Comparison between the prevalences of herpes simplex virus type 1 and 2 among Libyan women with spontaneous abortion in Ajdabiya city

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مقارنة بين معدل انتشار فيروس الهربس البسيط من النوع الأول والثاني بين النساء الليبيات اللاتي يعانين من الإجهاض التلقائي في مدينة أجدابيا

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Abstract:
Herpes simplex virus (HSV) is a significant human pathogen that causes various infections such as genital herpes. Infection during pregnancy poses a significant risk to pregnant women, primarily affecting outcomes such as miscarriage. High seroprevalence of HSV-1 and HSV-2 is observed globally, with significant differences across countries. So aims of this study to compare between infections of two types of herpes simplex virus among women with unexplained spontaneous abortion in Ajdabiya city, eastern Libya, in terms of prevalence, severity, immune responses, and assess related risk factors critical to better managing the infection.
Methods: Blood samples were collected from 60 Libyan women with unexplained spontaneous abortion aged 19 to 48 years, attributed to the central hospital of Ajdabiya, Serum was separated then analysed using HSV 1-IgG/IgM and HSV 2-IgG/IgM, a lateral flow chromatographic immunoassay. The results obtained were processed in SPSS Version 25.
Results of 23.3% and 35% of total cases were seropositive for IgM of HSV1 and HSV2, respectively, while 83.3% and 6.7% were seropositive for IgG of HSV1 and HSV2, respectively. There was a weak positive correlation between the occurrence of acute infections of HSV1 and HSV2 (P=0.048, R=0.258), while there was no significant correlation between past infections of HSV1 and HSV2 (P=0.363).
Conclusion: Herpes simplex virus type 2 is more prevalent than type 1 , suggesting that HSV-2 is more severe than HSV-1. A significant low seropositivity of HSV-2 IgG was found, in contrast to HSV-1, suggesting that HSV-2 is better able to evade the immune system than HSV-1. This highlights the need for informing women and their partners about herpes transmission, early symptoms, and preventive measures.

Keywords: Comparison, HSV-1, HSV-2, spontaneous abortion, Prevalence.

الملخص:
فيروس الهربس البسيط (*HSV*) هو مُمْرِضٌ بشريٌّ خطيرٌ يُسبِّبُ عدوى مُختلفة، مثل الهربس التناسلي. تُشكِّل العدوى أثناء الحمل خطرًا كبيرًا على النساء الحوامل، ويؤثر في المقام الأول على مخرجات الحمل مثل الإجهاض. يُلاحَظُ انتشارٌ مُصْلِيّ مرتفعٌ لفيروسي الهربس البسيط من النوعين 1 و2 عالميًا، مع وجود اختلافاتٍ كبيرةٍ بين البلدان. لذا، تهدف هذه الدراسة إلى مُقارنةِ حالاتِ الإصابةِ بنوعين من فيروس الهربس البسيط بين النساءِ اللاتي يُعانين من إجهاضٍ تلقائيٍّ غير مُفسَّرٍ في مدينة أجدابيا، شرق ليبيا، من حيثِ الانتشارِ والشدةِ والاستجاباتِ المناعية، وتقييمِ عواملِ الخطرِ ذاتِ الصلةِ الحاسمةِ لإدارةِ العدوى بشكلٍ أفضل.
الطريقة: جُمعت عينات دم من 60 امرأة ليبية مصابات بإجهاض تلقائي غير مُفسَّر، تتراوح أعمارهن بين 19 و48 عامًا، من المستشفى المركزي بأجدابيا. فُصلت عينات المصل ثم حُلِّلت باستخدام اختباري *HSV-1-IgG/IgM* و*HSV-2-IgG/IgM*، وهو اختبار مناعي كروماتوغرافي ذو تدفق جانبي.
عولجت النتائج باستخدام برنامج *SPSS* . كانت نتائج 23.3% و35% من إجمالي الحالات إيجابية مصليًا لـ *IgM* من *HSV1* و*HSV2*، على التوالي، بينما كانت نتائج 83.3% و6.7% إيجابية مصليًا لـ *IgG* من *HSV1* و*HSV2*، على التوالي. كان هناك ارتباط إيجابي ضعيف بين حدوث الإصابات الحادة بفيروسي الهربس البسيط من النوعين 1 و2 (*P=0.048*، *R=0.258*)، بينما لم يكن هناك ارتباط يُذكر بين الإصابات السابقة بفيروسي الهربس البسيط من النوع 2 و1 (*P=0.363*).
الاستنتاج: يُعد فيروس الهربس البسيط من النوع 2 أكثر انتشارًا من النوع 1، مما يشير إلى أن فيروس الهربس البسيط من النوع 2 أشد من فيروس الهربس البسيط من النوع 1. وُجدت إيجابية مصلية منخفضة بشكل ملحوظ لأجسام *IgG* الخاصة بفيروس الهربس البسيط من النوع 2، على عكس فيروس الهربس البسيط من النوع 1، مما يشير إلى أن فيروس الهربس البسيط من النوع 2 أكثر قدرة على التهرب من الجهاز المناعي من فيروس الهربس البسيط من النوع 1. وهذا يُبرز الحاجة إلى توعية النساء وشركائهن حول انتقال الهربس والأعراض المبكرة والإجراءات الوقائية.

الكلمات المفتاحية: الإجهاض التلقائي، الانتشار، الهربس البسيط1، الهربس البسيط 2، مقارنة.

1.Introduction

Herpes simplex virus (HSV) is a significant human pathogen that causes many infections, such as oral and genital herpes, ocular infections, and may develop into serious complications such as encephalitis. [1][2] Infection with the virus during pregnancy can lead to serious complications for pregnant women such as spontaneous abortion [3]. There are two main types of herpes simplex virus: type 1 (HSV-1) and type 2 (HSV-2). Both cause latent infections, where the virus remains dormant in the ganglia and can reactivate and cause recurrent infections. [2][4] (HSV-1) typically infects the oral cavity and remains latent in the ganglia of the trigeminal facial nerve, while (HSV-2) primarily infects the genitals and remains latent in the ganglia of the lumbosacral nerves in the lower back. Although both HSV-1 and HSV-2 can infect either the oral or genital regions [5] The impact of herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) on miscarriage has been studied in various studies, suggesting a possible link between these viral infections and increased rates of spontaneous abortion. Both types may contribute to adverse obstetric outcomes, but their effects differ in frequency and severity. Herpes simplex virus type 2 is frequently associated with recurrent spontaneous abortion (RSA). [6][7] Herpes simplex virus type 1 and 2 infections have been found to be associated with increased rates of miscarriage in women, especially during the first trimester of pregnancy.[8][9]) In a previous study, herpes simplex virus type 2 was detected in placental tissue of women who had experienced spontaneous abortion, revealing potential pathogenic mechanisms.[10] On the other hand, there is limited data on HSV-1 in the context of abortion, with a lower prevalence, while studies focus more on HSV-2 and other viruses such as parvovirus B19 and cytomegalovirus. [6][8] The two types have differing transmission routes, immune responses, and clinical manifestations. Understanding the differences between herpes simplex virus types and how and when they are transmitted is critical to better managing the infection.

1.1. Transmission

HSV-1 is primarily transmitted in early childhood through non-sexual contact, while HSV-2 is primarily transmitted through sexual contact and is the leading cause of genital herpes.[11] Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) show preferred sites of infection in the body. HSV-1 typically infects the oral cavity and is associated with cold sores or fever blisters. It latent in the facial trigeminal ganglia. While herpes simplex virus type 2 (HSV-2) primarily infects the genitals and is associated with genital herpes, it latent in the lumbosacral nodes in the lower back. Although each type has a preferred location, both can cause infections in the oral or genital areas. At the beginning of herpes virus attachment to cells, both types bind to the same receptors on cell surfaces, such as heparan sulphate receptors, but they differ in their ability to compete for these receptors. [5] HSV may compete for cell receptors to enter and infect them. The competition may lead to interference or co-infection, .this competition reflects and determines the severity of the virus, with the virus that binds more tightly and grows more rapidly prevailing. The stronger competitor virus prevents the other from binding, leading to interference, as the stronger competitor prevents the weaker virus from entering. In co-infection, the herpes simplex virus binds to the same receptor, and both enter the cell. HSV entry mechanisms involve complex interactions between viral glycoproteins and host cell receptors, which not only facilitate virus binding but also disrupt normal immune signalling and weaken antiviral responses [12][13][14] These competitive dynamics between HSV, its glycoproteins, and host immune regulators suggest strategies for the virus's survival and prevalence.[15] Herpes simplex virus (HSV) can be transmitted to the foetus during birth when it passes through the birth canal or during vaginal delivery. In very rare cases, the virus can cross the placenta and infect the developing foetus, which can lead to serious complications. On the other hand, herpes simplex virus type 2 can cause recurrent infections in the genital area, which may affect the embryo's attachment to the placenta, potentially leading to serious complications such as miscarriage.[16] Interaction between HSV-1 and HSV-2 has been observed in different populations, with specific trends among populations at lower risk of coinfection.[17] There is evidence suggesting that herpes simplex virus type 1 (HSV-1) and (HSV-2) can recombine or exchange genetic material, resulting in new strains with different tropisms, which hinders the development of candidate vaccines and causes ongoing frustration.[19][18] The annual incidence of herpes simplex virus type 1 and type 2 infection among sexually active adults is 1.6 and 5.1 cases per 100 people, respectively. [20] Co-infection with HIV-1 and HSV-2 heightens the risk of HIV-1 transmission, but antiviral therapy to manage HSV-2 can reduce this risk. [21] The risk of transmitting the herpes virus to the foetus or neonate increases during the second half of pregnancy, which can lead to serious outcomes. [23][22] The differentiation between herpes simplex virus type 1 (HSV-1) and HSV-2 in causing genital lesions is significant, as HSV-2 is primarily associated with this infection. There are studies that show co-infection with HSV-2 leads to an increase in recurrence rates of genital lesions, suggesting that HSV-2 has a higher propensity to reactivate in the genital region than HSV-1 after initial infection. [24] Thus, herpes simplex virus type 2 (HSV-2) causes the majority of genital herpes cases (71%), compared to herpes simplex virus type 1 (29%), indicating a greater predilection toward genital lesions. [25] Genital HSV-1 infections tend to occur through oral-genital contact, particularly among specific demographics like men who have sex with men, but HSV-2 remains the primary type responsible for genital herpes transmission [27][26]. Although HSV-2 is more prevalent in genital herpes, the prevalence of HSV-1 in genital herpes has been observed to be significantly higher among specific population groups, suggesting that both types deserve increased attention in surveillance and prevention efforts. Whereas genital herpes type 1 is usually transmitted through oral sex and is most prevalent among homosexuals (men who have sex with men), genital herpes type 2 remains the primary type responsible for transmission [27].

1.2. Epidemiology

There are risk factors that influence the spread of herpes simplex virus types 1 (HSV-1) and 2 (HSV-2) and cause marked differences in virus prevalence across population groups and geographic regions. HSV-1 seroprevalence increases with the number of sexual partners, while HSV-2 seroprevalence is associated with riskier sexual behaviours , such as prostitution and having male partners with genital herpes.[29][28] In comparison, the prevalence of herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) across different countries reveals significant regional differences in their epidemiology. Whereas the prevalence of HSV-1 and HSV-2 is high worldwide, there are significant differences between countries, particularly in Europe and regions such as Africa and the Americas.[31][32][30] A significant difference was observed between the prevalence of HSV-1 and HSV-2 in developing and developed countries, indicating differences in transmission mechanisms and public health factors. Studies confirm that the prevalence of herpes simplex virus type 2 (HSV-2) is high in some regions, particularly developing countries, while HSV-1 causes genital infections in developed countries. Meanwhile, the prevalence of HSV-2 is highest in Africa and the Americas, with a lower prevalence in Europe and Asia.[29] The prevalence of HSV-1 as a cause of genital herpes is increasing in developed countries, which poses a challenge to the association of HSV-1 and oral herpes infection.[11] Despite conservative sexual norms, cases of herpes simplex virus type 2 infection are increasing, indicating the need for enhanced surveillance.[34] Considering demographic characteristics, prevalence rates of both viruses tend to be higher among women and increase with age [31].[30] A higher prevalence of HSV-1 infection has also been observed in lower socioeconomic backgrounds.[34]

1.3.Immune response and related diagnostic tests

Available data indicate significant differences in the serological profile of the two types of viruses, particularly with regard to their Immunoglobulin M (IgM) responses. The study emphasizes the need for type-specific serological assays, particularly highlighting the effectiveness of tests designed to differentiate between HSV-1 and HSV-2 IgM antibodies, which is essential for accurate diagnosis and treatment [36][37][35]. Studies showed significant differences in seropositivity between Immunoglobulin G (IgG) for HSV-1 and HSV-2 in terms of prevalence and responses, suggesting the importance of type-specific serology in understanding infections and guiding treatment options. The higher prevalence of HSV-1 IgG antibodies (90%) compared to HSV-2 IgG antibodies (63.1%) in asymptomatic pregnant women shows a notable difference in seropositivity rates [36]. Other studies supported the last findings, whereas they showed a significant decline in HSV-2 IgG antibody levels during pregnancy, contrasting with more stable HSV-1 IgG levels, suggesting different dynamics in immune response [38]. Serologic testing for both HSV types may aid in diagnosing viral infections associated with unexplained abortions, though HSV-2 appears to be the more prevalent concern in obstetric complications [6][39]. The newly developed dual Enzyme Immunoassay (EIA) system demonstrated high sensitivity (100%) and specificity (> 95%) for detecting HSV-specific IgG antibodies, outperforming conventional EIAs [40]. The utilization of (EIA) technologies for the diagnosis of Herpes Simplex Virus (HSV) types 1 and 2 plays a significant role in clinical settings, exhibiting various strengths and challenges in identifying type-specific antibodies. Recent studies highlight the performance, sensitivity, and specificity of different EIA systems relative to traditional methods and demonstrate that enzyme-linked immunosorbent assays can detect HSV effectively, achieving a detection rate of 94% to 100% when conditions are optimized [42][41]. The development of lateral-flow immunochromatographic assays (LFIA) for HSV-2 has been shown to provide 100% sensitivity and 97.3% specificity, ensuring timely results for better patient counselling and treatment [43].

1.4. Objectives of the study

Comparison between infections of two types of herpes simplex virus among women with unexplained spontaneous abortion in Ajdabiya city, in terms of prevalence, severity, immune responses, the relationship between them, and assessment of related risk factors for critical better management of the infection.

2.Methods

The study was conducted in the city of Ajdabiya, eastern Libya, on women with unexplained spontaneous abortion, attributed to the city's central hospital and private clinics. Their condition was followed up by a specialist doctor. A questionnaire was designed for the collection of data about these aborted women, including age, gestational age, number of miscarriages, whether the patient was suffering from risk factors for miscarriage such as uterine abnormalities, incomplete cervix, uterine cysts, uncontrolled diabetes, thyroid disorder, autoimmune diseases, hereditary diseases, and whether the patients were taking medications that could lead to miscarriage or were exposed to radiation or infection. All cases that had these conditions were excluded, and 60 cases were chosen without them. Blood samples of 5 ml were collected from the women, and the serum was separated using a centrifuge for five minutes at 5000 rotations per minute. The serum was stored in tubes free of any preservatives and frozen at -20 °C until tested. All sera samples were transferred to room temperature, then analysed by using HSV 1-IgG/IgM & HSV 2-IgG/IgM Cassette Test (serum)-based commercial kits and assayed according to the manufacturer's instructions. This test is a lateral flow chromatographic immunoassay for the qualitative detection and differentiation of antibodies (IgG and IgM) to two types of herpes simplex viruses. It is suitable for epidemiological investigation. In general, a positive IgM result indicates acute infection, and a positive IgG result only indicates past, chronic, and latent infection. The results obtained were processed in the SPSS Version 25

program to determine the prevalence of the two types of viruses, study the relationship between the two virus infections, detection of IgG and IgM antibodies seropositivity to determine the extent of the immune response to virus infections. and study other correlation between infections and age, recurrent abortion, gestational age A correlation test was used to study statistical differences under a confidence interval of 95%. A p-value of <0.05 was considered statistically significant.

3. Results

Study population included 6o cases with a maximum age 48 years, a minimum 19 years, mode 40 years, mean 34.3 and median 36.5. Although there was no significant difference between age and acute HSV1 infection according to Pearson's correlation test (P = 0.458 > 0.05), and also no significant difference between age and acute HSV-2 infection (P = 0.984 > 0.05) , However, the most common cases were four acute cases of HSV1 and other four of HSV2 at the age of 41 years.

Table 1- Age Distribution of total study population and acute infected cases with two types of herpes simplex virus

Age Distribution	HSV1 IgM		HSV2 IgM		Total cases	
	Negative	Positive	Negative	Positive	Frequency	Percent
(19-29) Years	18	4	14	8	22	36.7
(30-39) Years	11	2	10	3	13	21.7
(40-48) Years	17	8	15	10	25	41.7
Total	46	14	39	21	60	100

23.3% of total cases had acute infection of HSV1. 13.3% of them occur during the first trimester of gestation and 10% occur during the second trimester of gestation, while there were no cases infected with HSV1 during the third trimester of gestation. On the other hand, 35% of total cases had acute infection of HSV2. 31.7% of them occur during the first trimester of gestation and 3.3% occur during the second trimester of gestation, while there were no cases infected with HSV2 during the third trimester of gestation.

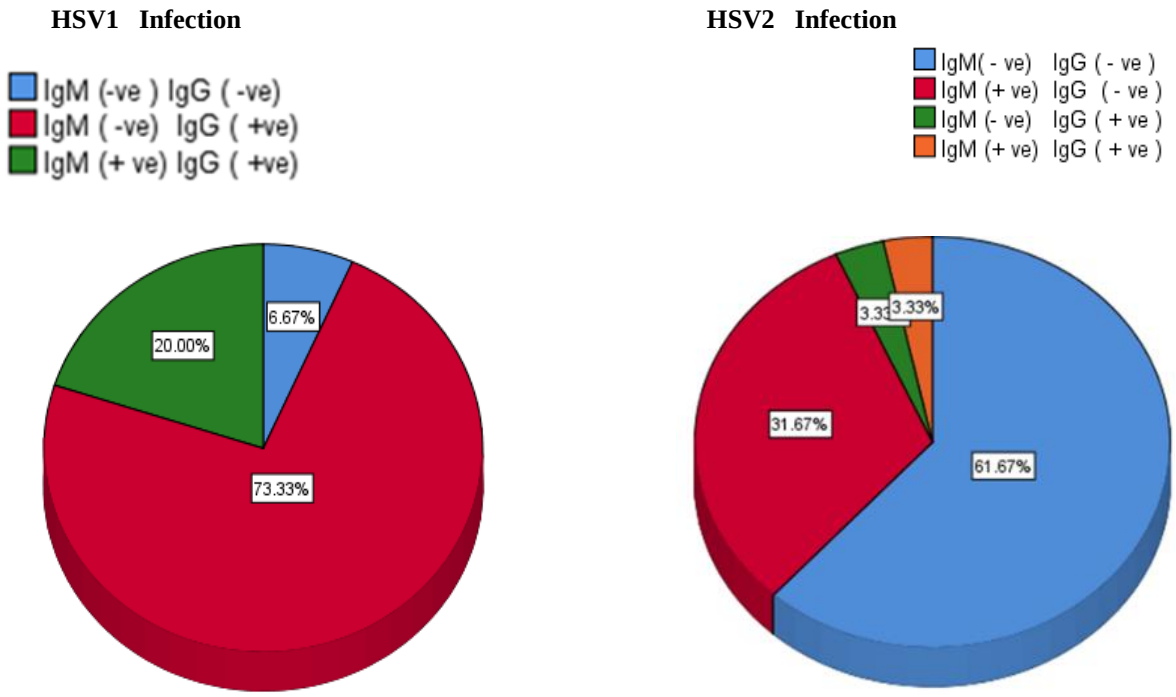

Figure .1. Seroprevalences of IgM and IgG antibodies of HSV1 & 2

Table .2. Seroprevalence of anti-IgM of HSV 1 & HSV2 according to trimesters

Percent of Trimesters		Seroprevalence of anti- IgM of HSV 1 & HSV 2			
		HSV1 IgM	Frequency	Percent	p. value
1st trimester	70.0%	Negative	34	56.6	0.048
		Positive	8	13.3	
2nd trimester	28.3%	Negative	11	18.3	
		Positive	6	10	
3rd trimester	1.7%	Negative	1	1.7	
		Positive	0	0	
Total	100%	Negative	46	76.6	
		Positive	14	23.3	
		HSV2 IgM			
1st trimester	70.0%	Negative	23	38.3	
		Positive	19	31.7	
2nd trimester	28.3%	Negative	15	25	
		Positive	2	3.3	
3rdtrimester	1.7%	Negative	1	1.7	
		Positive	0	0	
Total	100%	Negative	39	65	
		Positive	21	35	

83.3% and 6.7% of total cases were seropositive for anti-IgG HSV1 and anti-IgG HSV2, respectively. There was a weak positive correlation between the occurrence of acute infections of herpes virus type 1 and type 2 (P=0.048, R=0.258), while there was no significant correlation between chronic or latent infections of the two types of herpes simplex virus (P=0.363 > 0.05).

Table .3. Seroprevalence of anti-IgG of HSV 1 & 2

		Frequency	Percent
HSV1 IgG	Negative	10	16.7 %
	Positive	50	83.3 %
HSV2 IgG	Negative	56	93.3 %
	Positive	4	6.7 %

There was no significant difference between the occurrence of a first-time miscarriage and recurrent miscarriage in both cases of herpes simplex virus infection, types one and two, according to the correlation test. P-values were (0.460 > 0.05) and (0.541 > 0.05) during the acute infection of HSV1 and HSV2, respectively.

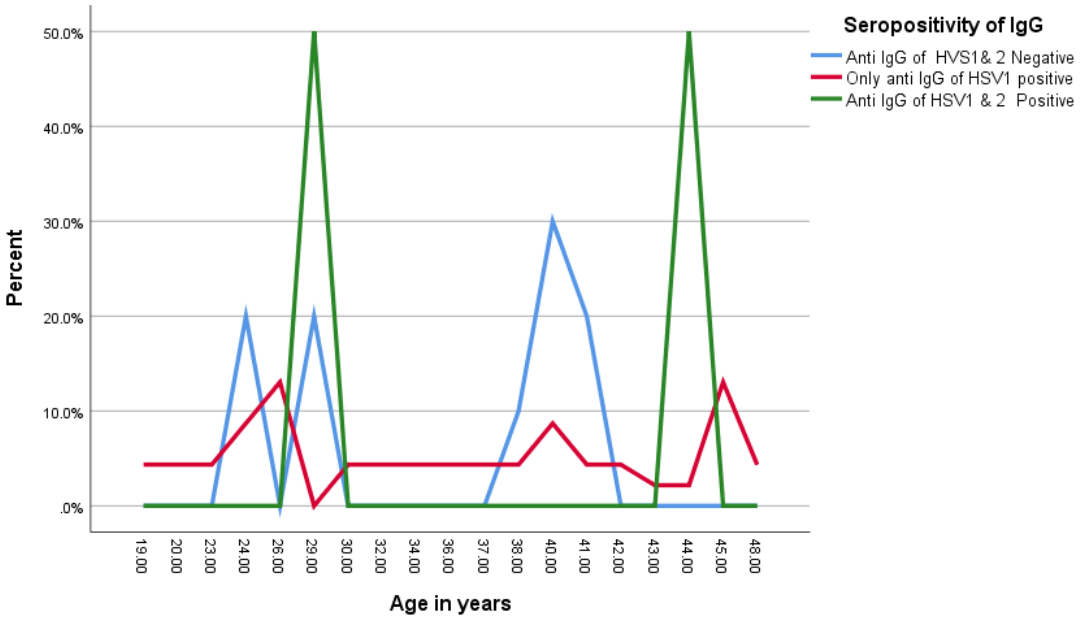

Figure .2. Seroprevalences of IgG antibodies of HSV1 & 2 according to age

Table .4. Seroprevalence of anti-IgM of HSV 1 & 2 according to number of mischarge.

Acute infection	Number of miscarriages	Seroprevalence of anti-IgM		P. value
		Negative	Positive	
HSV1	First Abortion	80.6 % (25/31)	19.5% (6/31)	0.460
	Recurrent Abortion	72.4 % (21/29)	27.6 % (8/29)	
HSV2	First Abortion	61.3 % (19/31)	38.7 % (12/31)	0.541
	Recurrent Abortion	69% (20/29)	31% (9/29)	

4.Discussion

The results of the current study show that herpes simplex virus type 2 is more prevalent than type 1 among women who have had unexplained spontaneous abortions in the city of Ajdabiya, eastern Libya. Our findings agree with previous studies conducted in Africa [28] ; these findings reflect the difference between the two types of virus in terms of severity, mechanisms of virus transmission, immune responses, and the public health factors of the study population. On the other hand, it became clear that age does not play a role in the prevalence of either type 1 or type 2 viruses,

whether the infection is recent or chronic, suggesting that other factors play a role in these prevalences. The prevalence of HSV-2 suggests the virus's strong tropism for the genital region, as well as its ability for reactivation and coinfection in this region. The frequency of HSV-2 is greater than that of HSV-1, suggesting HSV-2 is a strong competitor, may compete with HSV-1 for cell receptors to enter and infect them, lead interference, and block the entry of HSV-1. This competition reflects the virulence of the virus and illustrates its evolutionary adaptations, which enhance its infectious potential and ability to evade immune detection. The researcher monitored anti-IgG HSV-2 in the study population and found low seropositivity of HSV-2 IgG (6.7%), in contrast to anti-IgG HSV-1 (83.3%) ., this difference suggests a more subtle mechanism of the immune response to infection and that HSV-2 is better able to evade the immune system than HSV-1. Our study agrees with a previous study [38]. On the other hand, the prevalence of HSV-2 infection was more common in the first trimester of pregnancy, reflecting its virulence for causing of miscarriage, its impact on the foetus during this period of gestation, which is when organogenesis occurs. As for herpes simplex virus type 1, the prevalence of herpes simplex virus type 1 infection in the first and second trimesters of pregnancy was almost similar, and it is likely that the mother was infected accidentally, by direct contact with infected people or a contaminated environment. However, in very rare instances, the virus can cross the placenta and infect the foetus, and HSV-1 is rarely acquired through sexual contact, especially in areas where homosexuality is not widespread. Our findings showed that there is no significant difference between infection of HSV-2 in cases of first miscarriage and recurrent miscarriage; this may indicate a low reactivation of type 2 virus infection. The findings of a previous study are consistent with our findings that HSV-2 is more prevalent in communities with a lower risk of reinfection [19].

5. Conclusions and recommendations

Herpes simplex virus type 2 is more prevalent than type 1 among women who had unexplained spontaneous abortions in the city of Ajdabiya, eastern Libya, suggesting HSV-2 is more severe than HSV-1 and found a significantly low seropositivity of HSV-2 IgG in contrast to HSV-1. This suggests HSV-2 is better able to evade the immune system than HSV-1, highlighting the need for awareness and screening among pregnant women to reduce the risk of fetal transmission and prevent miscarriage. Abstaining from sexual contact during an active herpes outbreak reduces the risk of transmission. For any infected parents, daily antiviral medications, such as acyclovir, can reduce viral shedding. This can reduce the spread of the virus and decrease the likelihood of transmission by informing women and their partners about herpes transmission, early symptoms, and preventive measures. On the other hand, the prevalence of herpes simplex virus IgM-1 infection among pregnant women was found to be high, and generally, HSV-1 and HSV-2 infections are responsible for spontaneous abortion, highlighting the need for regular screening of women of reproductive age.

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