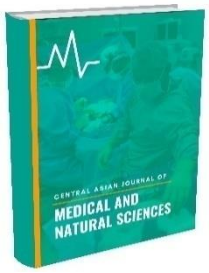
CENTRAL ASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES



Volume: 04 Issue: 04 | Jul-Aug 2023 ISSN: 2660-4159

http://cajmns.centralasianstudies.org

Serological and Molecular Detection of Herpes Virus From Vaginal Samples of Pregnant Women

1. **Alaa Farooq Rasool**
2. **Sarah Shareif Akool**
3. **Hawraa Ali Mahmood**

Received 2nd May 2023,

Accepted 3rd Jun 2023,

Online 29th Jul 2023

[1,2,3] Central Public Health Laboratory in Muthanna

Key words: HSV-1, HSV-2, Women, Pregnant, Molecular, Serological.

Abstract: This investigation was done for serological and molecular detection of herpes virus from vaginal samples of pregnant women. This study involved the enrollment of 210 pregnant women who were admitted to a private hospital in Baghdad. The data collection period spanned from 2021 to 2023. Samples of serum and cervicovaginal lavage were obtained from the participants and then transferred to the laboratory under refrigeration using ice. Virus DNA from lavage specimens was extracted by using kit. Two primers were used in this study for detection of HSV-1 and 2. The presence of antibodies specific to HSV-1 and HSV-2 was determined in serum samples using a competitive type-specific ELISA. IgG prevalence was assessed to be 91% (191 out of 210) and IgM prevalence was determined to be 5.7% (12 out of 210) based on results from serological tests of total HSV-1 and HSV-2 antibodies. Out of a total of 210 people, 96.7% (203 of 203) were affected. The estimated HSV-1 and HSV-2 frequencies in this research were 5.2% and 8.6%, respectively, based on molecular studies. The current study showed that age group 21-30 y were mostly affected.

Inconclusion, the primary observation derived from this study is the comparatively reduced prevalence of HSV-1 and HSV-2 among pregnant women, as opposed to the rates reported by the World Health Organisation (WHO). However, it is evident that there is a need for extensive research to be conducted in order to determine the prevalence of HSV infections among Iraqi women, particularly in pregnant women, and to analyse the trends over time. In addition, the utilisation of molecular and serological techniques can prove to be advantageous in carrying out such investigations.

Introduction:

The Herpesviridae family is a group of viruses with a reputation for causing long-lasting infections in humans. Five genera and 45 species make up what is called the subfamily Alphaherpesvirinae. Among these species, human alphaherpesvirus 1 and 2 are widely prevalent. They belong to the genus Simplexvirus and are commonly referred to as herpes simplex type 1 and 2 (HSV-1 and HSV-2) respectively (1-3).

These viruses have been linked to infections in the orofacial and genital regions, respectively. The herpes simplex virus (HSV) is widely acknowledged as a highly prevalent cause of sexually transmitted infections (STIs) on a global scale. It is estimated that between 60% and 95% of the adult population worldwide either carries HSV viruses or is affected by related infections, which typically manifest as subclinical or asymptomatic conditions. The severity and frequency of these infections are contingent upon the individual's immune system and the frequency of viral exposure (4,5).

The transmission of HSV-1 primarily occurs through non-sexual means, specifically mouth-to-mouth contact, resulting in the development of oral ulcers. On the other hand, HSV-2 is responsible for the occurrence of genital ulcers, which are transmitted through sexual contact (6, 7). Nevertheless, certain reports attribute the causation of genital ulcers to HSV-1 (8).

Based on the most recent report released by the World Health Organisation (WHO) in 2017, it was found that a significant proportion of individuals under the age of 50, specifically 67%, are infected with HSV-1 globally. Additionally, HSV-2 infection was observed in 13% of individuals aged 15-49 worldwide. According to estimates, the prevalence of HSV-2 infection among women in 2017 was approximately 313 million (8-9).

Both genital and oral herpes are very infectious during pregnancy, and they may be passed on to the baby either via the mother's viremia or, more often, through the birth canal. This may cause newborn herpes, which has been linked to an increased death rate (5, 10). Neonatal herpes is a relatively infrequent yet highly detrimental complication that arises from infection with the herpes simplex virus during the gestational period (11).

When compared to pregnant women who have already been exposed to HSV, uninfected women who come into contact with the virus for the first time in their third trimester have a much greater risk of transmitting it vertically. It's possible that some mothers get infected for the first time during their pregnancies (12,13). The acquisition of HSV during gestational status has been associated with various adverse outcomes, including spontaneous abortion, severe neurologic injuries, prematurity, stillbirth, preterm birth, as well as congenital and neonatal herpes (14, 15). The implementation of acyclovir therapy in pregnant women who are infected, along with the consideration of caesarean delivery after localising genital lesions, are effective strategies that should be considered in order to reduce the risk of congenital transmission of herpes simplex virus (HSV) (16, 17).

While the prevalence of HSV-1 is increasing worldwide, certain societies have experienced a reversal in this upward trend due to improvements in their hygienic conditions. Therefore, the implementation of enhanced sanitation measures would effectively reduce the likelihood of adolescents being exposed to this virus before reaching puberty, consequently decreasing the risk of post-puberty transmission of HSV (4, 18). Furthermore, according to Kahlon and Whitley (19), a significant proportion of mothers, approximately 80%, are not cognizant of their history of HSV infection when their newborns become infected. Overall, conducting research and monitoring women of reproductive age, particularly prior to and during pregnancy, is an essential strategy to prevent the occurrence of complications associated with HSV infection, such as neonatal herpes (20).

This investigation was done for serological and molecular detection of herpes virus from vaginal samples of pregnant women.

Materials and Methods:

This study involved the enrollment of 210 pregnant women who were admitted to a private hospital in Baghdad. The data collection period spanned from 2021 to 2023. Serum and cervicovaginal lavage specimens were collected from the participants and subsequently transported to the laboratory while being kept at a low temperature using ice. The specimens obtained from cervicovaginal lavage were subjected to centrifugation at a force of 1000 times the acceleration due to gravity for a duration of 10 minutes. Following centrifugation, the liquid portion above the sediment was removed and discarded. For either short-term or long-term storage, the cells were resuspended in 1 ml of PBS and frozen at -20 degrees Celsius. After drawing blood from the body's periphery, the serum was separated by centrifuging the blood at 2000 times the acceleration of gravity for around 10 minutes. The obtained serum aliquots were subsequently stored at a temperature of -20°C until they were subjected to serological analyses.

Virus DNA from lavage specimens was extracted by using Genaid kit and according to the manufacturer instructions.

The forward and reverse primers developed for UL5 and UL27 detection are listed in Table 1 (21). Denaturation at 95 degrees Celsius for 15 minutes was followed by 35 cycles of denaturation at 94 degrees Celsius for 45 seconds, annealing at 58.5 degrees Celsius for 45 seconds, extension at 72 degrees Celsius for 45 seconds, and a final extension step at 72 degrees Celsius for 7 minutes for the aforementioned genes.

The PCR products were subjected to analysis using ultraviolet (UV) light following electrophoresis at a voltage of 100 volts for a duration of 1 hour on a 1.5% agarose gel that had been treated with a DNA-safe stain.

Table 1. Type specific primers of HSV-1 and HSV-2

Gene	F	R	bp
HSV-1	5'-GACGTCACCGTTTCGCAGGTGT-3'	5'-CGTTGGCCGGTTTCAGCTCCAT-3'	412
HSV-2	5'-CGCGCCTCCGAAAGATGGTGTT-3'	5'-TCGTCCAGCCCGGCGAAGATAA-3'	217

The presence of antibodies specific to HSV-1 and HSV-2 was determined in serum samples using a competitive type-specific ELISA (Mybiosource company). Specimens were coded as seropositive or seronegative, and the data was entered in Microsoft Excel 2010.

Results:

Based on the results of the serological analysis of total HSV-1 and HSV-2 antibodies, it was found that the prevalence of IgG was 91% (191 out of 210) and that of IgM was 5.7% (12 out of 210) (Figures 1 and 2). The total prevalence was 96.7% (203 out of 210).

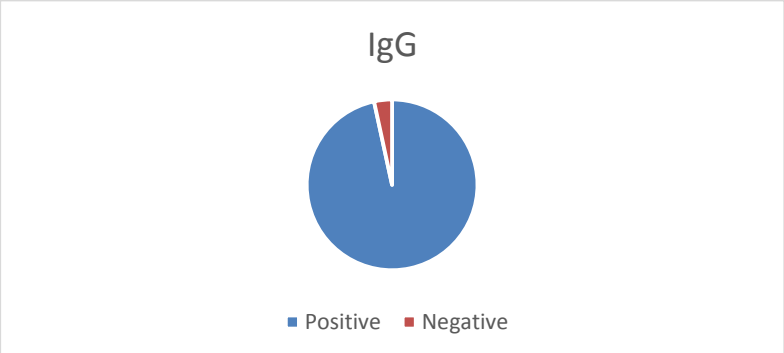


Figure 1. The prevalence of IgG in the study participants

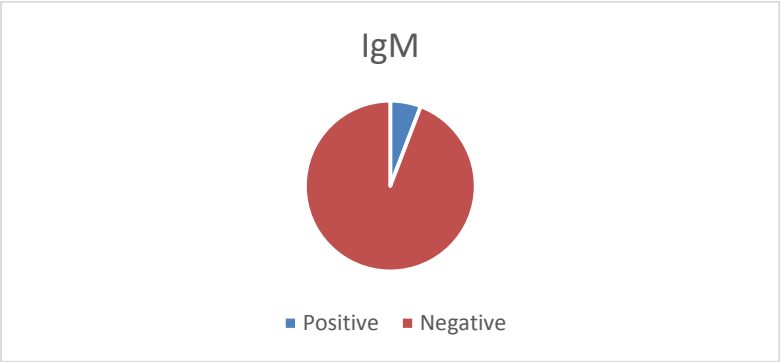


Figure 2. The prevalence of IgM in the study participants

The estimated HSV-1 and HSV-2 frequencies in this research were 5.2% and 8.6%, respectively, based on molecular studies (Figures 3, 4, 5, and 6).

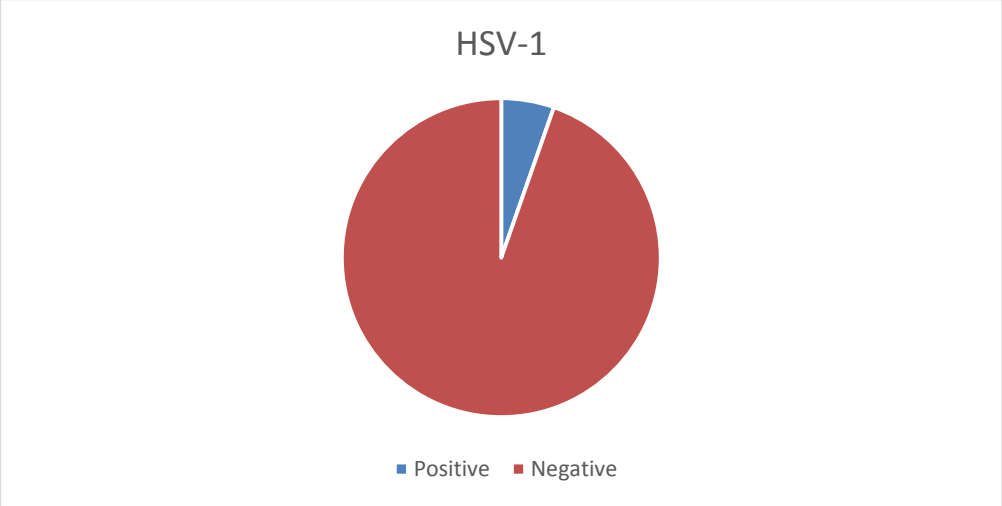


Figure 3. Molecular prevalence of HSV-1

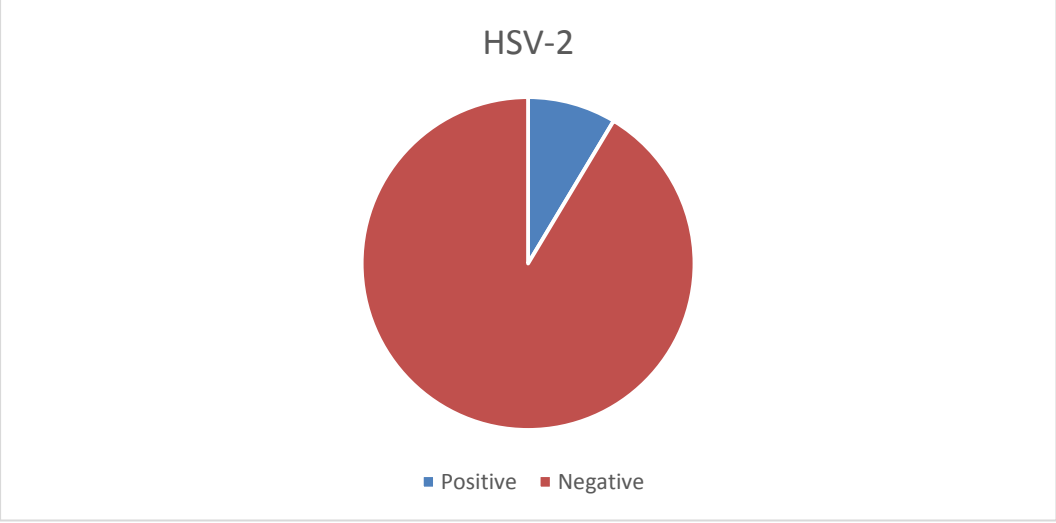


Figure 4. Molecular prevalence of HSV-2

The current study showed that age group 21-30 y were mostly affected (Table 2).

Table 2. Prevalence according to age

Age groups(y)	IgG	IgM	HSV-1	HSV-2
15-20 (52)	46 (88.5%)	3 (5.8%)	2 (3.8%)	4 (7.7%)
21-30 (113)	109 (96.5%)	7 (6.2%)	8 (7.1%)	13 (11.5%)
31-42 (45)	36 (80.0%)	2 (4.4%)	1 (2.2%)	1 (2.2%)
Total (210)	191 (96.7%)	12 (5.7%)	11(5.2%)	18 (8.6%)

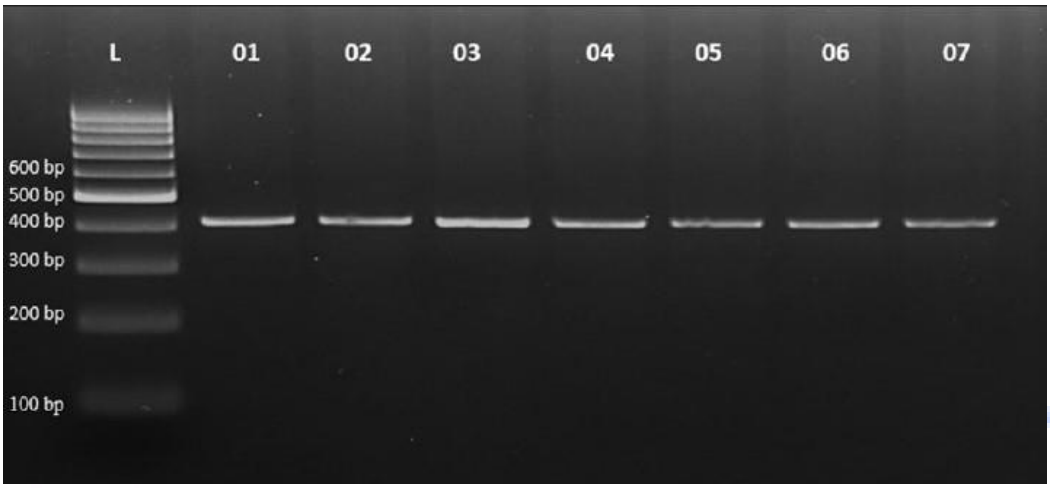


Figure 5. Gel electrophoresis for HSV-1 gene, showed positive results at 412bp

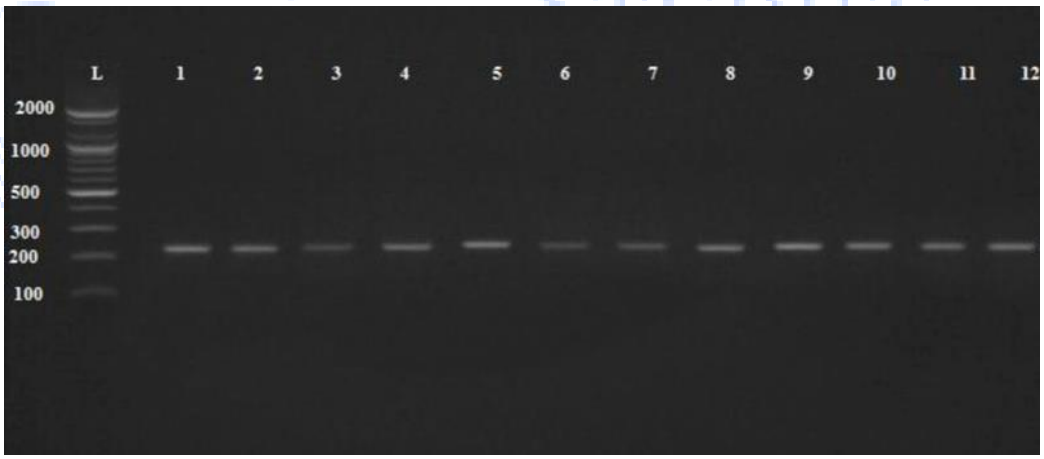


Figure 6. Gel electrophoresis for HSV-2 gene, showed positive results at 217 bp

Discussions:

According to a study conducted by Monavari et al., the prevalence rates of HSV1 and HSV-2 were reported to be 22.9% and 14.3% respectively (23). The findings of the present study align with a previous investigation conducted in Sudan, which examined a cohort of 100 pregnant women. In that study, the prevalence of HSV-1 and HSV-2 was reported to be 3% and 2% respectively (24), mirroring the results obtained in the current research. Within the scope of our investigation, it was determined that 5.2% (11 out of 210) and 8.6% (18 out of 210) of the clinical specimens analysed exhibited positive results for the presence of the HSV-1 and HSV-2 genomes, respectively. The aforementioned

variables may account for the extensive variability observed. Molecular typing results from this research show that HSV-2 was the most common type, with an 8.6% prevalence rate.

Furthermore, the enzyme-linked immunosorbent assay (ELISA) was employed to identify antibodies (specifically IgM, IgG, and total) targeting HSV-1 and HSV-2 in serum samples. A total of 800 women were surveyed for a research by Rezaei-Chaparpordi et al. (25) in Iran's northwestern provinces. The research found that the prevalence of HSV-1 was 58.4 percent, whereas that of HSV-2 was 3.5 percent. According to their findings, it has been reported that the prevalence of HSV-1 is higher than that of HSV-2. They also observed an age-related rise in HSV-1 sero-prevalence (25). However, when it comes to HSV-1 and HSV-2 infections, the ELISA results showed that the frequency of IgM and IgG antibodies were 5.7% and 91%, respectively.

Serological survey results showed a significant increase in the sero-prevalence of HSV infections among the pregnant women surveyed in this research (96.7%). One notable constraint of the current study was the absence of examination into the specific antibodies targeting HSV-1 and HSV-2 using the enzyme-linked immunosorbent assay (ELISA) method. However, prior studies have shown that knowing the sero-prevalence of both herpes kinds may aid in epidemiological studies and the creation of healthcare policy. The high sero-prevalence of HSV infections found in our research might be due to a number of causes, including widespread virus spread, low female education about the issue, and environmental influences (22).

According to the data collected on HSV-positive patients, the largest incidence of infection was seen in women between the ages of 24 and 33. Results from a study in South Africa were consistent with ours (26, 27). Consistent with earlier studies, this one found a favourable connection between HSV infectivity and younger ages. This association could potentially be attributed to heightened sexual activity among individuals within this age group. Furthermore, it was observed in the previous study (28) that the age group of 20-29 years was the most prevalent among the female participants.

Conclusion:

The primary observation derived from this study is the comparatively reduced prevalence of HSV-1 and HSV-2 among pregnant women, as opposed to the rates reported by the World Health Organisation (WHO). However, it is evident that there is a need for extensive research to be conducted in order to determine the prevalence of HSV infections among Iraqi women, particularly in pregnant women, and to analyse the trends over time. In addition, the utilisation of molecular and serological techniques can prove to be advantageous in carrying out such investigations.

References:

1. Szczubiałka K, Pyrć K, Nowakowska M. In search for effective and definitive treatment of herpes simplex virus type 1 (HSV-1) infections. RSC Advan. 2016;6(2):1058-75.
2. Ruderfer D, Krilov LR., Herpes simplex viruses 1 and 2. Pediatr Rev. 2015;36(2):86-90.
3. Hammad WAB, Konje JC. Herpes simplex virus infection in pregnancy–an update. Eur J Obstet Gynecol Reprod Biol. 2021;259:38-45.
4. Khadr L, Harfouche M, Omori R, Schwarzer G, Chemaitelly H, Abu-Raddad LJ. The epidemiology of herpes simplex virus type 1 in Asia: systematic review, meta-analyses, and meta-regressions. Clin Infect Dis. 2019;68(5):757-72.
5. Marchi S, Trombetta CM, Gasparini R, Temperton N, Montomoli E. Epidemiology of herpes simplex virus type 1 and 2 in Italy: a seroprevalence study from 2000 to 2014. J Prev Med Hyg. 2017;58(1): E27-E33.

6. Bradley H, Markowitz LE, Gibson T, McQuillan GM. Seroprevalence of herpes simplex virus types 1 and 2—United States, 1999–2010. J Infect Dis. 2014;209(3):325-33.
7. Pebody RG, Andrews N, Brown D, Gopal R, De Melker H, François G, et al. The seroepidemiology of herpes simplex virus type 1 and 2 in Europe. Sex Transm Infect. 2004;80(3):185-91.
8. Sert UY, Ozgu-Erdinc AS, Saygan S, Engin-Ustun Y. Herpes simplex infection during pregnancy, results of a tertiary referral center in Turkey. Z Geburtshilfe Neonatol. 2020;224(1):22-5.
9. Wang C, Zhou YH, Yang HX, Poon LC. Intrauterine vertical transmission of SARS-CoV-2: what we know so far. Ultrasound Obstet Gynecol. 2020;55 (6):724-5.
10. Lima L, Padalecki G, Castro C, Cordeiro J, de Paula V. Seroprevalence of human herpesvirus type 2 in a reference center for pregnant women in Rio de Janeiro, Brazil. Virus Rev Res. 2017;22(1): 20-1.
11. Kimberlin DW. Neonatal herpes simplex infection. Clin Microbiol Rev. 2004;17(1):1-13.
12. Patton ME, Bernstein K, Liu G, Zaidi A, Markowitz LE. Seroprevalence of herpes simplex virus types 1 and 2 among pregnant women and sexually active, nonpregnant women in the United States. Clin Infect Dis. 2018;67(10):1535-42.
13. James SH, Kimberlin DW. Neonatal herpes simplex virus infection. Infect Dis Clin North Am. 2015;29(3):391-400.
14. Tookey PA, Mahdavi S, Peckham CS. Surveillance of neonatal herpes in the British Isles 2004-2006. F1000Research, 2020;9(163):163.
15. Mahant S, Hall M, Schondelmeyer AC, Berry JG, Kimberlin DW, Shah SS. Neonatal herpes simplex virus infection among medicaid-enrolled children: 2009-2015. Pediatrics. 2019;143(4):e20183233.
16. Samies NL, James SH. Prevention and treatment of neonatal herpes simplex virus infection. Antiviral Res. 2020;176:104721.
17. Aggerholm BS, Ostenfeld EB, Andersen LH, Krogh RH, Arendt LH, Sandager P. [Genital herpes simplex virus infection in pregnancy]. Ugeskr Laeger. 2020;182(5):V09190527. Danish.
18. Looker KJ, Magaret AS, May MT, Turner KM, Vickerman P, Gottlieb SL, et al. Global and regional estimates of prevalent and incident herpes simplex virus type 1 infections in 2012. PloS One. 2015;10(10):e0140765.
19. Kahlon J, Whitley RJ. Antibody response of the newborn after herpes simplex virus infection. J Infect Dis. 1988;158(5):925-33.
20. Woestenberg PJ, Tjhie JH, de Melker HE, van der Klis FR, van Bergen JE, van der Sande MA, et al. Herpes simplex virus type 1 and type 2 in the Netherlands: seroprevalence, risk factors and changes during a 12-year period. BMC Infect Dis. 2016;16:364.
21. Yasaghi M, Hosseini SD, Moradi A, Hassanpour M, Tabarraei A. Molecular detection of HHV-1, HHV-2, HHV-5 and HBV in semen of fertile and infertile men by multiplex PCR method. Iran J Microbiol. 2022;14(6):921-7.
22. Malary M, Abedi G, Hamzehgardeshi Z, Afshari M, Moosazadeh M. The prevalence of herpes simplex virus type 1 and 2 infection in Iran: a metaanalysis. Int J Reprod Biomed. 2016;14(10):615- 24.

23. Monavari SH, Vaziri MS, Khalili M, ShamsiShahrabadi M, Keyvani H, Mollaei H, et al. Asymptomatic seminal infection of herpes simplex virus: impact on male infertility. J Biomed Res. 2013;27 (1):56-61.

24. Mohammed MS, Y.E. Yousif, and N. Hisham, Al Tayeb. Molecular Detection of Herpes Simplex-1 and 2 Viruses among Pregnant Women in Khartoum State (Sudan). African J Med Sci. 2020;5(7).

25. Rezaei-Chaparpordi S, Assmar M, Amirmozafari N, Modiri L, Massiha A, Shokri-Fashtali S, et al. Seroepidemiology of herpes simplex virus type 1 and 2 in northern iran. Iran J Public Health. 2012; 41(8):75-9.

26. Abbai NS, Govender S, Nyirenda M. Herpes simplex virus-2 infections in pregnant women from Durban, South Africa: prevalence, risk factors and co-infection with HIV-1. South African J Infect Dis. 2018;33(5):1-7.

27. Sharma P, Ganga RT. A study to assess the prevalence of Herpes simplex type 2 (HSV-2) infections in pregnant women in a tertiary care hospital. Int J Health Sci. 2022;6(S1):11938-45.

28. Mezher MN, Mejbel FA, Hussein HK. Detection of Herpes Simplex-2 Virus in Women with Spontaneous Abortion in Al-Najaf City/Iraq. J Pharm Sci Res. 2018;10(1):110-13.