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## DENGUE AND ITS CHALLENGES

Ahmed Mujadid Burki, Muhammad Luqman Satti

PNS Shifa Hospital, Karachi Pakistan

Dengue is not a new disease, it has reemerged in the past 20 years with an expanded geographic distribution of both the viruses and the mosquito vectors, increased epidemic activity, the development of hyperendemicity and the emergence of dengue hemorrhagic fever in new geographic regions. In 1998 this mosquito-borne disease was the most important tropical infectious disease after malaria, with an estimated 100 million cases of dengue fever, 500,000 cases of dengue hemorrhagic fever, and 25,000 deaths annually.<sup>1</sup> The reasons for this resurgence and emergence of dengue hemorrhagic fever in the waning years of the 20th century are complex and not fully understood, but demographic, societal, and public health infrastructure changes in the past 30 years have contributed greatly. Dengue is a common arboviral infection with a diverse spectrum of clinical manifestations. Dengue hemorrhagic fever is a more severe form of infection characterized by plasma leak and hemoconcentration. Although hepatic dysfunction is common in dengue illness, massive liver necrosis is rarely reported.<sup>2</sup> Lactic acidosis is a poor prognostic marker in liver failure related to dengue. Management of acute renal injury in dengue hemorrhagic fever due to prolonged shock is challenging as the fluid reabsorption during the recovery phase expands the intravascular volume and precipitates heart failure and pulmonary edema. Dengue fever is characterized by its clinical polymorphism ranging from asymptomatic to severe forms, which are rare in travelers. Its definite diagnosis is based on virological tests selected according to the stage of the disease and the kinetics of the virus.

Several attempts in developing an effective vaccine to protect individuals from dengue infection and the stage of clinical trials are gathered in the present work as well. It has 4 serotypes of epidemiological importance. The classification denotes two clinical spectrums- dengue fever (DF) and dengue haemorrhagic fever (DHF). Most cases are stereotype and amenable to fluid resuscitation. Dengue is an

extremely challenging infection to treat in the globe today.<sup>3</sup> Unusual presentation and complications could be fatal, if not detected early where therapeutic window period is very short. Clinicians need awareness of these problems which are not uncommon, but underreported and often overlooked. Dengue virus infection is now a global problem affecting tens of millions of people.<sup>4</sup> The spread of the four dengue virus serotypes had led to increased incidence of dengue haemorrhagic fever (DHF) reported and with 2.5 billion people at risk, efforts towards the development of safe and effective vaccines against dengue must be accelerated.<sup>5</sup>

The possible reasons for rapid deterioration and deaths in recent dengue outbreak could be multi factorial. First, due to global warming and change in climatic conditions, the disease severity and rapid progression might have changed. Second, may be due to heavy burden of disease in this season, early warning signs in some patients might have been missed in the initial screening and triage of febrile patients leading to dehydration and then rapid deterioration. Third, in young patients as reported in some studies, there may be an exaggerated immune response which can be confirmed by tests such as IL-6 and serum ferritin levels. Fourth, the patients could have a possible second exposure, which can lead to a fatal outcome as reported in many studies.<sup>6,7</sup> However some studies conclude that primary infection can have high mortality than secondary infections.<sup>8,9</sup> Fifth, some patients might have a high viral load leading to fatal outcome as shown in many studies.<sup>10,11</sup>

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**CORRESPONDENCE AUTHOR**

**Dr Ahmed Mujadid Burki**

**Consultant Intensivist**

**PNS SHIFA Hospital, Karachi**

Email: [ahmedburki2004@yahoo.com](mailto:ahmedburki2004@yahoo.com)

# TRICHURIS TRICHIURA INFECTION DIAGNOSED BY HISTOPATHOLOGY

Summiya Nizamuddin, Maliha Latif, Asad Hayat Ahmad

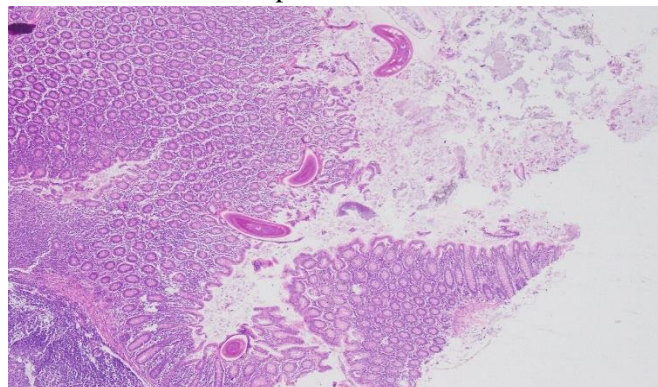
Shaikat Khanum Memorial Cancer Hospital and Research Centre, Lahore Pakistan

## DEAR EDITOR,

We would like to highlight a case of a young female patient who was accidentally diagnosed with a *Trichuris trichiura* infection, when her abdominal resection samples were sent for histopathological studies.

The patient was a 16-year-old female patient who presented to her local hospital in Temergara, Lower Dir district with a history of abdominal pain along with abdominal distention for the last 6 months. The patient subsequently underwent a right hemicolectomy procedure and her terminal ileum, cecum, appendix and ascending colon were resected. Suspecting tuberculosis or a malignancy, portions of resections were sent for histopathological studies, to the histopathology section at the Shaikat Khanum Memorial Cancer Hospital and Research Centre, Lahore.

Gross examination of specimen revealed interloop fibrous adhesions between small bowel, large bowel and appendix with some twisting of bowel, but no discrete tumor or polyp was identified. Microscopically, on hematoxylin and eosin stain (H&E) one section of large bowel showed cross-sections of multiple intestinal parasites. (Picture 1) To confirm the identification of the parasite, the case was discussed with microbiology and parasitology experts and on closer inspection, the worm was identified as *T. trichiura* and hence, reported.



**Picture 1: 40x H&E stain on a section for the large bowel showing multiple worm in cross section of the, embedded in the mucosa.**

On the cross sections of the worm, one of the most prominent structures observed were the bacillary bands (Picture-2). Bacillary band is a basophilic layer beneath the muscular layer of the worm that is specific to this genus of worms.



**Picture-2: 400x H&E Cross section of the worm, with prominent bacillary bands.**

Another helpful feature in its identification was the presence of the worm in the large bowel (which is the most typical location for the whipworm), and the fact that its medial end was embedded within the mucosa, and its distal end partially loose in the lumen. Whipworms are known to embed their thinner anterior end into the intestinal mucosa, allowing the larger end to hang free in the gut lumen. This lets eggs to be shed into the lumen from the adult female.

We made multiple attempts to contact the patient's guardians to advice stool examination for ova and parasites and to seek treatment for trichuriasis, unfortunately, all in vain.

*T. trichiura* is as soil-transmitted helminth infection and is among the most common infections affecting the poorest and most deprived communities, worldwide. They are spread by eggs present in human faeces which contaminate soil in places with poor sanitation. The eggs hatch into larvae in the small intestine, which then grow into their mature forms and localize in the colon.<sup>1</sup>

These are nematode parasites and cause diarrhoea and dysentery in humans. They are also known to cause rectal prolapse in heavy infections.<sup>2</sup>

*T. trichiura*, a round worm, popularly known as the human whipworm, gets its name from its characteristic shape with a short thicker end and a longer thinner end, giving the worm a 'whip-like' shape. The thinner, anterior end lies buried in the mucosa of the ileocecal region. The size of these worms varies from 3 to 5 cm. The female usually larger than the male.<sup>3</sup>

A quarter of the world's population is infected with *T. trichiura*, which is characterized by a relative absence of symptoms and generally go undetected. Often only patients with heavy infections become symptomatic. In those with heavy parasitic infection, symptoms include anaemia, diarrhea, and intestinal bleeding. Definitive diagnosis is made by identifying *T. trichiura* eggs in stool specimens. However, several reports have described the detection of *T. trichiura* during colonoscopy, where colonoscopy was performed for evaluation of nonspecific gastrointestinal symptoms, such as abdominal pain, diarrhea, and anemia.

Bowel resection is not usually indicated for *T. trichiura* infection and resection in this case was likely due to subacute intestinal obstruction secondary to fibrous interloop adhesions causing some degree of mechanical obstruction. Etiology of adhesions could not be ascertained by histopathologic examination in

this case but is unlikely to be due to *T. trichiura* infection.

The purpose of this case is to highlight the accidental finding of *T. trichiura* infection on histopathological examination which is the first encounter in our setting.<sup>4</sup>

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## CORRESPONDENCE AUTHOR

**Dr Summiya Nizamuddin**  
Department of Pathology,  
Shaukat Khanum Memorial Cancer Hospital  
and Research Centre, Lahore Pakistan  
Email: [summiyan@skm.org.pk](mailto:summiyan@skm.org.pk)



# RESPONSE TO INTRADERMAL ROUTE OF HEPATITIS B VACCINATION IN CHILDREN ON MAINTENANCE HEMODIALYSIS: A SINGLE CENTER EXPERIENCE

Kalimullah Khan, Aasia Zubair, Madiha Aziz, Sanaullah Agha, Pawan Kumar, Seema Hashmi

Sindh Institute of Urology and Transplantation, Karachi Pakistan

## ABSTRACT

**Background:** To determine the response of intradermal route of hepatitis B vaccine (HBV) in pediatric dialysis patients.

**Material and Methods:** Prospective, observational study carried out in the Hemodialysis Unit, Sindh Institute of Urology and Transplantation (SIUT), Karachi, from October 2021 to September 2022. Patients younger than 18 years, Hepatitis B surface antigen negative irrespective of primary vaccination status, were tested for anti-Hepatitis B antibody titers. Those with levels less than 10IU/L were recruited for intradermal vaccination at 0,1 and 6 months and titers rechecked in the 2<sup>nd</sup> and 7<sup>th</sup> month of study.

**Results:** Of the 168 children screened, 81 (48%) patients were eligible for vaccination, however, 52 agreed to participate. Males were 27 (52%). Mean age was  $12.18 \pm 2.61$  years. End-stage kidney disease due to unknown etiology was the most common cause in 27 (52%) patients with mean dialysis duration being  $1.43 \pm 1$  years. Most, 48 (92%) patients were hypertensive and majority, 47 (90%) patients tested negative for hepatitis C. Final analysis was done on 38 patients. Thirty-four (89.4%) patients responded; 25 (65.8%) being good and excellent responders. Mean antibody titers before intradermal vaccination of  $2.87 \pm 3$  IU/L improved to  $383 \pm 397$  IU/L. Ages 11-15 years showed a statistically significant association with the development of anti-Hepatitis B antibody titers (p value: 0.02) while gender, dialysis duration, pre-vaccination HBs titers, hepatitis C status, hypertension and cause of end-stage kidney disease did not have any effect.

**Conclusion:** ID route of HBV is efficacious in producing seroprotective anti-HBs titers in pediatric ESKD patients. Children 11-15 years had more robust response.

**Keywords:** Children, Hemodialysis, Hepatitis B, Intradermal, Vaccination

## BACKGROUND

Exposure to blood borne viral infections like hepatitis B (Hep B) remains an accompaniment risk to the life-saving treatment modality of hemodialysis, for end stage kidney disease patients (ESKD) including children. The highly infectious Hep B virus can be acquired via the percutaneous or mucosal route by contact with central dialysis lines, contaminated blood products and potentially infected instruments or surfaces. Chronically infected patients and health care workers, and an impaired immunity in ESKD patients also form an important part of the equation.<sup>1</sup> Resultantly, the reported prevalence of Hep B ranges

from 6%- 15% in the various pediatric hemodialysis populations studied. Shah SR *et al* documented the prevalence of Hep B to be 2.5% in pediatric hemodialysis patients in Pakistan.<sup>1</sup> Long- term carriers have an increased risk of progressive chronic liver disease, cirrhosis, and hepatocellular carcinoma.<sup>3,4</sup> Immunization and robust infection control practices are the two strategies to contain Hep B. The Centre for Disease Control recommends double the dose of hepatitis B vaccine (HBV) in adults via intramuscular (IM) route to account for the low seroconversion rates in ESKD patients.<sup>1,5</sup> In patients younger than 20 years of age, higher doses might be more immunogenic, but no specific recommendations have been made. The Southwest Pediatric Nephrology group found seroprotection rate of 91% in pediatric dialysis patients with an augmented intramuscular vaccine dose.<sup>6</sup> However, titers reported by Sonia JF *et al* after secondary IM HBV vaccination were lower in ESKD patients even after 100% seroconversion.<sup>7</sup> Measures including intradermal (ID) route, use of third


**Correspondence:** Dr. Asia Zubair, Sindh Institute of Urology and Transplantation, Karachi Pakistan

Email: [aasiazubair@gmail.com](mailto:aasiazubair@gmail.com)

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generation vaccines with newer adjuvants or immunostimulants have reported better response rates.<sup>8</sup> Administration of ID HBV in healthy children<sup>9</sup> and in those with conditions like celiac disease<sup>10</sup> has shown comparable seroprotection rates of more than 90% similar to that of the primary Hep B vaccination. While, multiple studies in adults<sup>11</sup> have shown better efficacy of ID HBV route in non-responders to the IM route; no study to the best of our knowledge has been conducted in pediatric hemodialysis patients. Hence, the aim of this study was to determine the seroprotection rate of the ID route of HBV in previously vaccinated pediatric hemodialysis patients with inadequate hepatitis B virus surface antibody (anti-HBs) titers. Secondly, it also aimed to determine if any of the clinical features correlated with the response to the ID vaccine.

## MATERIAL AND METHODS

This prospective interventional study was conducted at the hemodialysis unit of Sindh Institute of Urology and Transplantation (SIUT), Karachi, over 12 months from October 2021 to September 2022, after approval from the ethical and scientific committee (SIUT-ERC-2021/A-329).

Considering the approximate number of pediatric patients on hemodialysis in 6 months to be 200, with a margin of error of 10%, a confidence interval of 95%, and a reported response rate of 75.8%<sup>12</sup> to ID HBV, a sample size of at least 53 patients was calculated using the Epi Info<sup>13</sup> sample size calculator. A consecutive, non-probability sampling technique was used.

Written and informed consent was taken from parents/guardians, and assent was obtained from children more than 12 years of age. Demographic and clinical details were recorded. Patients younger than 18 years and on maintenance hemodialysis for at least 1 month were screened for Hepatitis B surface antigen (HBsAg) and hepatitis C antibody (HCV) using the Micro-Particle Enzyme Immunoassay (MEIA) ARCHITECT SYSTEM. Patients with negative HBsAg and HCV were subsequently tested for anti-HBs titers using the same immunoassay and those with titers <10 IU/L were recruited in the study. Patients who tested HCV positive but were not on anti-retroviral therapy were included in the study. Patients with a history of hepatitis B infection, localized skin

infection, and human immunodeficiency virus (HIV) positive patients were excluded from the study.

Participants were vaccinated with low dose (2µg) ID recombinant HBV (AMVAX 10µg/0.5ml, AMSON VACCINES AND PHARMA (PVT) Islamabad) at 0,1, and 6 months<sup>9</sup> by the principal investigator using a 1ml tuberculin syringe and "wheal" formation was documented as evidence of the ID injection.

Side effects at the injection site were noted at the time of vaccination and, at home by parents. They were recorded on the subsequent dialysis session. They included pain at the time of injection till a maximum of 5 minutes, dark pigmentation, skin nodules, itching and fever.

Blood samples for anti-HBs titers were collected in the 2<sup>nd</sup> and 7<sup>th</sup> month of the study. Patients with titers <10 IU/L were considered non-responders, and those with titers >10 IU/L were considered responders. The responders were further categorized on the basis of their Anti-HBs titers as very poor: 10 to 50 IU/L, poor: 50 to 100 IU/L, good: 100 to 1000 IU/L, and excellent > 1000 IU/L.<sup>15</sup>

Statistical analysis was performed using SPSS version 20. Categorical variables were expressed as frequency and percentages, while continuous variables were represented by mean and standard deviation. Fisher exact test was used to determine the association between the categorical variables. A p-value of <0.05 was considered statistically significant

## RESULTS

Among the 168 screened patients as shown in Figure-I, 81(48%) patients were eligible for vaccination, of which, only 52 (31%) agreed to participate in the study. Fourteen (27%) patients were further excluded from the final analysis as 3 (6%) died from disease-related complications, 6 (11.5%) transferred to other facilities, 1 (2%) patient received a live-related kidney transplant and 4 (7.6%) more patients withdrew from the study.

Of the 52 enrolled participants, 27 (52%) were males with a mean age of  $12.18 \pm 2.61$  years. Table-I shows the demographic and clinical characteristics of the participants. Most patients had end-stage kidney disease (ESKD) due to unknown etiology and had been on dialysis for a mean time period of  $1.43 \pm 1$  years. Majority i.e., 48 (92%) were hypertensive and most patients, 47 (90%) tested negative for hepatitis C.

Overall, 34 (89.4%) out of 38 patients who completed the study showed response to ID vaccination, of which, 25 (65.8%) patients were good and excellent responders. The mean anti-HBs titers before ID vaccination were  $2.87 \pm 3$  IU/L while after vaccination they improved to  $152.31 \pm 249.24$  IU/L after the second dose and to  $383 \pm 397$  IU/L at the end of the study as shown in Table-II. Of the clinical variables studied, patients of the age group 11-15 years showed a

statistically significant association with the development of anti-HBs titers ( $p$  value 0.02) as shown in Table-III. None of the other variables including gender ( $p = 0.53$ ), duration of dialysis ( $p = 0.56$ ), pre-vaccination HBs titers ( $p = 0.20$ ), hepatitis C status ( $p = 0.45$ ), hypertension ( $p = 0.46$ ) or cause of ESKD ( $p = 0.24$ ) had any statistically significant effect on the development of seroprotection.

**Table-I: Demographic and Clinical characteristics of study participants (n=52\*)**

Variable	n (%)
<b>Gender</b>	
Male	27 (52)
Female	25 (48)
<b>Age (years) median (IQR)</b>	12 (10-14)
<b>Age Categories (years)</b>	
5-10	11 (21.2)
10-15	36 (69.2)
>15	5 (9.6)
<b>Duration of hemodialysis (years) mean<math>\pm</math>SD</b>	1.43 $\pm$ 1
<b>Cause of ESKD</b>	
Glomerular Disease	7(13.5)
CAKUT	13 (25)
Renal Stones	5 (10)
Unknown	27 (52)
<b>Hypertension</b>	
Yes	48 (92%)
No	4 (8)
<b>Anti HCV Status</b>	
Negative	47 (90)
Positive	5 (10)
<b>Side Effects</b>	
None	6 (11)
Pain at injection site and anxiety	28 (54)
Skin pigmentation	16 (31)
Fever	1 (2)

\*14 children were excluded from final analysis. Details in text

ESKD: end stage kidney disease, CAKUT: congenital abnormality of kidney and urinary tract

**Table-II: Hepatitis B surface antibody (Anti-HBs) Titers (n=38)**

Variable	n (%)
Pre-vaccination titres (IU/L) mean $\pm$ SD	2.87 $\pm$ 3
Response	
Responder	34 (89.4)
Non-Responders	4 (11)
Post-vaccination titres (IU/L) at 2 months mean $\pm$ SD	152.31 $\pm$ 249.24
Post vaccination titers at 2 months	
Non-Responder	
<10 IU/L	9 (24)
Responder	
Very Poor (10-50 IU/L)	13 (34)
Poor (50-100 IU/L)	5 (13)
Good (100-1000 IU/L)	9 (24)
Excellent (>1000 IU/L)	2 (5)
Post vaccination titers at 7 months	
Non-Responder	

<10 IU/L	4 (10.5)
<b>Responder</b>	
Very Poor (10-50 IU/L)	4 (10.5)
Poor (50-100 IU/L)	5 (13.2)
Good (100-1000 IU/L)	18 (47.4)
Excellent (>1000 IU/L)	7 (18.4)
Post-vaccination titres (IU/L) at 7 months <i>mean±SD</i>	383±397

**Table-III: Association of clinical and demographic features with response to Hepatitis B vaccine.**

Variable	Responder	Non-responder	P value
<b>Age in years</b>			
5-10 yrs	9	0	0.02
11-15 yrs	23	2	
>15 yrs	2	2	
<b>Gender</b>			
Male	20	3	0.53
Female	14	1	
<b>Pre-vaccination titers</b>			
0-5 IU/L	24	4	0.20
6-9.9 IU/L	10	0	
<b>Duration on Dialysis</b>			
<1yr	17	1	0.56
2-5 yrs	16	3	
>5 yrs	1	9	
<b>Anti HCV Status</b>			
Negative	30	3	0.45
Positive	4	1	
<b>Hypertension</b>			
Yes	30	4	0.46
No	4	0	
<b>Cause of ESKD</b>			
Glomerular diseases	6	0	0.24
CAKUT	9	3	
Renal Stones	4	0	
Unknown Etiology	15	1	

## DISCUSSION

In this prospective, interventional study, the ID route of HBV was found to be efficacious in inducing seroprotection in the majority of our dialysis dependent children who were previously vaccinated but had anti-HBs titers less than 10 IU/L.

Our study found a seroprotection rate of 90% after 3 doses of ID vaccine. In contrast, Kamath *et al* found seroprotection of only 72% after 3 doses of IM vaccine in chronic kidney disease (CKD) children who had previously received primary immunization.<sup>15</sup> In fact, Drachman *et al* observed a response rate of 86% after giving 5 injections of augmented dose of 40 micrograms IM HBV in dialysis- dependent children.<sup>16</sup> Better seroprotection via ID route is likely due to the abundance of antigen presenting Langerhans cells in the dermis that elicit an enhanced immune response in the otherwise low immunity state of ESKD.<sup>12</sup> A German study demonstrated vaccine-reactive T cells in previously vaccinated Hep B patients but having inadequate anti-HBs antibody titers. These T cells can

possibly be induced to produce seroprotection via the ID route of vaccination.<sup>17</sup> While, Barraclough KA *et al* from Australia found the ID route of vaccination to be the only factor predictive of seroprotection in ESKD patients.<sup>3</sup>

Response rate of 76% was seen at 8 weeks in our study after the second ID dose and that of 90% after the third dose at 28 weeks. In comparison, the adult hemodialysis population at our institute reported a seroprotection of 76% after 18 weeks of the ID dose as reported by Hanif F *et al*.<sup>12</sup> It appears that children mount a better antibody response as compared to adults. Age less than 30 years appears to be a non-modifiable factor against HBV vaccine response.<sup>18</sup>

In this regard, Chanchairujira T *et al* found 92% patients to have good and excellent response and titers greater than 100 IU/L with ID vaccination as compared to 69% patients developing good seroprotection with the IM route.<sup>19</sup> A slightly lesser percentage (69%) of our patients developed anti-HBs titers more than 100IU/L. A possible explanation is that in the aforementioned cohort of Chanchairujira T

*et al*, 7 doses of ID vaccine were given compared to 3 doses in our study.

The age group of 11-15 years was found to have the most response rate to ID vaccine. An African study group similarly showed maximal seroprotective rates after primary HBV in children less than 15 years of age.<sup>20</sup> Children younger than 10 years made up a smaller proportion of patients needing revaccination in our cohort. This could likely be because of residual titers from the effect of primary immunization series.

No gender predilection was seen in our study. Conversely, the pediatric study from Bangladesh showed better seroconversion rates in girls.<sup>7</sup> However, a predominance of males is usually noted in ESKD patients as reported by Preka *et al*.<sup>21</sup> This could perhaps be because of greater prevalence of congenital abnormalities of the urinary tract in boys.

Hepatitis C infection has been proposed to impair dendritic cell antigen presentation. Co-infection with hepatitis C in 10% of our studied patients did not hamper vaccine response. This finding has been supported by other studies too.<sup>22</sup> However, in a duo of studies conducted by Navarro *et al*, induction of seroprotection by HBV was low in hemodialysis patients with HCV co-infection.<sup>23</sup>

Hypertension has also been implicated in decreased response rates as it appears to cause mechanical and oxidative injury of vessels which leads to modification of the immune system.<sup>24</sup> While most of our patients were hypertensive, possibly because measurements were taken just before a dialysis session, it did not appear to affect antibody formation.

Injection site pain and anxiety were the most common side effects in 54% of our patients. Bunapuradah *et al*, however, reported pain in only 7.7% of their patients.<sup>25</sup> On the contrary, Egemen A *et al* documented skin pigmentation in 26% of the infants and 35% of the preschoolers as the most observed side effects in their study.<sup>26</sup>

In conclusion, our study has documented a robust seroprotective response of ID route of HBV in ESKD patients with low anti-HBs titers. The limitations of our study include a single-center analysis of a small cohort of patients with a short-term follow up. Our drop-out rate was also high due to several reasons, including switching of dialysis facility to a center closer to patients' area of residence. Large scale studies comparing augmented IM dose with ID dose and with longer follow up to determine the duration of persistence of adequate anti-HBs titers are needed.<sup>14</sup>

## CONCLUSION

The ID route of HBV can be used as an alternative vaccination method in pediatric ESKD patients who

have low anti-HBs titers. It appears to induce good protective antibody levels against Hep B in the uremia-associated immune suppressed and high-risk pediatric dialysis population who were previously vaccinated but have low seroprotection.

## CONFLICT OF INTEREST

Authors declare no conflict of interest

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## AUTHOR CONTRIBUTION

**Kalimullah Khan:** Conception, the acquisition, analysis, interpretation of data and manuscript writing

**Aasia Zubair:** Conception, the acquisition, analysis, interpretation of data and manuscript writing

**Madiha Aziz:** Conception, analysis, interpretation of data and manuscript writing

**Sanaullah Agha, Pawan Kumar:** Data collection and analysis

**Seema Hashmi:** Revised critically for important intellectual content

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# COMMON BACTERIOLOGICAL PATHOGENS AND THEIR ANTIBIOTIC SENSITIVITY PATTERN IN BILE OF PATIENTS WITH CHOLELITHIASIS

Kanwal Hameed, Asif Ali Amir Ali, Sughra Parveen, Imran Khan, Jehangir Ali, Abdul Waheed

Jinnah Postgraduate Medical Center (JPMC), Karachi Pakistan

## ABSTRACT

**Background:** To determine the pattern of antibiotic susceptibility of common bacteriological pathogens of bile in patients with Cholelithiasis.

**Material and Methods:** A prospective cross-sectional study was conducted in the department of surgery, Jinnah Postgraduate Medical Center, from January 2020 to July 2020. All patients between the ages of 16 and 65, with symptomatic cholelithiasis operated by open or laparoscopic cholecystectomy were included in this study. Patients with acute cholecystitis, obstructive jaundice, and common bile duct stones were excluded from the study. Cultures and Sensitivity tests were performed for aerobes and anaerobes pathogens in JPMC laboratory. Socio-demographic variables and clinical parameters were recorded in a predefined proforma. Data was entered and analyzed using Statistical Package for Social Sciences version 26.

**Results:** Out of a total of 610 samples, 314 cultures were positive for bacteria. Bacteriological investigation revealed *Escherichia coli* was isolated in 97 (30.89%) patients, *Escherichia coli* and *Klebsiella pneumoniae* in 66 (21.02%), *Escherichia coli*, *Klebsiella pneumoniae*, and *Shigella sonnei* in 18 (5.73%), *Escherichia coli*, *Salmonella enterica* in 18 (5.73%), only *Klebsiella pneumoniae* in 64 (20.38%), *Salmonella enterica* in 15 (4.78%), *Salmonella enterica* and *Klebsiella pneumoniae* in 32 (10.19%), and *Shigella sonnei* in 4 (1.27%) patients. The most susceptible antibiotic for microorganisms on the whole was Imipenem (66.7%). The highest resistance was shown against Cefradine (76.4%).

**Conclusion:** The current study concludes that the most common bacteria of symptomatic cholelithiasis are *Escherichia coli* and *Klebsiella pneumoniae* followed by *Salmonella enterica* and *Shigella sonnei*. These bacteria showed an overall susceptibility to Imipenem.

**Keywords:** Bacteriological investigation, Symptomatic cholelithiasis, Culture, Sensitivity, Antibiotics

## BACKGROUND

Bile is a necessary fluid required by the body since it consists of nutrients such as proteins, phospholipids, cholesterol and bile acids. It is produced by the liver but the gallbladder stores it. Bile helps in the absorption of fat in the intestines.<sup>1</sup> The gallbladder is supplied by the cystic artery which comes from the right hepatic artery.<sup>2</sup>

However, sometimes developmental problems are seen in the gallbladder such as multiple gallbladders, agenesis, bi-lobed and double cystic.<sup>3,4</sup> Double cystic gallbladder may be seen with its own cystic duct or the cystic duct may be in the form of a common cystic duct.<sup>5</sup> 14 to 30% of cholecystectomies are done as a

result of acute cholecystectomies.<sup>6,7</sup> Often, certain bacteria infect the gallbladder which is minimized by a healthy function of bile which excretes out harmful organisms. However, this function is limited when stones are developed in the gallbladder or in the common bile duct.<sup>8,9</sup> Gallstones are a common finding in individuals, 7.9% of men in the United States were found to be diagnosed with gallstones and 16.6% of women during a recent survey.<sup>9,10</sup>

More research is required to identify the reasons for sensitivity of certain bacteria towards antibiotics in patients who present symptomatically with cholelithiasis in order to reduce mortality and morbidity. Due to limited data from the local population, the current study was undertaken.

## MATERIAL AND METHODS


A prospective cross-sectional study was conducted at the department of surgery, Jinnah Postgraduate Medical Center, from January 2020 to July 2020. Ethical approval was obtained from the institutional review board of JPMC. A non-randomized consecutive sampling technique was applied to enroll the

**Correspondence:** Dr. Asif Ali Amir Ali, Postgraduate Trainee, Jinnah Postgraduate Medical Center (JPMC), Karachi Pakistan

**Email:** [aakhawaja09@gmail.com](mailto:aakhawaja09@gmail.com)

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participants in the study. The frequency of common bacteria i.e. *Shigella sonnei* was 6.35% [11] in patients with cholelithiasis at margin of error 4% with confidence interval 95%. After putting the values in the formula  $n = (Z_{1-\alpha/2})^2 P q / d^2$  sample size was =143 no. of specimens using WHO software for sample size calculation.

All patients between the ages of 16 and 65 year with symptomatic c+66holelithiasis operated by open or laparoscopic cholecystectomy were included in this study. All patients gave informed verbal and written consent to take part in the study. Patients with acute cholecystitis, obstructive jaundice, and common bile duct stones were excluded from the study. Cholecystitis was suspected in patients with TLC count greater than 12000 WBCs per microliter, high-grade fever, tenderness in the right upper quadrant.

Once patients were diagnosed with cholelithiasis on ultrasound imaging, they underwent surgery or laparoscopic cholecystectomy and then specimens from bile were sent for microbiological evaluation in sterile syringe. Patients were prescribed first prescribed empirical antibiotics postoperatively then changed the antibiotics according to the bacteria isolated. Duration of antibiotics varied from five to seven days. During the surgery, operative findings were documented and bile was aspirated in a 5-milliliter disposable and sterile syringe. Gallbladder was excised after cystic artery and duct were ligated while in laparoscopic cholecystectomy sample collected in 5cc syringe, after removing gallbladder from abdominal cavity. The collected specimen of the bile was labeled and sent to a JPMC laboratory. Sociodemographic (Age and gender) variables and clinical parameters (duration of hospital stay, duration of fever and total number of doses used) were recorded in a predefined proforma.

Data was entered and analyzed using Statistical Package for Social Sciences version 26. Common bacteriological investigation and their antibiotic sensitivity pattern in bile, in terms of drug sensitivity or susceptibility, was the unit of analysis. Descriptive statistics of age, duration of hospital stay and duration of fever, were computed in terms of Mean  $\pm$  SD and calculated. Frequency and percentages were also computed for gender. Family history of cholelithiasis, surgical approach (Laparoscopic/ Open cholecystectomy), were also recorded. Effect modifier was controlled through stratification like age, gender,

duration of hospital stays, and surgical approach to see the impact on of common bacteriological investigation and their antibiotic sensitivity pattern. Appropriate Chi-square test or Fisher exact test was applied. Two-sided tailed test at 95% confidence interval was applied at 5% level of significance was taken as significance.

## RESULTS

A total of 610 samples were studied out of these, 314 (51.47%) cultures were positive for bacteria. The mean age of patients was  $45.2 \pm 8.6$  years. The mean duration of hospital stay was  $2.5 \pm 1.3$  days and the mean duration of antibiotic treatment was  $2.5 \pm 1.1$  days (2.33 - 2.70). There were 81 (25.80%) male and 233 (74.20%) female patients. See Table-II for socio-demographic and clinical characteristics. Laparoscopic surgery was done in 108 patients (75.5%) while 35 (24.5%) were managed via open cholecystectomy.

Bacteriological investigation revealed *Escherichia coli* was isolated in 97 (30.89%) patients, *Escherichia coli* and *Klebsiella pneumoniae* in 66 (21.02%), *Escherichia coli*, *Klebsiella pneumoniae*, and *Shigella sonnei* in 18 (5.73%), *Escherichia coli*, *Salmonella enterica* in 18 (5.73%), only *Klebsiella pneumoniae* in 64 (20.38%), *Salmonella enterica* in 15 (4.78%), *Salmonella enterica* and *Klebsiella pneumoniae* in 32 (10.19%), and *Shigella sonnei* in 4 (1.27%) patients (Table-III).

The most susceptible antibiotic for microorganisms on the whole was Imipenem (66.7%). The highest resistance was shown against Cefradine (76.4%). The overall susceptibility pattern of the isolates is summarized in Table-IV.

**Table-I: Mean age, hospital stay, and duration of fever among study participants.**

Variables	Mean (standard deviation)
Age (years)	45.23 $\pm$ 8.67
Hospital stay (days)	2.59 $\pm$ 1.33
Duration of Fever (days)	2.68 $\pm$ 1.29

**Table-II: Characteristics of patients in the study**

Variables	N (percentage)
<b>Gender</b>	
Male	81 (25.80%)
Female	233 (74.20%)
<b>Family History of Cholelithiasis</b>	
Positive	248 (78.98%)

Negative	66 (21.02%)
<b>Surgical approach</b>	
Laparoscopic	237 (75.48%)
Cholecystectomy	77 (24.52%)

**Table-III: Bacteriological pathogens (n=314).**

Bacteriological Pathogen	N (%)
<i>Escherichia coli</i>	97 (30.89%)
Both <i>Escherichia coli</i> and <i>Klebsiella pneumoniae</i> isolated	66 (21.02%)
Mixed infection ( <i>Escherichia coli</i> + <i>Klebsiella pneumoniae</i> + <i>Shigella sonnei</i> )	18 (5.73%)
<i>Escherichia coli</i> + <i>Salmonella enterica</i>	18 (5.73%)
<i>Klebsiella pneumoniae</i> only	64 (20.38%)
<i>Salmonella enterica</i> only	15 (4.78%)
<i>Salmonella enterica</i> + <i>Klebsiella pneumoniae</i>	32 (10.19%)
<i>Shigella sonnei</i> only	4 (1.27%)

**Table-IV: Antimicrobial susceptibility patterns in the study population**

Antimicrobial Susceptibility Patterns	Resistant	Sensitive
Cefradine zone diameter > 16	13 (76.4%)	4 (23.5%)
Cefuroxime zone diameter > 30	44 (58.6%)	31 (41.3%)
Ceftriaxone zone diameter > 30	33 (62.2%)	20 (37.7%)
Ciprofloxacin zone diameter > 25	64 (58.1%)	46 (41.8%)
Amoxicillin zone diameter > 30	24 (64.8%)	13 (35.1%)
Cefoxitin (FOX) zone diameter > 22	9 (56.25%)	7 (43.75%)
Imipenem (IMP) zone diameter > 10	2 (33.3%)	5 (66.7%)

## DISCUSSION

Literature has shown that gram negative bacteria are the most frequently isolated pathogens from bile, specifically *Escherichia coli*.<sup>12-13</sup> Due to the increasing frequency of multidrug-resistant Gram-negative pathogens, the preference of appropriate antibiotics is limited. Unnecessary and inadequate use of antibiotics, are two most important factors contributing to the current resistance patterns. Thus, it is important to be aware about the common pathogens and their sensitivity and susceptibility patterns in the local population. This is to ensure that patients are receiving the most optimum empirical antibiotic therapy, postoperatively.

In the present study, we found that the majority of the patients were in their forties. This is according to the

study conducted by Manan *et al.*,<sup>11</sup> who reported a mean age of 45.95±10.14 years. The mean number of doses in our study was close to 5 days. Bacteriological investigation of bile reported that *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella enterica*, and *Shigella sonnei* were the most frequently isolated pathogens. For patients with *Salmonella enterica*, the antibiotic course varied from 5 to 7 days, and they most frequently presented with a history of typhoid fever. Manan F, *et al.*<sup>11</sup> noted that the most common bacteria found was *Escherichia coli* 28 (22.22%) followed by *Klebsiella pneumoniae* 22 (17.46%), *Salmonella enterica* 16 (12.70%) and *Shigella sonnei* 8 (6.35%). The study also reported *Escherichia coli* to have high resistance to Amoxicillin in 17 (60.71%) patients followed by resistance to Ciprofloxacin in 12 (42.86%) patients whereas *Klebsiella pneumoniae* showed high sensitivity to Ciprofloxacin in 13 (72.22%) patients. Al Harbi M, *et al.*<sup>14</sup> noted that the most common organism isolated was *Escherichia coli* as 9 (28.1%) while *Klebsiella pneumoniae* was 2 (6.3%). He reported 88.8% sensitivity of *Escherichia coli* with Cefuroxime.

Moazeni-Bistgani M, *et al.*<sup>15</sup> reported that the most common isolated organism was *Escherichia coli*, i.e., 26% followed by *Salmonella enterica* 14% and *Klebsiella pneumoniae* 4%. Kaya M, *et al.*<sup>16</sup> reported that *Escherichia coli* (28.2%) was the most frequently encountered bacteria. Sahu MK, *et al.*<sup>17</sup> further stated in his study about the prevalence of *Escherichia coli* and *Klebsiella pneumoniae* as 34 (19.1%) and 7 (3.9%) respectively. Furthermore, he also claimed that the most effective antibiotics were sequentially Amikacin, Ceftriaxone, and Clindamycin as *Escherichia coli* was 92.3% susceptible to Amikacin, while it was nearly resistant to Erythromycin (90%). A study by Moazeni-Bistgani and Imani presents valuable information on the antimicrobial susceptibility patterns of bacteria isolated from bile samples of patients with cholelithiasis. The study shows that a majority of the bacteria isolates exhibited resistance to at least one antibiotic, indicating a high prevalence of antimicrobial resistance among these bacteria which is in line with our findings.<sup>15</sup>

Thapa SB, *et al.*<sup>18</sup> reported that *Escherichia coli* showed high sensitivity to Imipenem (100%) and least to ciprofloxacin (70%). Kaya M, *et al.*<sup>16</sup> also reported that most susceptible antibiotics were Imipenem



(79%), Ciprofloxacin (52%) and Cefotaxime (14%). Sahu MK, *et al*<sup>17</sup> noted that *Escherichia coli* showed sensitivity to Cefotaxime in 15.7% patients followed by Ciprofloxacin in 14.3% and Imipenem in 100% patients whereas *Klebsiella pneumoniae* showed sensitivity to Cefotaxime in 10% patients followed by Ciprofloxacin in 20% and Imipenem in 100% patients. Adequate antibiotics are necessary for patients who are operated for symptomatic cholelithiasis and its complications. It is of great importance to periodically assess the common patterns of resistance and sensitivity against common antibiotics to provide the optimum antibiotic therapy to hospitalized patients.<sup>19,20</sup> Noncompliance of antibiotic drugs and overuse of antibiotics are major contributing factors in increasing the resistance against bacteria in our population. Therefore, antimicrobial activity against potential causative organisms, the severity of the cholecystitis, and the local susceptibility pattern must be taken into consideration when prescribing drugs.

## CONCLUSION

It is to be concluded that the most common bacteria of symptomatic cholelithiasis are *Escherichia coli* and *Klebsiella pneumoniae* followed by *Salmonella enterica* and *Shigella sonnei*. These bacteria showed an overall susceptibility to Imipenem. Additional studies are required to confirm our findings, probably with a larger sample size and with more parameters in multiple study centers in Pakistan are needed to confirm the findings of the present study.

## CONFLICT OF INTEREST

Authors declare no conflict of interest

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None

## AUTHOR CONTRIBUTION

**Kanwal Hameed:** Conception, analysis, interpretation of data and manuscript writing

**Asif Ali Amir Ali:** Conception, the acquisition, Analysis and interpretation of data and manuscript writing

**Sughra Parveen:** Data analysis, data Interpretation

**Imran Khan:** Revised critically for important intellectual content

**Jehangir Ali, Abdul Waheed:** Data Collection

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# ASSESSMENT OF THE LEVEL OF CONCERNS REGARDING COVID-19 PANDEMIC AMONG HOSPITAL BASED HEALTHCARE WORKERS IN QUETTA, PAKISTAN

Anjum Zia<sup>1</sup>, Farah Ahmad<sup>1</sup>, Akhtar Ali<sup>2</sup>

<sup>1</sup>College of Physicians and Surgeons of Pakistan, Karachi Pakistan

<sup>2</sup>Ziauddin University, Karachi Pakistan

## ABSTRACT

**Background:** The study was aimed to assess the level of concerns regarding the COVID-19 Pandemic among hospital-based healthcare workers in Quetta, Pakistan.

**Material and Methods:** It was a cross-sectional study carried out between August to December 2020 among frontline healthcare workers who work in the emergency department of different public sector hospitals of Quetta. The questionnaire was distributed through online social forums and physically at some places. The calculated sample size was n=400.

**Results:** The most significant findings were that n=179 (44.8%) felt themselves at risk to get Covid 19 from workplace; n=168 (42%) respondents had limited their social activities; n=125 (58.14%) respondents had access to complete Personnel Protective Equipment (PPEs) n=290 (72.5%) cumulative respondents had access to some -PPE items.

**Conclusion:** The healthcare workers suffered much mental anguish, trauma and isolation during the covid-19 pandemic. Scarcity of resources preventing access to complete PPE further added to it.

**Keywords:** Covid-19, Concern, Healthcare workers, Quetta

## BACKGROUND

*Coronavirus* is a single-stranded RNA enveloped, positive-sensed, and non-segmented virus that belongs to the Corona Viridae family. About six known coronaviruses can cause human diseases, including four viruses that cause mild respiratory infections.<sup>1</sup> The other two types are recognized as Middle East Respiratory Syndrome (MERS) and severe acute respiratory syndrome (SARS). The novel type of *coronavirus*, also called *COVID-19*, originated in December 2019 from extracted samples of lower respiratory tract infections of several patients in Wuhan, China.<sup>2</sup> The symptoms shown in the patients of *COVID-19* include severe pneumonia with dry cough, respiratory distress, fever, and fatigue. The novel *COVID-19* outbreak continued and finally WHO declared the disease as pandemic on March 11, 2020.<sup>4</sup> Emergency was announced after this outbreak.<sup>5</sup> The

whole scientific committee joined hands for research of newer of anti-viral drugs and vaccines for *COVID-19*. During pandemic, healthcare workers suffered from two main factors.<sup>6</sup> One was the increased burden of diseases that exhausted the health care systems, and the other was limited resources that prevented access to complete PPE making them feel vulnerable.

The objective of our study was to assess the concerns regarding the *COVID-19* Pandemic among hospital-based healthcare workers in Quetta, Pakistan.

## MATERIAL AND METHODS


It was a cross-sectional study conducted from August to December 2020. The maximum sample size was 384 calculated at 50% proportion with a bound error of 5%, and at 95% confidence level. The sample size was increased to 400 to increase the participation of healthcare workers. The setting was public and private healthcare facilities of Quetta, managing patients with covid-19 cases. The target population was the frontline healthcare workers, including doctors and other allied staff working in emergency departments of different healthcare facilities. A self-structured questionnaire was used based upon the previous survey studies with the concerns of healthcare assessment of medical personnel regarding the novel *COVID-19* pandemic.

**Correspondence:** Dr. Anjum Zia, MCPS HCSM Trainee, College of Physicians and Surgeons of Pakistan, Karachi Pakistan

**Email:** [anjumdr01@gmail.com](mailto:anjumdr01@gmail.com)

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The questionnaire comprised of two parts. Part one included demographic details, including age, gender, marital status, qualification level, designation, and working place—the second part comprised three domains related to self-satisfaction, social status, and workplace. The questionnaire was validated by a pilot on a small group of targeted participants. Since data was collected during the lockdown, the questionnaire was circulated through online social media resources, including WhatsApp, Facebook, and Instagram; informed consent was taken from each participant. The Statistical Package for the Social Sciences software (SPSS version 21.0; IBM Corporation, Armonk, NY, USA) was used for data analysis. Chi-square analysis was performed to check the association at a 95% confidence interval, and a p-value less than 0.05 was considered significant.

## RESULTS

A total of 400 healthcare workers, including physicians, laboratory staff, nurses, X-ray technicians, respiratory technicians, and working staff from government hospitals, submitted their responses to the questionnaire. There were 220 (55%) doctors, 145 (65%) male and 75 (35%) females, enrolled in the study—paramedical staff and other supporting staff comprised 45% of the study participants. Concerning self-satisfaction (Table-I.), most of the participants responded to be anxious and concerned about their health at the workplace, worried during receiving and managing the febrile patients with higher risks of viral

transmission in the absence of personal protective equipment (PPE), and hopeless to get appropriate care from any kind of administration.

To identify the exposure of study participants concerning dealing with *COVID-19* patients either directly or indirectly (Figure-I), most of the participants, 215 (53.8%), responded as “Yes” as they were dealing with the patients in isolation or quarantine facilities directly. With this, (n=185 (46.3%)) participants responded as “No” as they were not now receiving or dealing with the Covid-19 patients in their healthcare settings. After asking about their engagement with patients either directly or indirectly, who replied as yes (Figure-I), they were asked about the provision of preventive materials, trainings from management about the use, donning, and doffing of the materials. As shown in Figure-II, most front-line doctors, paramedic staff, and other supporting staff were not given PPE, and they were dealing with Covid-19 patients endangering their and their near one’s lives. When asked about trainings provided to them about PPE donning and doffing protocols, 70 (32.56%) responded that they were not given any training and 54 (25.12%) responded as they learned by themselves using YouTube videos (Table-III). Those who were not involved in managing COVID-19 patients directly (Figure-II) were asked about the provision of Semi-Personal Protective materials, and similar results were observed in Figure-III.

**Table-I: Responses about self-satisfaction & social status related questions.**

Statements regarding self-satisfaction	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
<b>A</b> I feel unsafe working at my workplace.	40 (10%)	37 (9.3%)	59 (14.8%)	123 (30.8%)	141 (35.3%)
<b>b</b> I feel worried while receiving and managing a febrile patient nowadays	43 (10.8%)	32 (8%)	58 (14.5%)	124 (31%)	143 (35.8%)
<b>C</b> I feel at risk to contract Covid 19 at workplace	30 (7.5%)	14 (3.5%)	34 (8.5%)	143 (35.8%)	179 (44.8%)
<b>d</b> I feel obliged to care for Covid 19-infected patient, but if I am protected well with Personal Protective Equipment (PPE)	19 (4.8%)	27 (6.8%)	46 (11.5%)	136(34%)	172(43%)
<b>E</b> I feel hopeless because I might eventually get a Covid 19 at work	30 (7.5%)	14 (3.5%)	73 (18.3%)	143 (35.8%)	140 (35%)
<b>f</b> I feel threatened if one of my colleagues have contracted Covid 19	33 (8.3%)	19 (4.8%)	55 (13.8%)	161 (40.3%)	132 (33%)
<b>g</b> If I get Covid 19, I don’t feel confident an employee will care for me	60 (15%)	44 (11%)	88 (22%)	107 (26.8%)	101 (25.3%)
<b>Statements regarding social status</b>					
<b>a</b> I have limited my social activities due to Covid 19 Pandemic	20 (5%)	25 (6.3%)	44(11%)	143(35.8%)	168(42%)
<b>b</b> I may transmit Covid 19 to my family members	28 (7%)	18 (4.5%)	31 (7.8%)	103 (25.8%)	220(55%)



if I get the infection						
<b>c</b>	I have feeling that my family members avoid me since I work in a hospital	69 (17.3%)	92(23%)	77 (19.3%)	89 (22.3%)	73(18.3%)
<b>d</b>	I have avoided leaving my home unnecessarily due to Covid 19	10 (2.5%)	21 (5.3%)	57 (14.2%)	142 (35.5%)	170(42.5%)
<b>E</b>	I feel my family will not look after me if I am infected	166 (41.5%)	102(25.5%)	60 (15%)	36 (9%)	36(9%)
<b>f</b>	I don't feel confident telling my family and friends if I am infected.	143 (35.8%)	71(17.8%)	64 (16%)	62 (15.5%)	60(15%)

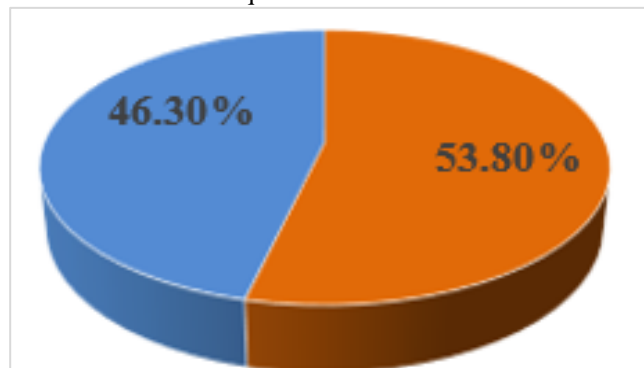
**Table-II: Responds about using the same PPE kit multiple times.**

Comparison based on designation		How many times have you been asked to use the same PPE kit?			
Designation	One Time	Once A Week with repeated sanitizing shower spray every day	if it is infected with any material like blood etc., it can be changed the next day	it has been asked by authorities to use the kit multiple times, and no new equipment will be given	Total
Doctor (Healthcare Administrator, Consultant, MO, PG, HO, etc.)	33 (15.39%)	53 (24.65%)	42(19.53%)	45 (20.93%)	173
Other Paramedical Staff (Nurse, MT, FMT, LHV, Laboratory staff, OT Staff, etc.)	7 (3.26%)	6 (2.79%)	9 (4.19%)	9 (4.19%)	31
c) Any other, please mention:	3 (1.40%)	3 (1.40%)	5 (2.32%)	0	11
Total	43(20%)	62(28.84%)	56 (26.05%)	54 (25.12%)	215

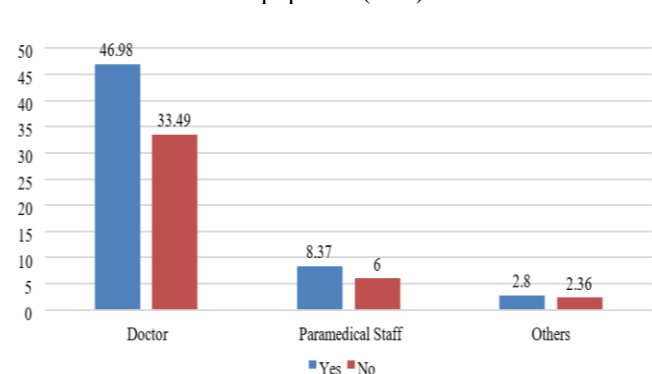
**Table-III: Responses about protocols of PPE use**

Training regarding donning and doffing of PPE				Total
		Have you been trained on donning (Put on) and doffing (put off) the PPE as per International Protocols?		
		Yes	No	No Training was given, but I watched it on YouTube and applied the method myself.
Designation				
Doctor (Healthcare Administrator, Consultant, MO, PG, HO, etc.)		49(22.79%)	70(32.56%)	54(25.12%)
Other Paramedical Staff (Nurse, MT, FMT, LHV, Laboratory staff, OT Staff, etc.)		10(4.65%)	10(4.65%)	11(5.12%)
Any other, please mention:		5(2.36%)	3(1.40%)	3(1.40%)
Total		64(29.77%)	83(38.60%)	68(31.63%)

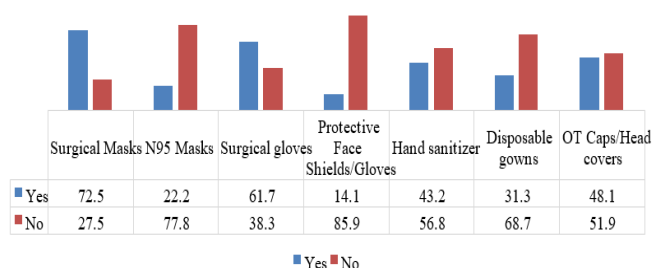
Do you directly deal with corona virus patients in isolation/quarantine ward?


**Figure-I: Responses regarding the direct involvement of staff in the management of COVID-19 patients.**

Have you been provided Full Personnel Protective Equipment (PPE)?


**Figure-II: Comparison based on designation.**

Provision of Semi- personal protective equipment



**Figure-III: Responses about Semi- personal protective equipment (PPE).**

## DISCUSSION

The analysis of the research literature related to the intensity of transmission and the health risks associated with the *COVID-19* pandemic promoted the concept of performing this research to assess the concerns of the frontline health care workers serving in the private and government health care centers of Quetta, Baluchistan. The current study identified that the healthcare workers working as frontline warriors were not given preventive equipment that could have protected them from infection and decreased their anxiety. The lack of resources and poor health care infrastructure contributed significantly towards the increased mortality and morbidity rate among the health care workers.<sup>10,11</sup>

Protective gears such as masks, gloves and other precautionary measures at workplaces can significantly reduce the risk of transmitting the virus.<sup>12,13</sup> As the virus droplets persist on the surfaces for longer, use of disinfectants in environmental cleaning is also important to deactivating the virus.<sup>13</sup> The present study highlighted that during pandemic very little community efforts were seen to ensure safety of frontline workers. They were made to use single use PPE for more than one time. In developing countries like Pakistan, the poor infrastructure of basic health facilities, feeble governance, inadequate health resources, and unawareness in public to adopt safety measures further aggravated the concern and anxiety of the medical practitioners.<sup>14,15</sup>

The government and other shareholders failed to organize the genuine requirements in taking care of an enormous number of clinical staff from minor and acute patients facing respiratory problems exposed to COVID.<sup>15</sup> In Pakistan, during *COVID-19* pandemic the

frontline physicians were mentally and physically exhausted, as there was no support system in sight in case of infection. Unlike other studies<sup>16,17</sup> the HCW of Quetta looked up to their families for supporting them in case of infection.

Smartphones are highly embedded in people's lives, and thus mobile applications are the best ways to spread awareness. It also enabled us to recognize that the majority of the people were in favor of strict guidelines and measures taken by the government and other shareholders in the healthcare system.<sup>18</sup> Though the awareness and education were provided to the population for taking preventive measures, leaving the healthcare workers helpless without preventive equipment showed the reluctant attitude of the government towards the healthcare system, which led to an increase in the level of concern among healthcare workers towards the *COVID-19* pandemic.

## CONCLUSION

The concerns among healthcare workers increased during the *COVID-19* pandemic as they were not given the essential protective equipment.

## RECOMMENDATIONS

Sympathetic and clear communication can contribute to a proper healthcare delivery system. All administrative authorities and medical staff must concentrate on the immediate needs of *COVID19* management and care. Likewise, food provision, rest breaks, decompression time, and sufficient duty times may be as vital as the availability of protective equipment and protocols. Compliance with the WHO recommendations are essential to ensure adequate support for frontline healthcare workers. Supplies must be made to protect them via infection-control methods, personal-protection equipment, and vaccines in the future.

## CONFLICT OF INTEREST

Authors declare no conflict of interest

## GRANT SUPPORT / FINANCIAL DISCLOSURE

None

## AUTHOR CONTRIBUTION

**Anjum Zia:** Conception, the acquisition, analysis, interpretation of data and manuscript writing

**Farah Ahmad:** Conception, Analysis and interpretation of data

**Akhtar Ali:** Data collection and analysis

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# ADVERSE EFFECTS FOLLOWING IMMUNIZATION OF COVID-19 VACCINES IN ISLAMABAD

Muhammad Yar Subhan Qadir<sup>1</sup>, Huzaifa Akram<sup>2</sup>, Farah Ahmed<sup>3</sup>, Basharat Ullah Baig<sup>4</sup>, Zaeem Zia<sup>4</sup>

<sup>1</sup>Jhpiego- A John Hopkins University Affiliate NSTP-NUST, Islamabad Pakistan

<sup>2</sup>Health Services Academy, Islamabad Pakistan

<sup>3</sup>College of Physicians and Surgeons Pakistan, Karachi Pakistan

<sup>4</sup>Ministry of National Health Services Regulations and Coordination, Pakistan

## ABSTRACT

**Background:** The COVID-19 vaccines were developed by different countries and organizations and were rays of hope towards the end of the COVID-19 pandemic. However, the concerns related to adverse effects following immunization was a constant source of hesitancy toward vaccination. The research used cross-sectional study for four months to understand the different adverse effects following COVID-19 vaccination in Islamabad.

**Material and Methods:** Around 400 participants were selected through a purposive sampling strategy. We included persons who received the selected COVID-19 vaccine at least 5 days ago and had a complete understanding of adverse effects. The 24th version of SPSS was used to analyze the data.

**Results:** The results showed that among participant no one experienced life-threatening adverse effects. However, about 52.5% (n=210) of participants showed adverse effects following COVID-19 vaccination. Among the participants showing adverse effects 59% (n=72) were in the age group of 18-30 years, 64% (n=105) were in the age group of above 30 to 50 years and 34% (n=33) were in the age group of above 50-70 years. The results showed that 70% (n=100) of recipients of the Cansino vaccine reported different adverse effects and Sinopharm recipients showed least adverse effects at 43% (n=100).

**Conclusion:** None of the participants showed any life-threatening adverse effects. However, recipients of all types of vaccines reported various adverse effects.

**Keywords:** COVID-19; Adverse effect following immunization; COVID-19 vaccine

## BACKGROUND

The COVID-19 pandemic was declared in 2020 on 11 March<sup>1</sup> and it resulted in millions of deaths around the globe. A number of efforts were made globally to counter pandemic of COVID-19<sup>2</sup> and multiple types of vaccines were developed by different nations. The success of the COVID-19 vaccine is only possible when the majority of the population is vaccinated in the world.<sup>3</sup> Most of the COVID-19 vaccines had minor adverse effects in most populations worldwide<sup>4</sup> but severe adverse effects had also been reported by COVID-19 vaccine recipient in a few countries. The hesitancy toward vaccines was observed in many countries.<sup>5</sup> There were many factors for this hesitancy<sup>6</sup> and one major reason for this was the adverse effects of COVID-19 vaccines.

COVID-19 vaccination started in Pakistan on 2nd February 2021 by the Prime Minister of Pakistan.<sup>8</sup> Initially, only Sinopharm was provided to healthcare workers and to elderly people, then new vaccines such as Sinovac, Cansino, Astrazeneca, and Pfizer were also available. Pakistan also started its own vaccine with help of China; the Pakvac.<sup>9</sup> The issue of adverse effects had been haunting the general public of Pakistan. The Government used different strategies to advocate the safety of COVID-19 vaccines.

Since different vaccines were administered in Pakistan, this study was done to assess the adverse effects following vaccination of Sinopharm, Sinovac, Astrazeneca, and Cansino in Islamabad, Pakistan. The study is helpful in understanding the different adverse effects experienced by vaccine recipients in different vaccines and in different age groups.

## MATERIAL AND METHODS

A cross-sectional study was conducted on people getting the COVID-19 vaccine in two vaccine centers in Islamabad. The vaccine recipients equal to and above age 18 years of age were enrolled for assessing the adverse effects. The data collection was carried out


**Correspondence:** Dr. Muhammad Yar Sobhan Qadir  
National Technical Advisor IPC in Jhpiego- A John Hopkins  
University Affiliate NSTP-NUST, Islamabad Pakistan

**Email:** [sobhanqadir@yahoo.com](mailto:sobhanqadir@yahoo.com)

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between March and June 2021. The sample size was 400 hundred (100 samples for each type of vaccine Sinopharm, Sinovac, Astrazeneca, and Cansino). The subjects were selected by purposive sampling technique who were coming for a second dose or those who had received a single dose of the COVID-19 vaccine at least 5 days ago to have a clear understanding of adverse effects. The data was collected by using a simple easily understandable questionnaire that include questions regarding sociodemographic factors (age, sex, primary employment, smoking status), use of aspirin, source of information about vaccine and reason for getting a vaccine, type of vaccine received, and side effects experienced following immunization. The reason for the study was explained by the doctor to the subjects and informed consent was taken for inclusion in the study. The data was collected for Sinopharm, Sinovac, and Astrazeneca on the day of the second dose and for Cansino data was collected telephonically after 5-7 days of vaccination. The questionnaire was filled out by the doctor after taking the history of adverse effects. It was initially piloted on 20 vaccine recipients and then a modified questionnaire was used for final data collection. The cases of coagulations, anaphylactic shocks, myocarditis, and altered state of consciousness or death were termed as severe adverse effect. The data was analyzed using the 24th version of SPSS.

## RESULTS

It is observed that a total of 52.5% (n=210) out of 400 recipients complained about different types of adverse

effects following immunization. Total of 44% (n=44) recipients of Sinopharm, 72% recipients (n=72) of Cansino, 46% recipients (n=46) of Sinovac, and 48% of recipients (n=48) of AstraZeneca presented with different types of adverse effects.

Total of 8.3% smoker presented with adverse effect while p value was calculated as 0.25, similarly 5.6% of people using Aspirin presented with adverse effect and p value calculated as 0.6 making both variables as non-significant.

A total of 67.7% (n=271) percent recipients mentioned that they wanted to get a vaccination in order to prevent COVID-19 infection, 30.7% (n=123) mentioned they got the vaccination only because it is required at an office/job place, and 1% (n=4) recipient mentioned it as a requirement to travel, and other of 0.5% (n=2) mentioned selected other options as a reason to get a vaccination.

A total of 35% (n=140) recipients of the vaccine mentioned that they got information about COVID-19 from the Health department, 33% (n=132) recipient mentioned that they got information regarding COVID-19 vaccination from electronic media, 14.7% (n=59) recipient's source of information was social media, similarly 14.7% (n=59) got information about COVID-19 vaccination from friends and relatives, a total of 1.5% (n=6) get information from the website of the Ministry of National Health Services, Regulations and coordination Pakistan, and 1% (n=4) recipients got information from Newspapers.

**Table-I: Age and Gender Based distribution of adverse effects following immunization**

Frequency of AEFI in age groups				
Age group (years)		Yes	% (n)	No % (n)
18-30	(n=122)	59%	(72)	41% (50)
Above 30-50	(n=164)	64%	(105)	36% (59)
Above 50-70	(n=97)	34%	(33)	66% (64)
Above 70	(n=17)	0%	(00)	0% (00)
Frequency of AEFI in gender groups				
Type of Side effects		Out of 225 Males recipients (%)		Out of 175 females' recipients (%)
Headache		16%		19.4%
Fever		30.6%		28%
Body ache		25.3%		27.4%
Tiredness		6.3%		9.1%
Dizziness		0.45%		1.14%
Vertigo		0.45%		1.14%
Palpitation		00 %		00%
Change in blood pressure		00 %		00%
Any allergic reaction		00%		00%
Any other complaint		0.45%		0.57%

**Table-II: Frequency of AEFI in different COVID-19 vaccines.**

Types of Side effect	Sinopharm (n=100)	Cansino (n=100)	Sinovac (n=100)	Astrazeneca (n=100)
Headache	18%	16%	19%	19%
Fever	21%	46%	22%	18%
Body Ache	21%	36%	22%	23%
Tiredness	8%	10%	7%	10%
Dizziness	1%	0%	2%	2%
Vertigo	1%	1%	1%	1%
Palpitation	0%	0%	0%	0%
Change in blood pressure	0%	0%	0%	0%
Allergic reaction	0%	0%	0%	0%
Any other side effects*	1%	0%	1%	0%

\*2 subjects reported that they got COVID-19 after vaccination, although both agreed that they were not following the SOPs of COVID-19 completely. There were many recipients who showed more than one symptom too.

**Table-III: Association of smoking status and use of aspirin with Adverse effect following immunization**

Variable	Response	Adverse effect
Smoker	Yes	8.3%
	No	91.7%
Use of Aspirin	Yes	5.6%
	No	94.4%

## DISCUSSION

The side effects following immunization with the COVID19 vaccines (Sinopharm, Sinovac, Astrazeneca, and Cansino) which were administered in Pakistan were assessed. The major side effects reported were fever, headache, body ache, lethargy, and a few presented with dizziness, vertigo, and allergic reaction. No one presented with severe adverse reactions as per study findings. Another study was conducted in the United Arab Emirates in 2021 which results showed that fatigue and headache were more common side effects post-first dose of the Sinopharm vaccine.<sup>10</sup> A study which was conducted in Saudi Arabia results shows that 62 % of participants experienced headache and 66% fever. Fatigue and fever were most commonly experienced post first dose of Astrazeneca.<sup>11</sup> Similarly, a study was conducted in Iran in 2021 which results showed that most common adverse effect following vaccination were chill/fever (26.86%) fatigue (28.37%) and skeletal pain (22.38%).<sup>12</sup>

The study results also show that the 30 above-50 year's age group experienced more side effects than the younger and older age groups. While no one above 70 years of age showed any side effects as per the study findings. In the gender-based distribution of side effects, headache, fever, and body aches were most common in males while dizziness and vertigo were most common in females. Similarly, a cross-sectional study conducted in Malaysia in 2021 shows similar results that the age group > 60 years experienced no side effects, and the likelihood of side effects were 7.4 higher in the younger age group 18-30 years.<sup>13</sup>

However, results also showed that males were less likely to experience side effects, especially those who received Sinovac. A study conducted in Eastern Ethiopia in 2021 results show that the magnitude of adverse effects experienced was higher in males than females.<sup>14</sup>

The study results showed that most participants experienced mild to moderate adverse effects following immunization and no severe adverse effect was experienced by participants. Similarly, a study conducted in Malaysia in 2021 showed that participants who experienced minor adverse effects were 40.9%, 48% experienced mild to moderate and 0.8% experienced severe adverse effects.<sup>13</sup>

About 67.7% of participants who were getting vaccination mentioned that they were willing for the vaccination for their safety without any compulsion. 30.7% of people mentioned that they got the vaccine due to their official requirement and 01% were those who needed it for traveling purposes at the time of data collection and 0.7% were in another category.

As far as the sources of information were concerned, 33% of people mentioned electronic media as the main source of information, 14.7% showed social media as the main source of information and 35% of people showed health department as main source of information. Similarly, A study conducted in Malaysia in 2021 results showed that mostly source of information on COVID-19 vaccine in participants was the official website of the ministry of health 53.7%,

social media 22.1%, 12% internet source such as Google and YouTube and 8.7% WHO.<sup>13</sup>

## CONCLUSION

Different types of vaccines had different side effects and on the basis of this data, we can find out which vaccine should be preferred in which particular age group in Pakistan. However, it is clear that as per data, no life-threatening adverse reactions were observed in people getting vaccination in Pakistan. As new variants are emerging in the country and no serious adverse effects were observed after vaccination, it is high time to push the vaccination campaign even further to save majority of the population. The people getting vaccination should know that they can experience mild to moderate side effects and no serious effects following immunization.

## CONFLICT OF INTEREST

Authors declare no conflict of interest

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None

## AUTHOR CONTRIBUTION

**Muhammad Yar Subhan Qadir:** Conception, the acquisition, Data Collection, Data Analysis

**Huzaifa Akram:** Manuscript writing, Interpretation of the data

**Farah Ahmed:** Revised critically for important intellectual content

**Basharat Ullah Baig, Zaeem Zia:** Data Collection

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# ACCURACY OF AUTOMATED CELL ENUMERATION METHOD FOR VARYING CONCENTRATION OF WBCS FOR VARIOUS BODY FLUID SAMPLES

Sana Brohi, Muhammad Shariq Shaikh, Bushra Moiz

Aga Khan University, Karachi Pakistan

## ABSTRACT

**Background:** Body fluids (BF) including peritoneal, pericardial, pleural, synovial and cerebrospinal fluids are now being analyzed by fully automated methods that are replacing the manual methods. The aim of this study is to assess the accuracy of automated instrument at varying concentrations of WBCs of various body fluid samples.

**Material and Methods:** This cross-sectional study was conducted at the section of Hematology, Department of Pathology and Laboratory Medicine, the Aga Khan University, Karachi, Pakistan from November 2020 to April 2021. Forty body fluid samples with suspicion of infection including peritoneal, pericardial, pleural, synovial and cerebrospinal fluids (CSF) were analyzed to verify accuracy of white blood cell counts on fully automated XN-1000™ hematology analyzer (Sysmex Corporation, Kobe, Japan) against Neubauer chamber method (a manual method of differentiating WBCs via microscopy). The culture results of the body fluid samples were also analyzed. EP Evaluator version 10.3.0.556 (Data Innovations, LLC, VT, US) was used for statistical analysis with other supporting statistics such as Correlation Coefficient (R), Bias, Mean and Standard Deviation were included.

**Results:** Eleven (n=11) of forty (n=40) body fluid samples showed high WBC count above the normal range. Samples with normal WBC (n=29) were also included in accuracy study to assess instrument performance at varying concentration of analyte. An average Error Index of -0.27 (-0.99 to 0.74) for WBC was obtained. Microbiological cultures grew *Escherichia coli* in 1 and *Acinetobacter* species in 1 CSF samples and *Staphylococcus aureus* in 1 pleural sample.

**Conclusion:** The study verified accuracy of varying concentration of WBC counting by fully automated Sysmex XN-1000 analyzer for various body fluid samples.

**Keywords:** Body fluids, validation, automated analyzer, performance specifications, accuracy verifications, high white blood count, infectious.

## BACKGROUND

Body fluids (BF) including peritoneal, pericardial, pleural, synovial and cerebrospinal fluids are a source of nutrition, waste disposal and necessary movement of enclosed organs. Each of these fluids has its own unique biochemical characteristics.<sup>1</sup> Over decades, hemocytometer (counting chamber) has been used for enumerating cells in body fluids. However, with the availability of automated cell counters, conventional hemocytometers are being readily replaced in clinical laboratories.<sup>2</sup> Several analyzers are available nowadays by various manufacturers that claim high accuracy.<sup>3-5</sup> Although manufacturers usually provide performance specifications in their instruction manuals, it is the responsibility of each lab to verify these in their own setting before initiating patient

reporting. Accrediting bodies such as Clinical Laboratory Standard Institute (CLSI), International council for standardization in hematology (ICSH) and college of American pathologists (CAP) recommend each lab to verify performance specification including accuracy, for each quantitative test offered by the laboratory.<sup>1,6,7</sup>

Understanding the difference between validation and verification is essential.<sup>8</sup> Validation is carried out by the manufacturer and provides performance characteristics of the method being used. If validation has not been carried out by the manufacturer, then it must be carried out by the laboratory by following any local guidelines and recommendations for establishing a laboratory-developed test (LDT).<sup>1,8</sup> However, verification is defined as a confirmation of the validation performed by the manufacturer that gives evidence that the analyzer can meet the specific requirements within a given test site.<sup>8</sup> Hence, this verification must be carried out by each laboratory before the analyzer is used for testing.<sup>8</sup> Accuracy study, one of the most important performance specifications verifies closeness of the measured value to the true value of an analyte.<sup>8</sup>

**Correspondence:** Dr Sana Brohi, Resident, Aga Khan University, Pakistan, Islamabad Pakistan

**Email:** sanamumtazbrohi@gmail.com

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White cell count is routinely analyzed in various body fluids. Causes of elevated WBC count in body fluids include infection, inflammation, hemorrhage, malignancy, seizures etc.<sup>9</sup>

The aim of our study was to verify accuracy of varying concentration of WBC counting by fully automated Sysmex XN-1000 analyzer against traditionally used manual method in various body fluid samples as automated method decreases the turn-around time of body fluid sample reporting.

## MATERIAL AND METHODS

Forty body fluid samples received in Hematology department; Aga Khan University Hospital Karachi from November 2020 till April 2021 were randomly included. Sixteen cerebrospinal, eight peritoneal, seven pleural, six pericardial, three synovial fluids with suspicion of meningitis, peritonitis, pneumonia, pericarditis and synovitis were included in the study. The body fluid samples were collected without anticoagulant in plain glass or plastic tubes, syringes or with anticoagulant like EDTA. These samples are normally discarded after finalizing results and dispatching reports to the patients. Therefore, before discarding we analyzed these samples on fully automated XN-1000<sup>TM</sup> hematology analyzer (Sysmex Corporation, Kobe, Japan) as well. No additional samples were drawn from the patients. Bile drainage, bronchoalveolar lavage and other fluids likely to be contaminated by external material were excluded. These fluids might be unsuitable for evaluation because of the presence of clots, crystals and high viscosity.

The data was analyzed on EP Evaluator software version 10.3.0.556 (Data Innovations, LLC, VT, US). The CLIA limit of 15% for WBC was set as an acceptable Allowable Total Error (TEa). Deming Regression Statistics (slope and intercept) and other supporting statistics including Correlation Coefficient (R), Bias, Mean  $\pm$  SD (Standard Deviation) were used to measure agreement between 2 methods i.e.

Automation versus manual method.

The study was exempted from ethical approval by ethical review committee of Aga Khan University Hospital, Karachi (2020-5531-14621).

## RESULTS

A total of 40 patients' body fluid samples were analyzed. Specimens were compared over a range of 0.007 to 25.94  $\times 10^3/\mu\text{L}$  for WBC. The difference between the two methods was within allowable error (i.e. 15%) for 40 of 40 specimens (100%). The average Error Index (Y-X)/TEa was -0.27 (-0.99 to 0.74). Correlation coefficient (R) is 0.9998 (-1.0 to 1.0) Mean  $\pm$  SD by automated method and manual methods were  $2.28 \pm 4.46$  and  $2.29 \pm 4.47$  respectively. Results of key statistics obtained and their acceptable limits are shown in Table-I, which confirms the accuracy of automated method. The study passed the criteria of accuracy. For lymphocytes and polymorphs; refer to Table-II.

Further, 11 out of 40 body fluid samples showed high WBC count (refer to Table-III). High WBC counts in different samples were as follows: 6 out of 16 CSF samples (i.e. WBC:  $> 0.005 \times 10^3/\mu\text{L}$ ), 3 out of 8 peritoneal samples (i.e. WBC:  $> 0.5 \times 10^3/\mu\text{L}$ ), 2 out of 7 pleural samples (i.e. WBC:  $> 0.5 \times 10^3/\mu\text{L}$ ), none of the pericardial and synovial samples showed high WBC counts. Also, the WBC reported by both methods i.e. Automation and manual method showed no significant difference between the two. The microbiological culture of all 40 samples was performed with suspicion of infection by primary physician. Total 3 (2 in CSF and 1 in pleural fluid) out of 40 body fluid samples were culture positive for bacterial growth. The 2 culture positive CSF showed *Escherichia coli* in one and *Acinetobacter species* in other CSF sample, whereas growth of *Staphylococcus aureus* was present in 1 pleural fluid sample.

**Table-I: Key statistics of accuracy study for WBC obtained with their acceptable limits.**

Statistics	Values obtained	Acceptable limits	Results: Acceptable or Unacceptable
Average error index	-0.27	-0.99 to 0.74	Acceptable
Correlation coefficient (R)	0.9998	-1.0 to 1.0	Acceptable
Slope	0.9998	0.991 to 1.005	Acceptable
Intercept	-7.9	-40.7 to 24.8	Acceptable
Bias	-12.4	$< -0.5 \%$	Acceptable
Mean $\pm$ SD (Automated method range)	$2.28 \pm 4.46$	0.007 to 25.94 ( $10^3/\mu\text{L}$ )	Acceptable
Mean $\pm$ SD (Manual method range)	$2.29 \pm 4.47$	0.008 to 25.939 ( $10^3/\mu\text{L}$ )	Acceptable

**Table-II: Key statistics of accuracy study for lymphocytes & polymorphs obtained with their acceptable limits.**

Statistics	Values obtained for Lymphocytes	Values obtained for Polymorphs	Acceptable limits	Results: Acceptable or Unacceptable
Correlation coefficient (R)	0.9368	0.9375	-1.0 to 1.0	Acceptable
Slope	0.861	0.861	Lymphocytes: 0.76 to 0.96 Polymorphs: 0.76 to 0.96	Acceptable
Intercept	11.09	3.09	Lymphocytes: 3.61 to 18.57 Polymorphs: -0.78 to 6.97	Acceptable
Bias	1.31	<-0.99%	Lymphocytes: < 1.85 % Polymorphs: <-3.44	Acceptable
Mean $\pm$ SD (Automated method range)	0.0727 $\pm$ 0.0228	0.006 $\pm$ 0.097		Acceptable
Mean $\pm$ SD (Manual method range)	0.0704 $\pm$ 0.261	0.005 $\pm$ 0.009		Acceptable

**Table-III: High WBC cut-off for various body fluid samples.**

Body Fluid	Total no: of specimen	Total no: of specimen with high WBC count	Cut-off for reportable high WBC count (10E3/uL)	Total no: of culture positive specimen
CSF	16	6	>0.005	2
Peritoneal	8	3	>0.5	0
Pleural	7	2	>0.5	1
Pericardial	6	0	>0.5	0
Synovial	3	0	>0.2	0

## DISCUSSION

Analysis of body fluids provides valuable insight in the diagnosis of many medical conditions such as meningitis, joint pathology, peritonitis and malignancies.<sup>10-12</sup> There are many challenges to manual assessment of body fluids such as skilled handling and subjectivity in interpretation making automated analyzers to be regarded as a fast and accurate tool for assessing body fluids.<sup>13-14</sup> Automated analyzers like Sysmex have body fluid mode which helps in analysis of body fluids with rapid and accurate enumeration of WBCs.<sup>15</sup>

The instrument manufacturers provide specifications such that users' i.e., laboratories have to perform only verifications to justify the manufacturer's intended use.<sup>5</sup> In case manufacturers do not provide specifications then the responsibility lies on laboratories to validate their specifications using international guidelines.<sup>16</sup> In this study, we have verified Sysmex XN -1000's body fluid mode against Manual Neubauer chamber via microscopy for accuracy using patients' samples. Previous study by DD Castellone *et al.* (2010) proved that automated analyzers like ADVIA® 2120/2120i compared well to manual methods of enumeration of RBC and WBC in various body fluids like, pleural and peritoneal.<sup>17</sup>

In our study, accuracy or trueness was verified by method of comparability that was performed using split sample testing, with retained patients' samples. Our

results passed the criteria of accuracy verification. Both methods were equally effective, but keeping in view the time consumed by manual method, the automated analyzer proved to decrease the turnaround time in less than 4 hours to more than 4 hours in manual method. Also being a manual mode of counting, the chances of errors such as pipetting errors, faulty sample preparations, volume of sample introduced into the chamber are high with it.

Sirin Lohajaroensub *et al.* (2015) studied 253 body fluid samples and compared the results between automated analyzer Sysmex XT-4000i and manual methods and found correlation of RBC and WBC count was high in ascitic fluid followed by pleural fluid, CSF and synovial fluid.<sup>18</sup> Manual microscopy of body fluid are time-consuming and labor-intensive and subjective while material handling, but fully automated analyzers meet the demand of increase in turnaround time, quality requirements and are objective in material handling.<sup>18</sup>

The Deming regression analysis didn't yield significant constant or proportional bias between the manual and the automated methods. The slope and intercept were computed assuming that the two methods have comparable precision (i.e., the same representative SD). In our study, the Mean  $\pm$  SD by automated method and manual methods for WBC were  $2.28 \pm 4.46$  and  $2.29 \pm 4.47$  respectively. Method comparison studies usually limit statistical calculation to

correlation coefficient between the two methods and in some instances, correlation does not mean that the two methods agree. Therefore, we also tested our study based on acceptable allowable total error (TEa). Based on biological variations, TEa is a variable that expresses the degree of error in a test result that can be tolerated without negatively impacting patient care. For example, acceptable CLIA values of TEa for red blood cells, white blood cells, and platelets are 6%, 15%, and 25%, respectively. We used same TEa of 15% for WBC analysis. Error Index (EI) is the ratio of the difference between 2 methods to TEa. We obtained an average error index of -0.27 for WBC (Table-I). An index greater than 1.00 or less than -1.00 is unacceptable which means that the difference between methods exceeds TEa. If an excessive number of specimens have an unacceptable error index, the experiment fails. Excessive number of specimens occurs if the EI is unacceptable for at least one specimen if  $n = <20$  or if the EI for more than 5% of the specimens is unacceptable when  $n = >20$ . We also found in our study that 3 samples were positive and 37 were negative for any bacterial growth. Since cause of raised TLC can be infectious and noninfectious i.e. reactive, malignancy, autoimmune, drugs etc, therefore, it is better to analyze all body fluid samples by microbiological culture. Also, several factors affect positivity of culture such as antibiotic treatment and bacterial load affect the outcome. Additionally, infectious causes may be other than bacterial or fungal e.g. viral. So infectious etiologies cannot be completely ruled in or out for complete inclusion of infection.

## LIMITATION

The limitation of our study is the correlation of culture negativity of majority body fluid samples for infectious cause. Also, there are various other possibilities like fungal or viral cause, bacterial load, antibiotic use or resistance, so the infectious cause cannot be completely ruled out in culture negative cases. Hence, more diagnostic tests such as molecular tests are now available to test for multiple etiologies at one time. Further characterization of fluid is beyond the scope of this laboratory-based paper. It is at physicians' discretion to further characterize body fluids based on their patient's clinical history and other laboratory data while dealing with individual patients.

## CONCLUSION

The study has verified the accuracy of WBC counting by Sysmex XN-1000 analyzer in body fluids against manual method in various body fluid samples. Automated method is rapid, accurate and less labor intensive and must be adopted by laboratories for efficient patient management

## CONFLICT OF INTEREST

Authors declare no conflict of interest

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None

## AUTHOR CONTRIBUTION

**Sana Brohi:** Conception, analysis, collection and interpretation of data, manuscript writing and revision.

**Muhammad Shariq Shaikh:** Conception, interpretation of data, manuscript writing, critical revision.

**Bushra Moiz:** Conception and critical review

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## MANUSCRIPT CATEGORIES

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You CH, Lee KY, Chey WY, Manguy R. Electro gastrographic study of patients with unexplained nausea, bloating and vomiting. *Gastroenterol* 1980; 79: 311-314. Chapter in a book: Weinstein L, Swartz MN. Pathogenic properties of invading microorganisms. In: Sodeman WA Jr, Sodeman WA, eds. *Pathologic physiology: mechanisms of disease*. WB Saunders, Philadelphia 1974; 457-472.

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