Proceedings S.Z.M.C. Vol: 34(4): pp. 10-15, 2020. PSZMC-764-34-4-2020

Frequency of Occult Hepatitis B in Pregnant Women Attending Antenatal Care Unit of a Tertiary Care Hospital

[1]Sameen Bint Ali, [2]Mateen Izhar, [2]Chetan Lal, [3]Asma Yaqoob, [2]Hadiqa tul Hafsa, [4]Asma Akram

[1]*Department of Pathology, Services Institute of Medical Sciences, Lahore*
[2]*Department of Microbiology and Virology, Shaikh Zayed Medical Complex, Lahore*
[3]*Department of Pathology, Fatima Memorial Medical & Dental College, Lahore*
[4]*Department of Pathology, Continental Medical College, Lahore*

ABSTRACT

Introduction: Occult infections (OBIs) caused by hepatitis B virus (HBV) are detected by the presence of HBV DNA without surface antigens. The prevalence of HBsAg in general population of Pakistan is around 3%, but there is no data regarding the occurrence of OBIs. **Aims & Objectives:** The goal of this research was to assess the frequency of occult hepatitis B infection in pregnant females presenting to antenatal care unit of Shaikh Zayed Hospital, Lahore. **Place and duration of study:** These samples were handled at the Microbiology Department of Shaikh Zayed Hospital, Lahore. The duration of study was one year after the approval of synopsis. **Material & Methods:** Four hundred and sixty three pregnant women were included in this research. Serum was separated from their blood samples. HBsAg, Anti-HBcand Anti-HBs tests were performed using the ELISA technique on all the samples. Real time PCR was performed to find HBV DNA. **Results:** The frequency of occult hepatitis B was < 0.25 in this research. Among the 463 women tested during pregnancy, hepatitis B surface antigen was positive in 2 (0.4%) women. These two were excluded from the study. Out of 461 samples, hepatitis B surface antibody was positive in 61 (13.2%) samples and they were negative for hepatitis B core antibody. These 61 samples were also omitted from the research. 25 (5%) samples were positive for both Anti-HBc and Anti-HBs. 11 samples (2%) were Anti-HBc positive and Anti-HBs negative. In 364 (79%) samples, both Anti-HBs and Anti-HBc were absent. Polymerase Chain Reaction was performed on 400 samples. **Conclusion:** The frequency of occult hepatitis B is very low < 0.25% in pregnant women. It is not recommended to routinely screen pregnant women for hepatitis B virus DNA.

Key words: HBV DNA, Occult hepatitis, Pregnant women

INTRODUCTION

Occult hepatitis B (OBI) is defined when hepatitis B virus DNA (HBV DNA) is present in the liver or serum of individuals who are negative for HBV surface antigen (HBsAg).[1] In cases of true OBI the amount of viral DNA in the serum is typically very low that is less than 200 IU/mL. Since it is not always practical or possible to test liver tissue, serum HBV DNA and viral marker tests are often used to diagnose OBI.[2]

As the endemic level of HBV is different in different parts of the globe so it makes the prevalence of OBI quite variable. Around the world it is stated to fluctuate from 1% to 95%.[3] This pervasiveness rate relies upon geographic contrasts (endemicity) and diverse patient qualities. OBI has important clinical significance because HBV can be transmitted through transfusion, organ transplantation and perinatal route.[4] In patients with low immunity or those who are having chemotherapy occult HBV infection can reactivate and lead to acute hepatitis which may develop hepatic fibrosis.[3,5,6] By its proto-oncogenic effect it is also a risk factor that can develop HCC.[7]

OBI is related in some cases to virus genetic variants. A modified surface antigen may be produced by the virus that is not detected by the diagnostic assays. According to different studies it may be due to host immune system which strongly suppress replication-competent viruses in their replication and transcriptional activities.[8,9] In OBI, absence of HBsAg may also be because of the screening done during very initial stage of acute infection before the development of antibodies and in the serum HBsAg is also not present. This also

happens during chronic HBV infection when HBsAg is declines to a very low level that cannot be detected.[10]

On the basis of the hepatitis B virus antibody profile, OBI can be distinguished into seropositive-OBI and seronegative-OBI. In seropositive-OBI Anti HBc is positive irrelevant with the positivity of Anti HBs. In seronegative-OBI antibodies of both Anti HBc and Anti HBs are negative.[11] Approximately 10% of the patients have shown occult HBV infection (OBI) in whom HBsAg is negative and Anti HBc is positive, in which serum HBV DNA is measurable. Detection of HBsAg alone is not sufficient for identifying all chronic HBV infection. Because it may effect patient HBV screening, blood donation, follow-up of patients who have cleared the infection.[1,12]

Various clinical contexts are seen in which occult hepatitis B virus may occur including patients with immunosuppresion from endemic areas of HBV. Pregnancy is a state related with immune suppression. Cellular immunity is disturbed during pregnancy. In normal pregnancy, to evade elimination of the fetus, the maternal Th 1 immune response may be suppressed. As a result of this, for the continuation of pregnancy Th2 phenotype becomes predominant. Cytokines such as interleukin (IL)-4 and (IL)-10 which are anti-inflammatory and are present during this time may lead to increased risk of infection. It has significant clinical implication not only to the mother's wellbeing but also because there is a chance of transmission of occult HBV from mother to fetus. One research demonstrates that HBV can be transferred from mother to child even if the mother is negative for HBsAg and positive for anti-HBc.[13]

To examine infants at danger for vertical transmission of HBV infection, serologic screening of pregnant women includes test for HBsAg. It is not enough being unable to perceive occult hepatitis B virus infection. Recently it was manifested that individuals with occult HBV infection can infect others, even with low viremic level. In highly endemic areas, mother to child (perinatal transmission) spread of hepatitis B is most common.[14] Approximately 90% of infants born with HBV infection always lead to a chronic carrier state. Moreover, vertical transmission of HBV leads infected infants to liver cirrhosis and carcinoma of liver in young adulthood. So it is very important to prevent vertical transmission of HBV.[15,16]

Viral hepatitis during pregnancy is related with a great possibility of maternal problems and it has been reported as the important reason of maternal demise.[17] According to a study conducted in District Kohat, prevalence of hepatitis B was found to be 6% among pregnant women.[18] In another study conducted in Rawalpindi military hospital, the prevalence was 4.69% in pregnant women.[19] Whereas, in normal population HBsAg positivity was 2.5% according to the national survey done by the Pakistan Medical Research Council (PMRC).[20] In all these studies, patients were tested for HBsAg but they were not investigated for HBV DNA by PCR. As pregnancy itself is a state of immune suppression we think it is under detected and we may be missing occult hepatitis B infection. Moreover, there is the risk of vertical transmission of HBV infection it is important to diagnose occult hepatitis B virus infection. This study is planned to detect HBV DNA by PCR in pregnant women screened negative by HBsAg surface marker.

MATERIAL AND METHODS

It was a cross sectional descriptive study. 369 samples of blood were collected based on international data.[21] Informed consent and history was taken. HbsAg negative pregnant women of all ages and parity were included in this examination visiting Shaikh Zayed Hospital from August to October 2017. Pregnant women with positive Anti-HBs and negative Anti-HBcIgG antibody were excluded from this research. These samples were processed for serum at the Department of Microbiology of Shaikh Zayed Hospital, Lahore.

HBsAg and Anti-HBc was estimated using ELISA kit manufactured by Roche Diagnostics USA (Cobas e 411).

Further, the real time polymerase chain reaction was performed to detect hepatitis B virus DNA to diagnose occult hepatitis B by Roche cobasTaqMan 48 kit USA.

Statistical analysis:

Data was entered and analyzed using SPSS version 23. P value <0.05 considered significant.

RESULTS

Four hundred and sixty three pregnant women were registered in this research. Their mean age with standard deviation (mean $\pm$ SD) was 27.26 ± 4.264. The minimum and maximum age of the patient was 17 and 40 years respectively. The highest proportion of participants (43%) was in the age group 26-30 years, followed by 33.5% in the group for 21-25 years. (Table-1)

The mean standard deviation of the duration of pregnancy was 25.14 weeks $\pm$ 6.926. The minimum

duration of pregnancy was 4 weeks and maximum duration of pregnancy was 37 weeks. (Fig-1)

In our study 7.1% of the patients were of high socioeconomic status. 74.1% belonged to middle socioeconomic class and 18.8% of the patients were of low socioeconomic status. (Fig-2)

Out of 463 patients 2 (0.4%) patients were positive for HBsAg serological marker and rest were negative (99.6%). And these 2 were excluded from the study. (Table-2)

Out of 461 samples, hepatitis B surface antibody was positive in 61 (13.2%) samples and they were negative for hepatitis B core antibody. These 61 samples were also omitted from the research. 25 (5%) samples were positive for both Anti-HBc and Anti-HBs. 11 samples (2%) were AntiHBc positive and Anti-HBs negative. In 364 (79%) samples both Anti-HBs and Anti-HBc were absent. And they were included in the study. (Table-3)

Polymerase chain reaction was done in all patients to identify HBV DNA in their serum samples. 400 study samples were run on Roche CobasTaqman 48 kit USA. HBV DNA was not detected in any of the study sample. Therefore, according to this study the frequency of occult hepatitis B ranges from 0% to 0.24%.

Age Groups	Frequency	Percentage (%)
Under 20	18	3.9
21-25	155	33.5
26-30	199	43.0
31-35	78	16.8
36 & above	13	2.8
Total	**463**	**100.0**

Table-1: Age Groups of the Patients

HBsAg	Frequency	Percentage (%)
Negative	461	99.6
Positive	2	0.4
Total	463	100.0

Table 2: Frequency and Percentage of HBsAg in Study Participants

Serological Markers	Number	(%)
Isolated Anti-HBs Positive (Anti-HBs Positive but Anti-HBc Negative)	61	13%
Isolated Anti-HBc Positive (Anti-HBc Positive but Anti-HBs Negative)	11	2%
Both Anti-HBs and Anti-HBc Positive	25	5%
Both Anti-HBs and Anti-HBc Negative	364	79%

Table 3: Serological Markers of Study Subjects

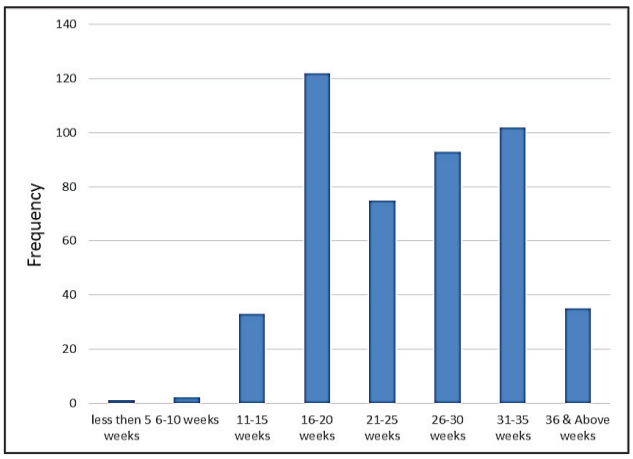

Fig-1: Gestational Age Groups of Study Participants(weeks)

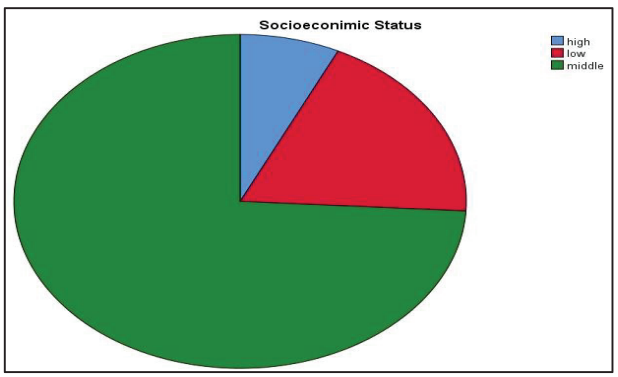

Fig-2: Socioeconomic Status

DISCUSSION

Viral hepatitis B is endemicaly present in Pakistan.[22] It is a major health problem not only in Pakistan but worldwide as well.[18] In Pakistan a study was done in 2016 at Rawalpindi Medical College to find the prevalance of Hepatitis B in pregnant women in Pakistan. The study inculded 519 pregnant females. Out of 519 pregnant females, 7 were posistive for HBsAg. The prevalance of hepatits B was found to be 1.3%.[23] In another study conducted in District Kohat, Khyber Pakhtunkhaw 100 pregnant females were tested from 8 December 2016 to 12 June 2017. Out of these 100 females 6 women were seropositive against HBV infection. The prevalance of hepatitis B was found to be 6%.[18]

Unidentified occult hepatitis B in pregnant women is a possible cause of infection from mother to fetus. The spread of hepatitis B virus from the pregnant women to her fetus can be stopped by a diagnosis of occult hepatitis B. The prevalance of occult hepatitis B has not been investigated in pregnant women in Pakistan. However, a study has been conducted in Pakistan in blood donors. 966 blood donors were selected from Armed Forces Institute of Transfusion, Rawalpindi. Eighteen (1.86%) donors were positive for HBsAg. Out of the remaining 948

HBsAg negative blood donors, HBV DNA was detected in 5 (0.53%) blood donors.[24] Prevalance rate of OBI among hemophalia patients in Pakistan was 1.7% as reported by Borhany and colleagues.[25] A study conducted in India showed 413 (19.8%) blood donors were positive for hepatitis B core antibody and negative for hepatitis B surface antigen. Of these 7.5% were positive for HBV DNA.[26]

Our results showed that the mean age of the study participants was 27.26 years and age did not seem to be an issue for HBV infection as there was no change in ages of women with HBV versus those without HBV.

Patients were selected randomly in our study. Their past history of hepatitis B and the hepatitis B core antibody and hepatitis B surface antibody levels were unknown. In our study 61 (13.3%) were positive for hepatitis B surface antibody but negative for hepatitis B core antibody. This indicated that they were self vaccinated. Out of these 61 ladies only 11 (18.03%) pregnant women gave history of vaccination rest 50 (81.96) were unaware of their vaccination status. In a study conducted in Karachi in 2015, 17% of the females had received HBV vaccination during childhood.[22] Routine HBV vaccination has been recommended by the World health Organization for all children.[21] In Pakistan, HBV vaccination was included in EPI since 2009. Because of this prevalance rate of hepatitis B surface antigen has declined and the positive rate of hepatitis B surface antibody has been on the rise.

The prevalance rate of occult hepatitis B in pregnant women was 6.6% in Botswana. Six hundered and twenty two HBsAg negative samples were tested for HBV DNA. 41 were positive for OBI.[27] In another study conducted in Korea, 4% pregnant women were positive for HBV DNA with the TaqMan PCR assay. 202 healthy pregnant women were enrolled in this study and HBV DNA was detected in eight of 202 individuals with TaqMan PCR assay.[21]

In our study 25 patients were positive for both Anti-HBc and Anti-HBs. This meant that these patients had recovered from past infection and were immune. Eleven individuals were positive for Anti-HBc and negative for Anti-HBs. Serological analysis of occult hepatitis B infection patients showed that they may b either seropositive (Anti-HBc alone or together with Anti-HBs positive) or seronegative (Anti-HBc and Anti-HBs negative). The subjects who are hepatitis B core antibody positive and hepatitis B surface antibody negative have the highest rate of detection of HBV DNA.[28]

So the actual rate of HBV infection in these women is 8.02%. Out of which 0.43% were carriers of HBV infection and 7.7% had some past infection. A study conducted at Ayub Teaching Hospital Abbotabad from December 2015 to May 2016. Through non probablity consecutive sampling 174 pregnant females were registered in the study. ELISA was the technique used to screen blood samples for hepatitis B surface antigen. Six patients were positive for HBsAg. So the prevalance rate was 3.4%.[29] 3.4% were carriers in comparison to 0.43% in our study.

The prevalance of occult hepatitis B infection varies from 1% to 87% from diverse areas of the sphere. In some geographical areas with little HBV endemicity, OBI was still reported. A number of factors are responsible for these variations. These include sensitivity of hepatitis B virus DNA detection assay, the sample size, and the detection of of hepatitis B virus DNA in liver tissue or serum sample.[30]

The actual prevalance of OBI is underestimated by detecting hepatitis B virus DNA in the serum. HBV DNA detection in liver biopsy is the best way for diagnosing occult hepatitis B infection. But the liver specimen are not easily available as liver biopsy is required and it is a procedure which require invasiveness. Moreover for finding HBV DNA in the liver tissue, FDA has not permitted to use any standardized and valid assay.[28]

In our study 79% of these women are negative for the antibodies of both HBsAg and HBcAg and they are the true negatives. And these women can get benefit from vaccination. As carriage rate of hepatitis B is very low in our study population that is why there was no occult hepatitis B detected in these pregnant women as endemicity of HBV infection associates with occurrence of OBI.[31]

CONCLUSION

The frequency of occult hepatitis B is <0.25% in pregnant women of Shaikh Zayed Hospital. It was not even present in Anti-HBc positive women or those who had no serelogical marker. Occult hepatitis B can be transmitted from mother to fetus but routine testing for HBV DNA in pregnant women is not recommended at this time from our study.

Limitations of the Study:

- The study participants from a single antenatal care centre of Shaikh Zayed Hospital were included in our study population. So the frequency of occult hepatitis B in the pregnant women from all over the country cannot be predicted from our research.

- The true frequency of OBI may have been under detected because hepatitis B virus DNA was detected on the serum samples and not in the PBMCs and liver.

Recommendations:

- We recommend before testing HBsAg, Anti-HBc screening in patients should be done. If Anti-HBc is negative, immunize the patient according to convention pursued by post immunization Anti-HBs levels. If Anti-HBc is positive, HBsAg should be tested further. If HBsAg is positive the patients should be evaluted for establishment of the stage of disease and treatment. If HBsAg is negative no further vaccination is required in patients as they had past hepatitis B virus infection.
- The study participants must be selected from different hospitals units.
- Along with the serum samples, the peripheral blood mononuclear cells and liver specimens (if possible) should be tested for detecting HBV DNA.

REFERENCES

1. Portilho MM, Nabuco LC, Villela-Nogueira CA, Brandão-Mello CE, Pilotto JH, Flores GL, et al. Detection of occult hepatitis B in serum and oral fluid samples. Memórias do Instituto Oswaldo Cruz. 2018; 113(1):62-5.
2. Seo DH, Whang DH, Song EY, Han KS. Occult hepatitis B virus infection and blood transfusion. World journal of hepatology. 2015; 7(3):600.
3. Kwak M-S, Kim YJ. Occult hepatitis B virus infection. World journal of hepatology. 2014; 6(12):860.
4. Rendon JC, Cortes-Mancera F, Restrepo-Gutierrez JC, Hoyos S, Navas M-C. Molecular characterization of occult hepatitis B virus infection in patients with end-stage liver disease in Colombia. PloS one. 2017; 12(7):e0180447.
5. Bajjou T, Tagajdid MR, Elkochri S, Abi R, Touil N, Rahoui J, et al. Recurrence of occult hepatitis B virus infection in a recipient of a liver transplant for HCV-related cirrhosis: full length genome, mutations analysis and literature review. International Journal of Research in Medical Sciences. 2017; 2(4):1294-301.
6. Yuen M-F. Clinical implication of occult hepatitis B infection. Annals of Blood.2017;2(4).
7. Makvandi M, Neisi N, Khalafkhany D, Makvandi K, Hajiani E, Shayesteh AA, et al. Occult hepatitis B virus among the patients with abnormal alanine transaminase. Jundishapur journal of microbiology. 2014; 7(8).
8. Raimondo G, Pollicino T. Occult HBV infection. Hepatitis B Virus in Human Diseases: Springer; 2016. p. 277-301.
9. Pollicino T, Raimondo G. Occult hepatitis B infection. Journal of hepatology. 2014; 61(3):688-9.
10. Maldonado-Rodriguez A, Cevallos AM, Rojas-Montes O, Enriquez-Navarro K, Alvarez-Muñoz MT, Lira R. Occult hepatitis B virus co-infection in human immunodeficiency virus-positive patients: A review of prevalence, diagnosis and clinical significance. World journal of hepatology. 2015; 7(2):253.
11. Said ZN, El Sayed MH, Salama II, Aboel-Magd EK, Mahmoud MH, El Setouhy M, et al. Occult hepatitis B virus infection among Egyptian blood donors. World journal of hepatology. 2013; 5(2):64.
12. Romero M, Madejón A, Fernández-Rodríguez C, García-Samaniego J. Clinical significance of occult hepatitis B virus infection. World Journal of Gastroenterology: WJG. 2011; 17(12):1549.
13. Chang P, Tu J, Chesterman A, Kim R, Robertson P, Rawlinson WD, et al. Development of occult hepatitis B viral infection in pregnancy: implications for antenatal screening in women from endemic areas. Obstetric medicine. 2010; 3(3):115-8.
14. Walz A, Wirth S, Hucke J, Gerner P. Vertical transmission of hepatitis B virus (HBV) from mothers negative for HBV surface antigen and positive for antibody to HBV core antigen. The Journal of infectious diseases. 2009; 200(8):1227-31.
15. Zenebe Y, Mulu W, Yimer M, Abera B. Sero-prevalence and risk factors of hepatitis B virus and human immunodeficiency virus infection among pregnant women in Bahir Dar city, Northwest Ethiopia: a cross sectional study. BMC infectious diseases. 2014; 14(1):118.
16. Khamduang W, Ngo-Giang-Huong N, Gaudy-Graffin C, Jourdain G, Suwankornsakul W, Jarupanich T, et al. Prevalence, Risk Factors, and Impact of Isolated Antibody to Hepatitis B Core Antigen and Occult Hepatitis B Virus Infection in HIV-1–Infected Pregnant Women. Clinical infectious diseases.2013;56(12):1704-12
17. Banks T, Kang J, Watts I, Tyrosvoutis MEG, Min AM, Tun NW, et al. High hepatitis B seroprevalence and risk factors for infection in pregnant women on the Thailand-Myanmar Border. The Journal of Infection in Developing Countries. 2016; 10(04):384-8.

18. Hussain R, Mansoor H, Iqbal T, Askar R, Shaheen U, Rehman HU. Prevalence of hepatitis b (HBV) infection In pregnant women in district Kohat, Khyber Pakhtunkhwa, Pakistan. 2018.
19. Anwar R, Razzaq K, Imran A. Frequency of Hepatitis B virus among pregnant women attending military hospital Rawalpindi. Pakistan Armed Forces Medical Journal. 2016; 66(6).
20. Ahmad I. Prevalence of hepatitis B and C viral infection among pregnant women in Peshawar, Pakistan. Hepatitis monthly. 2016; 16(6).
21. Kwon CI, Hwang SG, Shin SJ, Chang SW, Kim SY, Ko KH, et al. Occult hepatitis B virus infection in pregnant woman and its clinical implication.Liver International.2008;28(5):667-74
22. Shakeel S, Iffat W, Rehman H, Jamil N, Nazeer F, Naveed S, et al. Assessing the level of awareness and vaccination status of Hepatitis B among young female community of Pakistan. Bangladesh Journal of Medical Science. 2015; 14(4):376-83.
23. Farooq A, Rukhsar A, Talib N, Mahmood S, Rukhsar N, Asad M. Frequency of Hepatitis B and Hepatitis C in Pregnant Females of Rawalpindi. group.15:25.
24. Bhatti FA, Ullah Z, Salamat N, Ayub M, Ghani E. Anti–hepatits B core antigen testing, viral markers, and occult hepatitis B virus infection in Pakistani blood donors: implications for transfusion practice. Transfusion.2007;47(1):74-9
25. Arababadi MK, Ahmadabadi BN, Daredor HY, Kennedy D. Epidemiology of occult hepatitis B infection among thalassemic, hemophilia, and hemodialysis patients. Hepatitis monthly. 2012; 12(5):315.
26. Asim M, Ali R, Khan LA, Husain S, Singla R, Kar P. Significance of anti-HBc screening of blood donors & its association with occult hepatitis B virus infection: Implications for blood transfusion. Indian J Med Res. 2010; 132(312):7.
27. Mbangiwa T, Kasvosve I, Anderson M, Thami P, Choga W, Needleman A, et al. Chronic and occult hepatitis B virus infection in pregnant women in Botswana. Genes. 2018; 9(5):259.
28. Said ZNA. An overview of occult hepatitis B virus infection. World journal of gastroenterology: WJG. 2011; 17(15):1927.
29. Jadoon SM, Adeel M, Aslam S, Rasool A. Hepatitis B and Hepatitis C virus in women with first pregnancy. Journal of Ayub Medical College Abbottabad. 2017; 29(4):614-8.
30. Makvandi M. Update on occult hepatitis B virus infection. World journal of gastroenterology. 2016; 22(39):8720.
31. Raimondo G, Caccamo G, Filomia R, Pollicino T, editors. Occult HBV infection. Seminars in immunopathology; 2013: Springer.

The Authors:

Dr. Sameen Bint Ali
Demonstrator,
Department of Pathology,
Services Institute of Medical Sciences, Lahore.

Prof. Mateen Izhar
Head, Department of Microbiology and Virology,
Shaikh Zayed Medical Complex, Lahore

Dr. Chetan Lal
Assistant Professor,
Department of Microbiology and Virology,
Shaikh Zayed Medical Complex, Lahore.

Dr. Asma Yaqoob
Senior Demonstrator,
Department of Pathology,
Fatima Memorial Medical & Dental College,
Lahore.

Dr. Hadiqa tul Hafsa
Demonstrator,
Department of Microbiology and Virology,
Shaikh Zayed Medical Complex, Lahore.

Dr. Asma Akram
Senior Demonstrator,
Department of Pathology,
Continental Medical College, Lahore

Corresponding Author:

Dr. Sameen Bint Ali
Demonstrator,
Department of Pathology,
Services Institute of Medical Sciences, Lahore.
E-mail: sameenbinteali@gmail.com