL should be carefully weighed in the case of a critically ill patient.[7] Mini bronchoalveolar lavage (Mini-BAL) is an alternate approach for acquiring respiratory samples from mechanically ventilated patients that is straightforward,

less invasive, and inexpensive.[8,9] A study could not find any difference in the diagnostic frequency of the two procedures.[10]

Less invasive and new diagnostic techniques, like mini-BAL, has been investigated in the recent years based on above observation, notably in diagnosis of VAP "ventilator associated pneumonia". [7]However, there is inadequate evidence to evaluate the diagnostic usefulness of mini-BAL to BAL in ICU patients with predominant right lower lobe pneumonia. Feasibility and diagnostic usefulness of this less invasive technique, the mini-BAL, is gaining popularity. The aim of our study was to compare the results of mini–BAL and BAL among ICU patients with predominant right lower lobe pneumonia.

MATERIAL AND METHODS

After approval from ethical committee of institute the study was conducted at Medical ICU, Services hospital Lahore from 01-01-2020 to 31-12-2020. The study design was comparative cross sectional. The sample size of our study was 66, 33 in each group (ICU patients with predominant right lower lobe pneumonia). The patients were randomly assigned to each group. One group underwent BAL procedure and second group mini-BAL procedure.

Regarding BAL, under aseptic measures and local anesthesia with lignocaine, fibro optic bronchoscope was introduced through Endotracheal tube and then into the lavaged section. Two aliquots of 20ml saline were utilized to capture the return fluid in the mucous trap bottle. A minimum of 20ml of sample was sent to the lab for diagnosis.

In mini-BAL procedure; A sterile long suction catheter of size 12 French (Fr) was inserted through the ET and blindly progressed into the distal airways until resistance was encountered, at which point the tube was jammed. Suction was used to collect aspirate in a sterile mucous extractor container after 20 ml of Sodium Chloride 0.9 percent was given through the catheter. Following these procedures, the probe was carefully withdrawn utilizing turning movements.

The demographic, clinical, laboratory, bacteriological and mycological information for both groups were collected. The collected information was analyzed by using SPSS version 23. In descriptive statistics, mean, standard deviation, frequency with percentages were used. Chi square test was also used to compare outcome. P value less than 0.05 was set as significance level.

RESULTS

We enrolled 66 patients in our study 33 in each group. One group was BAL group and second group was Mini BAL group. Frequency and percentages of qualitative variables; gender, use of the antibiotic, diabetes mellitus, hypertension, atherosclerotic cardiac disease, chronic renal failure, sepsis, chronic liver failure are presented in Table-I. Descriptive statistics (mean+ SD) of Age, APACHE II, Temperature °C, Leukocytes/ mm3, CRP mg/dl, Procalcitonin ng/dl, Albumin, g/dl, Creatinine mg/dl are presented in Table-II. The outcome (bacteriological or mycological) was compared among BAL and mini-BAL group. Difference was not statistically significant Table-III.

Table-I: Qualitative Demographic, clinical and laboratory data of patients

		Groups	
		BAL Group	**Mini BAL Group**
Gender	Male	21 63.6%	24 72.7%
	Female	12 36.4%	9 27.3%
Use of the Antibiotic	Yes	30 90.9%	26 78.8%
	No	3 9.1%	7 21.2%
Diabetes Mellitus	Yes	12 36.4%	13 39.4%
	No	21 63.6%	20 60.6%

Hypertension	Yes	10 30.3%	8 24.2%
	No	23 69.7%	25 75.8%
Atherosclerotic cardiac disease	Yes	12 36.4%	9 27.3%
	No	21 63.6%	24 72.7%
Chronic Renal Failure	Yes	9 27.3%	8 24.2%
	No	24 72.7%	25 75.8%
Sepsis	Yes	7 21.2%	8 24.2%
	No	26 78.8%	25 75.8%
Chronic Liver Failure	Yes	1 3.0%	2 6.1%
	No	32 97.0%	31 93.9%

Table-II: Quantitative demographic, clinical and laboratory data of patients

	BAL Group	Mini BAL Group
Age	56.18±5.68	57.33±5.54
APACHE II	22.45±2.40	22.88±2.25
Temperature **°C**	36.97±0.92	36.70±0.85
Leukocytes, / mm3	11535.27±280.69	11551.79±321.46
CRP, mg/dl	22.73±4.68	21.79±4.57
Procalcitonin, ng/dl	4.18±1.69	4.61±1.89
Albumin, g/dl	2.52±0.51	2.61±0.50
Creatinine, mg/dl	1.55±0.51	1.48±0.51

(APACHE II "Acute Physiology and Chronic Health Evaluation", CRP "C-reactive protein")

Table-III: Bacteriological and mycological results of BAL and mini-BAL samples

		Groups		p value
		BAL Group	Mini BAL Group	
Bacteriological Results	Acinetobacter baumannii	3 9.1%	4 12.1%	
	Streptococcus pneumoniae	3 9.1%	2 6.1%	
	Staphylococcus aureus	3 9.1%	2 6.1	
	Enterococcus faecium	1 3.0%	2 6.1%	
	Pseudomonas aeruginosa	1 3.0%	1 3.0%	0.977
	Stenotrophomonas species	1 3.0%	2 6.1%	
	Klebsiella species	1 3.0%	2 6.1%	
	No agent	20 60.6%	18 54.5%	
Mycological results	Candida spp.	11 33.3%	12 36.4%	
	Aspergillus spp.	1 3.0%	2 6.1%	0.788
		21 63.6%	19 57.6%	

DISCUSSION

The study was carried out at Medical ICU Services Hospital to compare the outcomes of mini–BAL and BAL in ICU patients with predominate right lower lobe pneumonia.

Whether obtained in the community or through medical care, pneumonia is a serious condition. It has been established that mortality is reduced when antibiotic treatment is initiated promptly.[11] Due to the difficulty in identifying the causative organisms, selecting the appropriate antibiotic may be problematic. Because of this, broad-spectrum antibiotics are frequently prescribed, at least until microbiological testing is complete. Due to contamination from infected upper airways, endotracheal samples collected from intensive care patients may produce inaccurate results.[12] As a result, physicians must collect reliable lower respiratory tract samples that are free of contamination. Microbiological analysis of bronchoscopy material is regarded as a particular diagnostic technique for determining the causal bacteria in pneumonia.[13]

Bronchoscopy is an invasive, needs expertise, longer time for perform, expensive, disturb the respiratory mechanics, oxygenation and hemodynamics in intensive care unit during procedure. So, an easy method is needed to perform this.[14]

BAL has been linked to a number of problems in patients, particularly acute respiratory distress syndrome. Bleeding, cardiac arrhythmias, pneumothorax and Hypoxemia are some of the other problems. As a result, in the critical patient scenario, the benefits/risks of BAL should be carefully assessed before to application, with the procedure being used when less invasive techniques have limits or are not useful for diagnosis.[15]

Protected mini-BAL is less invasive, needs less expertise, requires less time to perform, inexpensive, has less impact on respiratory mechanics, oxygenation and hemodynamics in intensive care unit during procedure.[16]

In several investigations, the sensitivity and specificity of mini-BAL were reported to be 63-100 % and 66-96 %, respectively. These findings are comparable to those obtained with the fiberoptic BAL. Bronchoscopy is not recommended as a standard diagnostic procedure for some pneumonias, such as CAP, and should be reserved for people with severe types of pneumonia who have not responded to early medication and require further pneumonia investigations.[17]

In our study we find mini-BAL technique isolated bacteriological pathogens more than BAL technique similar in mycological pathogens results. The difference was present but the difference was not significant statistically. These results matched with the results of study.[1, 11]

Another study could not find any difference in the diagnostic frequency of the two procedures. [10]Rouby et al. utilized mini-BAL in patients with hospital-acquired pneumonia. In terms of microbiological correctness, mini-BAL was 74 percent comparable to pathologic examination of postmortem lung tissue. They proposed that instead of bronchoscopy, mini-BAL might be utilized.[11]

Limitations of the present study were small sample size; sample size was not calculated and use of single centre for data collection. We recommend large multi centre trials for confirmatory evidence so that guidelines can be developed and on the basis of evidence less invasive procedure may replace invasive procedure.

CONCLUSION

The frequency of isolation of bacteria and mycological pathogens is slightly higher in the mini-BAL group. Although there was no statistically significant difference, we propose that mini-BAL be used instead of BAL because it is less invasive, less expensive, requires less expertise, takes less time to perform, and causes less disruption to respiratory mechanics, oxygenation, and hemodynamics.

CONFLICT OF INTEREST

Authors declare no conflict of interest

AUTHOR CONTRIBUTIONS

Anam Ahmad: Data collection and interpretation, literature review

Syed Mazhar Ali Naqvi: Study design, concept and data analysis

Humaira Nazir: Data collection and interpretation

Noma Sarwar: Drafting and design of questionnaire

Waqas Aslam: Data collection and analysis

Rabia Tariq: Data interpretation and literature review

REFERENCES

1. Assefa M. Multi-drug resistant gram-negative bacterial pneumonia: etiology, risk factors, and drug resistance patterns. Pneumonia (Nathan). 2022;14(1):4. DOI: 10.1186/s41479-022-00096-z
2. GBD 2015 LRI Collaborators. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory tract infections in 195 countries: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Infect Dis. 2017; 17(11): 1133-61. DOI: 10.1016/S1473-3099(17)30396-1
3. Assefa M. Multi-drug resistant gram-negative bacterial pneumonia: etiology, risk factors, and drug resistance patterns. Pneumonia. 2022; 14(1): 4.
4. Medical News Today. What to know about bacterial pneumonia. [Internet]. 2019 [cited 2023 Jan 21]. Available from: https://www.medicalnewstoday.com/articles/312565.
5. Papazian L, Klompas M, Luyt CE. Ventilator-associated pneumonia in adults: A narrative review. Intensive Care Med. 2020; 46(5): 888-906. DOI: 10.1007/s00134-020-05980-0
6. Parashar D, Parashar R, Rani A, Kumar P. Role of bronchoalveolar lavage in undiagnosed lung diseases. J Cardiovascular Dis Res. 2022. 13(8) 451-62.
7. Geraci G, Pisello F, Sciumè C, Li Volsi F, Romeo M, Modica G. [Complication of flexible fiberoptic bronchoscopy. Literature review]. Ann Ital Chir. 2007; 78(3): 183-92.
8. Bacakolu F, Uysal FE, Kamaz Basoglu O, Aydemir S, Arda B. Diagnostic value of non-bronchoscopic mini-BAL in ventilator associated pneumonia. Eur J Pulmonol. 2007; 9(3): 139-46.
9. Neves CP, Costa AG, Safe IP, de Souza Brito A, Jesus JS, Kritski AL, *et al*. The role of mini-bronchoalveolar lavage fluid in the diagnosis of pulmonary tuberculosis in critically ill patients. BMC Infect Dis. 2020; 20(1): 229. DOI: 10.1186/s12879-020-04954-3
10. Vanbellinghen MC, Atasever B, van der Spoel HJI, Bouman CCS, Altenburg J, van Dijk K. Mini-Bronchoalveolar lavage for diagnosing coronavirus disease 2019-Associated Invasive Pulmonary Aspergillosis. Crit Care Explor. 2021; 3(12): e0601. DOI: 10.1097/CCE.0000000000000601
11. Gurlevik A, Urkmez S, Utku T, Dikmen Y. Comparison of bronchoalveolar lavage and protected mini-bronchoalveolar lavage in diagnosis of pneumonia in intensive care unit. J Anesth Crit Care Open Access. 2017; 7(2): 1-6. DOI: 10.15406/jaccoa.2017.07.00256
12. Ioanas M, Ferrer R, Angrill J, Ferrer M, Torres A. Microbial investigation in ventilator-associated pneumonia. Eur Respir J. 2001; 17(4): 791-801. DOI: 10.1183/09031936.01.17407910
13. Fagon JY. Diagnosis and treatment of ventilator-associated pneumonia: fiberoptic bronchoscopy with bronchoalveolar lavage is essential. Semin Respir Crit Care Med. 2006; 27(1): 34-44. DOI: 10.1055/s-2006-933672
14. Schnabel RM, van der Velden K, Osinski A, Rohde G, Roekaerts PM, Bergmans DC. Clinical course and complications following diagnostic bronchoalveolar lavage in critically ill mechanically ventilated patients. BMC Pulm Med. 2015 Sep 29; 15: 107. DOI: 10.1186/s12890-015-0104-1
15. Du Rand IA, Barber PV, Goldring J, Lewis RA, Mandal S, Munavvar M, Rintoul RC, et al. BTS Interventional bronchoscopy guideline group. summary of the british thoracic society guidelines for advanced diagnostic and therapeutic flexible bronchoscopy in adults. Thorax. 2011; 66(11): 1014-5. DOI: 10.1136/thoraxjnl-2011-201052
16. Fujitani S, Yu VL. Diagnosis of ventilator-associated pneumonia: focus on non-bronchoscopic techniques (non-bronchoscopic bronchoalveolar lavage, including mini-BAL, blinded protected specimen brush, and blinded bronchial sampling) and endotracheal aspirates. J Intensive Care Med. 2006; 21(1): 17-21. DOI: 10.1177/0885066605283094
17. EI-Shabrawy M, EL-Sokkary RH. Role of fiberoptic bronchoscopy and BAL in assessment of the patients with non-responding pneumonia. Egypt J Chest Dis Tuberc. 2016; 65(3): 613-20. DOI: doi.org/10.1016/j.ejcdt.2015.12.006
18. Tasbakan MS, Gurgun A, Basoglu OK, Ekren PK, Pullukcu H, Bacakoglu F. Comparison of bronchoalveolar lavage and mini-bronchoalveolar lavage in the diagnosis of pneumonia in immunocompromised patients. Respiration. 2011; 81(3): 229-35. DOI: 10.1159/000323176

CASE REPORT

SALMONELLA ENTERICA SEROVAR *TYPHI* (SALMONELLA *TYPHI*) BRAIN ABSCESS AFTER BRAIN SURGERY: A CASE REPORT

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ABSTRACT

Salmonella typhi (Salmonella enterica serovar Typhi) is one of the pathogens responsible for typhoid fever, a major public health problem in low and middle-income countries like Pakistan. Typhoid fever caused by *S. typhi* can result in severe disease with protean complications like gastrointestinal hemorrhage and perforation, endocarditis, pancreatitis, hepatitis, pneumonia or typhoid encephalopathy but focal intracranial infections like brain abscess is a rare occurrence. We are reporting a case of *S. typhi* brain abscess in an eleven-year-old boy who was operated for brain tumor and developed *S. typhi* brain abscess in the post-operative period.

Key Words: *Salmonella Typhi,* Gram negative rods, Brain abscess

BACKGROUND

Salmonella species are gram-negative, non–spore-forming, facultative anaerobic bacilli and are well known pathogens of gastroenteritis in humans. Intracranial infections are out of ordinary manifestations of salmonellosis in general and brain abscesses in particular[1]

Brain abscess is a focal pyogenic infection of the brain parenchyma; the major predisposing factors are an associated neighboring focus of infection, trauma, and hematogenous spread from a distant focus. The most common attributable microbial agents are anaerobic bacteria, aerobic and microaerophilic *Streptococci, Enterobacteriaceae,* Staphylococcus *aureus* while *Aspergillus* and *Nocardia* are commonly seen in immunocompromised patients. A variety of other microbial agents may also cause brain abscesses depending upon the site of primary infection, age, and immune status of the patient. [2]

Typhoid fever is an acute febrile illness caused by Salmonella enterica serovar *typhi* (*S. typhi)* and to a less extent by *Salmonella paratyphi A, B* and *C,* collectively called typhoidal salmonella. The typical presentation of clinical syndrome caused by *S. typhi* comprise of acute febrile illness with or without gastrointestinal symptoms. Intracranial involvement with meningitis and encephalitis can occur but focal intracranial infection like brain abscess is rare. We report a case of *S. typhi* related brain abscess in an 11-year-old boy after surgery for brain tumor.[3]

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CASE REPORT

An 11-year-old boy visited Shaukat Khanum Memorial Cancer Hospital and Research Center's emergency in Lahore with of history of fevers for the last fifteen days associated with seizures and altered mental status. Recently, he was diagnosed with a right temporoparietal lobe fibrous tumor and had tumor debulking surgery at some other hospital in Lahore Pakistan. Neurological examination revealed altered mental status, neck rigidity and mild dysarthria but no other focal neurological deficit.

He was empirically started on meropenem and vancomycin. An urgent computed tomography of the brain performed that revealed postsurgical hemorrhage, and multiple brain abscesses in the right parietotemporal lobe. Blood examination revealed white blood cells (WBC) 15700/µl with lymphocytes at 12 % of total leukocytes, hemoglobin 14g/dl, platelets 143000/µl, C-reactive protein (CRP) 154 mg/l and erythrocyte sedimentation rate 70 mm/hr. Blood culture also remained negative for any bacterial growth. Other laboratory workup was unremarkable.

Magnetic resonance imaging of brain confirmed the findings of brain abscess Figure-I. Surgical drainage of abscess was performed on 4th day of hospital admission by craniotomy, which confirmed the diagnosis of abscess at tumor resection site in right temporal lobe.

The pus specimen collected from the drained abscess was sent for culture to the hospital's onsite microbiology laboratory. Bacterial cultures from the pus grew non-lactose fermenting gram negative bacteria. This isolate was identified as *S. typhi* an API 20E (Bio Mérieux, Inc). Serotyping was done to confirm the isolate. This isolate was found to be susceptible only to meropenem, azithromycin and chloramphenicol on standard disk-diffusion testing. Evaluation of the cerebrospinal fluid drained during surgery revealed the following values; WBCs 0 /mm^3, RBCs 0 /mm^3, proteins 210 mg/d and glucose 22 mg/dl. Although CSF and blood cultures remained negative for any bacterial growth Table-I, the patient was treated with high dose meropenem. Unfortunately, the patient deteriorated during the post-operative period due to the large residual aggressive tumor and he died in next few days due to advanced intracranial disease and sepsis.

Table-I: Cerebrospinal fluid analysis report.

CSF parameters	Value	Normal Range
CSF glucose	22 mg/dl	66% of serum glucose
CSF proteins	210 mg/dl	15 -45 mg/dl
WBC	0	0-5 cells all lymphocytes
RBC	0	Not present
CSF culture	No bacterial growth	

CSF, cerebrospinal fluid WBC, White blood cells RBC, Red blood cells

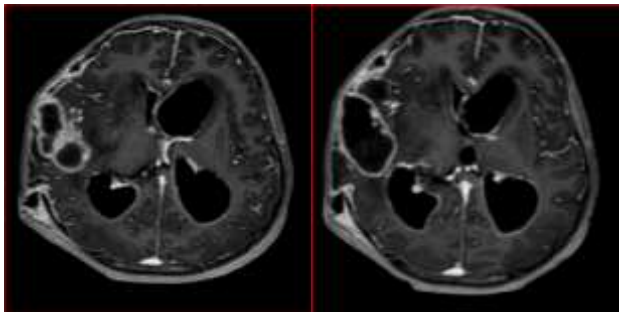

Figure-I: MRI brain with contrast of our patient showing brain abscess.

DISCUSSION

Salmonella species are an uncommon cause of post-operative intracranial infections such as meningitis, subdural empyema, encephalitis and brain abscess. We report a case of post-operative *S. typhi* brain abscess in a patient after surgery for brain tumor. McClintock reported the first case of *S. typhi* related brain abscess in literature in an autopsy report in 1902.[4,5]

In our literature review, we found twelve such cases in which patients with various intracranial malignancies had *S. typhi* related brain abscesses. Out of these twelve cases, eight patients developed *S. typhi* related brain abscess in the postsurgical period following surgery for brain tumor while in remaining four cases there was no history of recent brain surgery[6].

The review of those previously reported cases of *S. typhi* brain abscess further revealed that five cases had glioblastoma multiform; two cases were with craniopharyngioma, two cases were metastatic adenocarcinomas; two with astrocytoma's and one had oligodendroglioma as primary brain malignancy. Our case is unique in this aspect that we found *S. typhi* related brain abscess in association with an aggressive skull based fibrous tumor.[7]

Literature review also revealed that *S. typhi* species related intracranial infection usually occurs in those with various precipitating conditions such as immunocompromised states (e.g., HIV infection and AIDS, with prolong use of corticosteroids or chemotherapy etc.) blood disorders, previous brain surgery or infarcted brain tissue. [3] Among immunocompetent individuals, we also found one case report of *S. typhi* brain abscess following cerebral infarction. Based upon our review of literature related to salmonella brain abscess specifically in patients with brain tumor, the most important predisposing factors are immune compromised status and the presence of structural brain damage.[4]

Both innate and adaptive immune systems furnish resistance against salmonella infections in humans. Acidic pH of stomach, normal microbiota of intestine and mucosal lymphoid tissue provide innate immunity against infection after intestinal ingestion while T and B lymphocytes mediated adaptive responses are equally important. Our patient had uncontrolled malignancy and he was also on corticosteroids to mitigate the effects of tumor related brain edema, the factors that have high potential to impair both innate as well as adaptive immune responses.[8]

Intracranial salmonella infection tends to involve diseased tissue such as hematomas, fracture sites and neoplasm due to the organism tropism for dead tissue. In our case the tumor or postsurgical hematoma may had acted as a culture medium for salmonella typhi growth. The prolonged use of corticosteroids and presence of malignancy may have predisposed our

patient for the dissemination of salmonella typhi to brain after initial infection.[9]

Tissue cultures are most sensitive way to make microbial diagnosis while yields of blood and CSF cultures are low. Patients with *S. typhi* related brain abscess have poor prognosis without adequate drainage of abscess as in the previous literature almost all patient who died had inadequate drainage or did not undergo drainage of abscess. In our case, as already mentioned that he was treated with Meropenem but our patient died in few days, inadequate drainage of abscess and aggressive nature of primary tumor likely contributed to the patient poor clinical outcome. [10]

CONCLUSION

Although *S. typhi* related brain abscess is rare, and is usually associated with immunocompromised conditions it can occur in patients with intracranial malignancies as a result of chemotherapy or tumor related immune dysfunction, presence of necrotic tissue at tumor site or due to prolonged use of corticosteroids. Neuroimaging has a significant role in the diagnosis of such cases. Adequate drainage of pus, bacterial cultures along with culture guided postsurgical antibiotics therapy are the mainstay of treatment, although the nature of patient primary diagnosis also has significant impact over prognosis.

CONFLICT OF INTEREST

There is no conflict of interest to declare by any authors.

AUTHOR CONTRIBUTION

Muhammad Shehbaz: Primary author

Summiya Nizamuddin: Reviewer and contributed to manuscript writing as well

Azra Parveen: Contributed to manuscript writing as well

Salma Muhammad Abbas: supervisor. Reviewer and contributed to manuscript writing as well

REFERENCES

1. Mahapatra AK, Bhatia R. Salmonella intracerebral and subdural abscess--report of two cases. Postgrad Med J. 1987; 63 (739): 373-5. DOI: 10.1136/pgmj.63.739.373
2. Soni CR, Kumar G, Bollu PC. Salmonella brain abscess in a patient on chronic azathioprine therapy for myasthenia gravis: Report of an unusual case and review of literature in the post antibiotic era. J Neurovirol. 2010; 16: 83–92. Doi: 10.3109/13550280903493906.
3. Lu CS, Chiu CH, Lin TY, Lin SL. Salmonella typhimurium brain abscess in a six month old infant: A case report and review of literature. Changgeng Yi Xue Za Zhi. 1190; 219-25.
4. Wolfe MS, Louria DB, Armstrong D, Blevins A. Salmonellosis in patients with neoplastic disease. A review of 100 episodes at Memorial Cancer Center over a 13-year period. Arch Intern Med. 1971 :546-54. DOI: 10.1001/archinte.128.4.546
5. Rodriguez RE, Valero V, Watanakunakorn C. Salmonella focal intracranial infections: review of the world literature (1884-1984 and report of an unusual case. Rev Infect Dis.1986; 8: 31-41,
6. Hanel RA, Araújo JC, Antoniuk A, da Silva Ditzel LF, Flenik Martins LT, Linhares MN. Multiple brain abscesses caused by Salmonella typhi: Case report. Surg Neurol. 2000; 53(1): 86-90. DOI: 10.1016/s0090-3019(99)00161-5
7. Dadia SD, Modi RR, Shirwadkar S, Potdar NA, Shinde CA, Nair AG. Salmonella Typhi Associated Endogenous Endophthalmitis: A case report and a review of literature. Ocul Immunol Inflamm. 2018; 26(4): 527-32. DOI: 10.1080/09273948.2017.1306085
8. Kaur N, Varshney VK, Kombade SP, Yadav T, Suman S, Garg PK, Nag VL. Splenic abscess caused by salmonella typhi: An uncommon presentation. Jpn J Infect Dis. 2021; 74(2): 166-8. DOI: 10.7883/yoken.JJID.2020.518
9. Anandan Y, Antony T, Kumari S, Alexander N. Salmonella Typhi: a rare cause of parotid abscess. BMJ Case Rep. 2020; 13(11): e236423. DOI: 10.1136/bcr-2020-236423
10. Delori M, Abgueguen P, Chennebault JM, Pichard E, Fanello S. Un abcès du sein à Salmonella typhi et revue de la littérature [Breast abscess with Salmonella typhi and review of the literature]. J Gynecol Obstet Biol Reprod (Paris). 2007; 36(7): 709-12. DOI: 10.1016/j.jgyn.2007.04.013

CASE REPORT

SHIGELLEMIA IN A CHILD WITH GASTROENTERITIS: A CASE REPORT AND REVIEW OF MICROBIOLOGY AND MANAGEMENT

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ABSTRACT

Shigellemia is a rare entity of sepsis encountered in individuals having gastroenteritis caused by *shigella* spp. It is reported to be common in infants, immunocompromised, those having chronic illnesses and post-transplant patients on immunosuppressants. We present a case of a two and half year-old child who developed sepsis like illness during recovery from gastroenteritis. *Shigella* spp was isolated from blood culture and he responded well to oral antibiotics. This case illustrates the importance of blood and stool cultures that need to be sent in every febrile gastroenteritis case irrespective of immune status. Early detection and prompt treatment will likely improve outcome.

Keywords: Shigellemia, Gastroenteritis, Child

BACKGROUND

One of the major causes of childhood mortality is diarrheal diseases. Diarrhea related deaths in children are estimated to be 2 million, most of whom are under 5 years of age.[1,2] This preventable disease has astonishingly increased risk of mortality in children with severe malnutrition and sepsis which are also common in our setup.[1-8] Blood and stool cultures are often not done routinely in Pakistan as it could improve etiologic diagnosis. Pathogenic enteric isolates can be isolated in acute gastroenteritis (GE).

The burden of shigellosis is high in resource-poor countries. Multiple studies in Pakistan have documented the importance of *Shigella* as an important etiologic agent in GE. A study from Aga Khan University Hospital of 6670 stool samples from children and adults showed *Shigella flexneri* in 6% samples.[3] Similarly a prospective, population-based study in six Asian countries (including Pakistan) in >600,000 persons of all ages showed *Shigella* species in 2,927 (5%) of 56,958 diarrhea episodes with *Shigella flexneri* being the most frequently isolated.[4] We report here a child with dysentery and *Shigella spp.* blood stream infection (shigellemia) and discuss its importance of epidemiology, diagnosis and treatment.

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CASE REPORT

A two and half year-old male child resident of Kashmir presented in outpatient clinic with history of loose bloody motions 2 weeks ago which was treated by a local pediatrician with metronidazole after which his loose stool improved. However, the child continued to have decreased appetite, low-grade fever, foul smelling stool and a vesicular rash. He was born to non-consanguineous parents at full term uneventfully. He had regular follow up visits to our clinic for routine checkup and immunization. He had age-appropriate development. He was well fed and his height and weight were falling in normal range on centiles chart. His immunization status was up-to-date. He had no history of any past major illness or hospitalization.

On examination the child had weight of 12.5 kg, height of 88 cm, head circumference of 48 cm, pulse 120/minute and temperature of 37C˚. On systemic examination the child was comfortable, with no obvious distress but had pallor. Ear, nose and throat examination was normal. Abdominal examination showed soft non-tender abdomen with no distention and positive bowel sounds. Rest of systemic examination was unremarkable. Clinical impression of dysentery was made.

Laboratory reports showed: Hemoglobin of 10.30 g/dL, hematocrit 32.4%, White Blood Cell count of 14700/µL with Neutrophil 41%, Lymphocyte 45%, Monocytes 10%, and platelet count of 401000/µL, C-reactive protein 6.1 mg/L, alanine transaminase (ALT) 37 U/L, aspartate aminotransferase (AST) 48 U/L, serum albumin 3.93 mg/dL, serum creatinine 0.45 mg/dL and serum calcium of 9.6 mg/dL. Stool routine examination was negative for gross blood, mucus, ova,

cyst, worms and red blood cells but there were 2 WBC's/high power field. On third day blood culture showed growth of gram-negative rods, that later was verified as *Shigella* spp. susceptible to cefixime, ciprofloxacin, ceftriaxone, co-trimoxazole and was resistant to ampicillin and chloramphenicol.

The child was given intravenous ceftriaxone for 5 days and followed by oral cefexime for another 5 days. On 8th day of antibiotic parents told on telephonic inquiry that the child's general conditions had improved, was afebrile for the last 4-5 days and resolution of his anorexia and diarrhea. A follow-up at one month revealed that the child was well and had no further new symptoms.

DISCUSSION

Shigella remains an important public health problem and is the second commonest cause of morbidity and mortality accounting for upto 13% of all diarrheal deaths worldwide.[9] It is responsible for most deaths in children less than five-year-old especially malnourished in low-income countries.[9] Septicemia with *shigella* spp is a rare entity reported in literature but shigella gastroenteritis also called shigellosis or dysentery is a common illness.[4] In shigellosis there is invasion of gastrointestinal tract and infection often remains confined to gastrointestinal tract. Shigellosis prevention is difficult because only few organisms are required to cause the infection. Shigellosis is a public health problem and may be a self-limiting illness that usually resolves in about 14 days.[1,3,4]

Invasive diseases by *shigella* are rare except in immunocompromised patients, post-transplant patients and chronically malnourished children and its mechanism is unclear.[2,4] Septicemia with *shigella* is reported to occur in upto 4% among more than 2000 patients having shigellosis as underlying diagnosis.[10] These patients had significantly more severe manifestations such as dehydration, abdominal tenderness, lethargy, renal failure, leukocytosis and other hematologic findings compared to non-bacteremia patients. These bacteremic patients were also twice as likely to die as well with highest risk for infants less than one-year olds who were malnourished, non-breast fed or were afebrile at presentation.[10] Among children *Shigella* blood stream infection has been reported in literature mainly in infants.[11-14] Other vulnerable populations are also at increased risk such as travelers,[15] acquired immunodeficiency syndrome (AIDS)[16] and transplant patients.[17]

In Pakistan shigellemia has been reported in couple of case series and reports.[18,19] A study from Lahore reported among 45 patients with malignancy shigellemia in 4 (8.8%) patients including three children.[19] All of these were due to *shigella flexneri* and most isolates were susceptible to third generation cephalosporins. None of these patients died. Commonest species causing sepsis is *shigella flexneri* and the most virulent as well.[4]

Shigella resistant strains appears to have emerged over last few decades. A prospective, population-based study in six Asian countries (including Pakistan) was done in >600,000 persons of all ages showed *Shigella* species in 2,927 (5%) of 56,958 diarrhea episodes.[4] *Shigella flexneri* was the most frequently isolated. The majority of *Shigella flexneri* isolates in each site was resistant to amoxicillin and cotrimoxazole. Ciprofloxacin-resistant *Shigella flexneri* isolates were also identified including from Pakistan (3%).[4] Several resistant species from stool cultures are isolated from Pakistan reported in other studies.[5-7] One study from Karachi reported *shigella flexneri* to be the commonest species of *shigella* isolated.[5] It also showed that 100% were susceptible to nalidixic acid, 4% to ampicillin and 7% to co-trimoxazole.[5] Another study from the same city reported co-trimoxazole resistance of 56-89%, ampicillin resistance of 4-87% and no resistance to nalidixic acid was identified.[7] A cross-sectional study from slum areas in Karachi in children with gastroenteritis showed isolation rate of 4% (193/4688 stool samples) for *Shigella species* (*Shigella flexneri* 58%, *Shigella sonnei* 16%, *Shigella boydii* 15% and *Shigella dysenteriae* 11%).[6] All isolates were susceptible to ofloxacin and ceftriaxone. However, resistance was high to commonly used antibiotics (cotrimoxazole 88%, ampicillin 56% and nalidixic acid 39%). Another study from in Karachi in 2002-4 in which resistance to cotrimoxazole was 56-89%, ampicillin 4-87% but nalidixic acid was 0%.[7] A more recent study from Karachi in 199 different samples of *S. flexneri* showed high level of multiple drug resistant strains particularly serotype 2b.[20] All these isolates showed high resistance to amoxicillin/clavulanic acid (100%), quinolones (74.6%) and trimethoprim-sulphamethaxazole (54.4%). Also, multiple resistant genes were reported including *bla*OXA gene. [20] Multiple resistant isolates to trimethoprim-

sulphamethaxazole, cephalosporins, ciprofloxacin and azithromycin have also been reported from other parts of the world and is now part of the high priority so called "GLASS" pathogens.[21] Thus treatment options are becoming limited in cases of severe dysentery due to *Shigella*.

Shigellemia needs to be treated aggressively with appropriate antibiotics and fluid rehydration. Appropriate antibiotics therapy shortens the duration of illness, microorganism shedding as well as it reduces complications. Local resistance patterns should dictate empirical therapy. Recommended antibiotics include cephalosporins, ciprofloxacin and azithromycin. Current WHO guidelines continue to support the use of fluoroquinolones (first-line), β-lactams (second-line) and cephalosporins (second-line). Azithromycin is appropriate as a second-line therapy in regions where the rate of non-susceptibility of ciprofloxacin is known to be high.[22] A systemic review and meta-analysis in children also is in line with the WHO recommendations as well.[23] Our case responded well to cephalosporins to which the organism was susceptible. Outcome is good if diagnosis is suspected early and therapy initiated early. A high mortality rate has been associated with shigellosis including shigellemia.[9]

CONCLUSION

In summary Shigellemia is a rare entity of septicemia but always should be included in the differential of clinical sepsis associated with or followed by dysentery. Symptoms like poor appetite and fever after gastroenteritis not responding to other antibiotics should be investigated for associated underling septicemia. Sending blood and stool cultures should be an integral part of managing any febrile gastroenteritis. Early and accurate diagnosis can avoid morbidity and mortality associated with this condition.

CONFLICT OF INTEREST

There is no conflict of interest to declare by any authors.

AUTHOR CONTRIBUTION

Hamza Khan: Data acquisition, literature review, initial and final draft

Inayat Ullah: Data acquisition, literature review, initial and final draft

Ejaz Ahmed Khan: Conception, literature and data analysis, final draft

REFERENCES

1. Caulfield LE, Onis M de, Blössner M, Black RE. Undernutrition as an underlying cause of child deaths associated with diarrhea, pneumonia, malaria, and measles. American Journal of Clinical Nutrition 2004; 80: 193-8. DOI: 10.1093/ajcn/80.1.193
2. Bhutta ZA, Nizami SQ, Thobani S, Issani Z. Risk factors for mortality among hospitalized children with persistent diarrhoea in Pakistan. J Trop Pediatr 1997 Dec;43(6):330-6. DOI: 10.1093/tropej/43.6.330
3. Alam M, Akhtar YN, Ali SS. Seasonal variation in bacterial pathogens isolated from stool samples in Karachi, Pakistan. J Pak Med Assoc 2003; 53(3): 125-9.
4. von Seidlein L, Kim DR, Ali M. A multicentre study of Shigella diarrhoea in six Asian countries: disease burden, clinical manifestations, and microbiology. PLoS Med 2006; 3(9): e353. DOI: 10.1371/journal.pmed.0030353
5. Khalil K, Khan SR, Mazhar K, Kaijser B, Lindblom GB. Occurrence and susceptibility to antibiotics of Shigella species in stools of hospitalized children with bloody diarrhea in Pakistan. Am J Trop Med Hyg 1998 Jun;58(6):800-3. DOI: 10.4269/ajtmh.1998.58.800
6. Zafar A, Sabir N, Bhutta ZA. Frequency of isolation of shigella serogroups/serotypes and their antimicrobial susceptibility pattern in children from slum areas in Karachi. J Pak Med Assoc 2005;55(5):184-8.
7. Zafar A, Hasan R, Nizami SQ, von Seidlein L, Soofi S, Ahsan T, Chandio S, Habib A, Bhutto N, Siddiqui FJ, Rizvi A, Clemens JD, Bhutta ZA. Frequency of isolation of various subtypes and antimicrobial resistance of Shigella from urban slums of Karachi, Pakistan. Int J Infect Dis. 2009; 13(6): 668-72. DOI: 10.1016/j.ijid.2008.10.005
8. Soofi SB, Habib MA, von Seidlein L, Khan MJ, Muhammad S, Bhutto N, et al. A comparison of disease caused by Shigella and Campylobacter species: 24 months community based surveillance in 4 slums of Karachi, Pakistan. J Infect Public Health. 2011 Mar;4(1):12-21. DOI: 10.1016/j.jiph.2010.10.001
9. Khalil IA, Troeger C, Blacker BF, Rao PC, Brown A, Atherly DE, et al. Morbidity and mortality due to shigella and enterotoxigenic Escherichia coli diarrhoea: The Global Burden of Disease Study 1990-2016. Lancet Infect Dis. 2018; 18(11):1229-1240. DOI: 10.1016/S1473-3099(18)30475-4
10. Struelens MJ, Patte D, Kabir I, Salam A, Nath S, Butler T. Shigella Septicemia: Prevalence, Presentation, Risk Factors, and Outcome. The Journal of Infectious Diseases 1985;52(4):784-790. DOI: 10.1093/infdis/152.4.784
11. Panhotra BR, Panigrahi D, Agarwal KC, Walia BN. Shigella septicemia in children: report of three cases. Indian J Pathol Microbiol.1980;23:299-301.
12. Yen JB, Chang KW, Wu TL, Kuo AJ, Su LH. Shigella flexneri sepsis in an infant. Chang Gung Med J. 2003; 26(8): 611-4.
13. Jain S, Sharma M, Gupta R, Shree N, Kumar M. Multidrug resistant *Shigella flexneri*: A rare case of septicaemia in an infant. Journal of Clinical and Diagnostic Research. 2014;8(6):03–04. DOI: 10.7860/JCDR/2014/8434.4511
14. Nayyar C, Thakur P, Tak V, Singh A. Shigella sonnei sepsis in an infant: A Case Report. Journal of Clinical and Diagnostic Research. 2017:5,(5). DOI: 10.7860/JCDR/2017/25594.9796
15. Grondin C, Imbert P, Ficko C, Mérens A, Dutasta F, Bigaillon C, Rapp C. Shigella flexneri bacteremia in two immune-competent adult travelers. J Travel Med. 2012; 19(4): 258-60.

DOI: 10.1111/j.1708-8305.2012.00627.x
16. Huebner J, Czerwenka W, Gruner E, von Graevenitz A. Shigellemia in AIDS patients: Case report and review of the literature. Infection. 1993; 21(2):122-4. DOI: 10.1007/BF01710748
17. Appannanavar SB, Goyal K, Garg R, Ray P, Rathi M, Taneja N. Shigellemia in a post renal transplant patient: a case report and literature review. J Infect Dev Ctries. 2014;8(2):237-39. DOI: 10.3855/jidc.3000
18. Usman J, Aziz S, Karamat KA, Butt T. Shigella septicaemia in an infant. J Pak Med Assoc. 1997; 47(5): 150-1.
19. Raza A, Mahboob A, Nizammudin S, Nazeer SH, Sultan F. Shigella infections: A two year experience in cancer patients. J Pak Med Assoc. 2016; 66(1): 37.
20. Nisa I, Haroon M, Qasim M, Driessen A, Nijland J, Yasin N, et al. Association of serotype with antimicrobial resistance patterns among Shigella flexneri Isolates from Pakistan: The importance of serotype 2b. Pediatr Infect Dis J. 2020; 39(11): e352-e358-9. DOI: 10.1097/INF.0000000000002791
21. WHO. Media Centre. News Release. WHO publishes list of bacteria for which new antibiotics are urgently needed. 2017. Available from: http://www.who.int/mediacentre/news /releases/ 2017/ bacteria-antibiotics-needed/en/.
22. Williams PCM, Berkley JA. Guidelines for the treatment of dysentery (shigellosis): A systematic review of the evidence. Paediatr Int Child Health. 2018; 38(sup1):S50-S65. DOI: 10.1080/20469047.2017.1409454
23. Tickell KD, Brander RL, Atlas HE, Pernica JM, Walson JL, Pavlinac PB. Identification and management of Shigella infection in children with diarrhoea: A systematic review and meta-analysis. Lancet Glob Health. 2017 Dec; 5(12): e1235-e1248. DOI: 10.1016/S2214-109X(17)30392-3

SHORT COMMUNICATION

PATIENT'S PERCEPTION ABOUT CROSS-INFECTION PREVENTION IN NISHTER INSTITUTE OF DENTISTRY (NID), MULTAN, PAKISTAN

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ABSTRACT

Cross-contamination is the transmission of microbial agents from one subject to another. It can be spread from individual to individual or through infected agents. The source of cross-infection can be saliva, blood, body secretions, infected instruments with blood, and necrotic tissue debris**.** Every year lives are lost because of the spread of infections in hospitals becomes a serious cause of world public health issues. In this descriptive cross-sectional study, a consecutive sampling technique was applied in the periodontology department of Nishter Institute of Dentistry, Multan. 450 participants completed a questionnaire. Self-administered close-ended questionnaire in the English language was used and used local language to familiarize with the questionnaire. Results of the study revealed that 68 – 89% of the participants had a satisfactory level of knowledge about infection and infection prevention and most of them were well educated. 239 participants perceived that AIDS and Hepatitis can be transmitted during dental treatment. Regarding self-reported practice, most of the participants were aware of the fact that sterilized instruments should be used during dental treatment. 89.8% of participants stated that dentists should wear gloves and facemasks to prevent cross-infection from dentist to patient and vice versa. There was a statistically significant difference between occupation and personal protective equipment. There was enough knowledge and awareness of patients presented to the periodontology department of Nishter Institute of Dentistry regarding the infections which can spread in dental clinics like hepatitis and AIDS. There is an increased need in enhancing the awareness of the patients through social media, and also a need to provide knowledge of patients about cross-infection and how can it be prevented through educational programs both for the patients and service providers. It can also be done through awareness campaigns in different shopping places and educational institutes and public places.

Key Words: Infection control guidelines, Personal protective equipment, Health care workers, World Health Organization, Centre of Disease Control

BACKGROUND

Cross-contamination is the transmission of microbial agents from one subject to another. It can be spread from individual to individual or through infected agents. The source of cross-infection can be saliva, blood, body secretions, infected instruments with blood, and necrotic tissue debris.[1,2] Dental health care providers are directly exposed to blood-transmitted infections such as Hepatitis B (HBV) and Hepatitis C (HCV) viruses, staphylococci, streptococci, and HIV (human immunodeficiency virus), mumps, influenza, rubella, and Mycobacterium Tuberculosis[2,3] patients are ignorant of the disease process as antibodies produce against infection by the body is detectable after long incubation period.[4]

Knowledge and attitude also play a very important role in the prevention of transmission of infection in dental facilities**.**[5] The current study emphasized that the information regarding the cross-contamination practices of oral health professionals and assistants for dental facilities and patients' perceptions regarding to generated information will be helpful for the implementers to apply the guidelines for contamination control practices in the working places. In 1986, the Center for Disease Control and Prevention (CDC) issued a paper of recommendations for infection control in dental settings.[6] The Occupational Safety and Health Administration (OSHA) also published guidelines to prevent the spread of infection.[7] British

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Dental Association also issued guidelines for Infection Control in Dentistry.[8] In addition, other measures are also developed for infection control. CDC and WHO also developed guidelines for hand hygiene practices.[9] Most of the patients have good knowledge regarding wearing of gloves by dental health professional during dental procedures but have less knowledge of wearing of face masks during dental practices, Moreover, they were so much afraid of HIV infections in their community and they did not prefer those dental clinics where they knew that HIV patients were treated.[3] Dental infection is the main cause of the burden of communicable diseases in Pakistan. Increased awareness of patients about cross-infection control methods adopted by dental health care providers will help hopefully, to ask and remind them to adopt all essential precautions for the prevention of cross-infection and to save their lives as well. Hence, this survey helps to reveal the perceptions in dental patients about infection control practices visiting Nishter Institute of Dentistry Multan (NID), Pakistan

MATERIAL AND METHODS

A descriptive Cross-Sectional survey was conducted at Nishtar Institute of Dentistry, Multan. All OPD patients in NID who visited the Periodontology Department, constituted the sample of 450 patients between May 2019 to August 2019 were recruited in the study. A consecutive sampling Technique was applied. Self-administered close-ended questionnaire in the English language was used in this study, which was explained to the participants in local language by dental health professional. All the adult patients (more than 15 years) who visited for dental treatment were recruited for the study. The patients who refused to be a part of the study were excluded from the study.

A Statistical package, SPSS version 20 was used for data entry and Analysis. The mean age and frequency of different attitudes were analyzed by Descriptive analysis. Chi-square was also done to see the statistical significance.

RESULTS

Results showed that among the participants 60.7% were female while 39.3% of participants were male. 65.3% of participants were between the age group of 16-32 years. 27.3% of participants were between age group of 33-48 years. Only 7.3% of participants were in the age group of 49-65 years. 27.1% of the respondents were jobless. 12.4% of participants were doing government jobs while 24.2% were doing private jobs. 5.8% of participants were businessmen while 30.4% were doing other jobs. The majority of the participants 26.4% were bachelor's degree holders and 10% were below matric level. 38.4% of the respondents belonged to the socially deprived class.

Compared to males, female patients said that dentists should use personal protective equipment's. There was significance between gender and facemasks wear (P-Value 0.028). There was also an association between gender and glasses wear (P-Value 0.001).

Table-I: Degree of knowledge or agreement

Degree of agreement Statement	*Agree*	*Disagree*	*Don't Know*
Disease can be caused during the dental procedure via instruments	63.6%	20.4%	16%
AIDs and Hepatitis can be transmitted together	4.7	95.3	-
Only one disease can be transmitted at a time	15.1	84.9	-
Clean instruments should be used	90.1	-	9.9
Delayed next visit due to the fear of cross-infection	50.7%	49.3	
Dentist should always follow the cleanliness of instruments	76.4	10.4	13.2
Dentist should always wear new pair of gloves	89.8	10.2	-
Cross-infection can be prevented by using new pair of gloves	27.3	72.7	-

Table-II: Gender and personal protective equipment's

Question	Gender	Yes	No	P Value
Necessary to wear gloves	Male	158	19	**0.773**
	Female	246	27	
Necessary towear facemasks	Male	152	25	**0.028**
	Female	252	21	
Necessary to wear glasses	Male	104	73	**0.001**
	Female	203	70	

DISCUSSION

This is the first kind of short communication about patient's perception regarding cross-infection prevention in Pakistan which was conducted in Nishter Institute of Dentistry, Multan. Although many studies had been done on dentists and dental assistants, but this was the first study that was conducted to assess patient's perception.

89.8% of participants of this study perceived that gloves should be used in dental clinics to avoid cross-infection. This value was slightly higher than the study which was conducted in Nigeria[3] which showed that 88.8% of patients responded that dentists should wear gloves while caring their patients and in comparison with the study which was conducted in Egypt[10] which showed the percentage of 90% of respondents agreed that dentists should wear gloves to prevent cross-infection. These results were also higher than the studies conducted by Jorden[11], 87%, and Burke *et al*[12] 84% to wear the gloves during procedure[13] another study in which the percentage was 69%, 264 participants perceived that wearing gloves protected the transmission of infection from one patient to another patient, while 258 patients stated that it is used to prevent infection from dentist to patient. When it was asked from patients for facemask wear, 89.8% stated that it is necessary for dentists to wear facemasks and this value is same as for gloves wear. Studies conducted by Mousa *et al*[2] and Porter *et al*[14] showed the values 72% and 73% respectively for facemasks wear, which are lesser than the results of this study. The most important reason which was mentioned by the patients in this study was that facemasks prevent cross-infection from one patient to another patient. Response for the use of eyeglasses used by dentists during dental treatment was 68.2% which showed a higher percentage than a study conducted in Egypt[10] (37%) in and by Porter *et al*[14] in Hong Kong and Great Britain, i.e. 37% and 44%, respectively. This low value showed that there is less awareness among people about infection spread by splashes which were produced during dental treatment and cause infections via lachrymal route. 73% of patients were aware of the disease of HIV and Hepatitis. Statistical significance between gender and all other study variables was not found. This was accordance to the Nigerian study[3], that study also showed that there was no statistical significance between gender and transmission of HBV, HCV, HIV. for that appropriate and powerful practices of widespread precautionary measures complete HBV vaccination among dental health professionals are important to evade transmission of contaminations while performing various dental procedures.

However, statistical significance was found in the Egyptian study[2], among males and females and other study variables of cross-infection prevention. This difference was because females were more concerned about dental hygiene than the male participants who were least bother about dental hygiene. A statistical significance was found between education and knowledge regarding HIV and Hepatitis. Participants who had graduation were more knowledgeable than the other participants.

The current results reflect the self-reported cross-infection awareness among a population with a relatively high socioeconomic status (as judged by reported income and education). Therefore, caution is advised in generalizing as this is a rather unique population, not representative of the general Jordanian population. Future studies with a larger and more community-representative sample to assess the effect of patient knowledge of cross-infection control measure on the dentist's behavior and commitment to use infection control measures are warranted.

CONCLUSION

This cross-sectional study revealed that there is a satisfactory level of knowledge and awareness regarding cross infections that can spread in dental clinics like Hepatitis and AIDS, but have good knowledge that how these infections are avoided in dental clinical settings. There is an increased need in enhancing the awareness of this issue through educational programmes. There is limited knowledge of certain aspects of infection control among patients visiting he dental department of public sector NID, Multan. In the future, further studies are recommended to assess the dentist's behavior and commitment to using infection control practices.

LIMITATIONS

This study was conducted in public sector hospital and

cannot be generalized, for generalizing the results these types of studies required comparison with private sector hospitals with large sample size and mix socio-economic background.

RECOMMENDATIONS

A rational and practical schedule for the barrier of cross-contamination and cross infection to minimize the combination of transmission of infections should be followed. All medical staff should protect themselves by making it sure that they are fully vaccinated against Hepatitis B and other communicable diseases. In the working place, it is the liability of dental health professionals to make all employees conscious of the principles of infection control. Suitable infection control safety measures should be provided and should be in use regularly. Training of the assistants should be done annually; Immunization condition of all personnel should be maintained throughout service.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest

AUTHOR CONTRIBUTION

Qaisar Joiya: Qaisar was involved in data collection and drafted the outline of the article.

Shamima Abdullah: Shamima and Jawwad contributed in data analysis.

Tahira Gul: contributed in drafting, proof read and finalized the manuscript.

REFERENCES

1. Ibrahim NK, Alwafi HA, Sangoof SO, Turkistani AK, Alattas BM. Cross-infection and infection control in dentistry: Knowledge, attitude and practice of patients attended dental clinics in King Abdulaziz University Hospital, Jeddah, Saudi Arabia. J Infect Public Health. 2017; 10(4): 438-45. DOI: 10.1016/j.jiph.2016.06.002
2. Mousa AA, Mahmoud NM, Tag El Din AM. Knowledge and attitudes of dental patients towards cross-infection control measures in dental practice. EMHJ-Eastern Mediterranean Health J.1997; 3 (2): 263-3.
3. Sofola O, Uti O, Onigbinde O. Public perception of cross-infection control in dentistry in Nigeria. Int Dental J. 2005; 55(6): 383-7. DOI: 10.1111/j.1875-595x.2005.tb00051.x
4. McCarthy GM, Britton JE. A survey of final-year dental, medical and nursing students: occupational injuries and infection control. J Canadian Dental Assoc. 2000; 66(10): 561.
5. Puttaiah R, Shulman JD, Youngblood D, Bedi R, Tse E, Shetty S, *et al*. Sample infection control needs assessment survey data from eight countries. Int Dent J. 2009;59(5):271-6.
6. Control CfD. Reports on AIDS Published in the Morbidity and Mortality Weekly Report June 1981 Through May 1986: Department of Health and Human Services, Public Health Service, Centers for …; 1986.
7. Safety USO, Administration H. Ergonomics, the Study of Work: US Department of Labor, Occupational Safety and Health Administration; 1991.
8. Nield H. A short history of infection control in dentistry. Bdj Team. 2020;7(8):12-5. DOI: doi.org/10.1038/s41407-020-0402-1
9. Onyedibe KI, Shehu NY, Pires D, Isa SE, Okolo MO, Gomerep SS, *et al*. Assessment of hand hygiene facilities and staff compliance in a large tertiary health care facility in northern Nigeria: A cross sectional study. Antimicrob Resist Infect Control. 2020; 9(1):1-9. DOI: doi.org/10.1186/s13756-020-0693-1
10. El-Houfey AA, El-Maghrabi NM. Knowledge and attitudes of dental patients toward cross infection control measures in dental clinics at Assiut University Hospital. Int J Nursing Didactics. 2016; 6(8):14-20.
11. Barghout N, Al Habashneh R, Ryalat ST, Asa'ad FA, Marashdeh M. Patients' perception of cross-infection prevention in dentistry in Jordan. Oral Health Prev Dent. 2012; 10(1): 9-16.
12. Kearns H, Burke F, Cheung S-W. Cross-infection control in dental practice in the Republic of Ireland. Int Dental J. 2001; 51(1): 17-22. DOI: 10.1002/j.1875-595x.2001.tb00812.x
13. Woo J, Anderson R, Maguire B, Gerbert B. Compliance with infection control procedures among California orthodontists. Am J Orthod Dentofacial Orthop. 1992; 102(1): 68-75. DOI: 10.1016/0889-5406(92)70016-4
14. Porter S, Peake G, Scully C, Samaranayake L. Attitudes to cross-infection measures of UK and Hong Kong patients. Br Dent J. 1993;175(7):254-7. DOI: 10.1038/sj.bdj.4808292

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