Seroprevalence of Herpes Simplex Virus Type 2 (HSV-2) in Pregnant Women and its Relation to Some Blood Cells and IL-2 in Kirkuk, Iraq

Abdulla Kamil Abdulla

Correspondence:
Abdulla Kamil Abdulla
M.Sc. Medical Microbiology Medicine College
Tikrit University, Erbil Health Directorate, Iraq
Email: abdullakurdistan@yahoo.com

ABSTRACT

Background: The HSV-2, is a widespread viral pathogen. It has been described as an important etiological agent in uterus and during the intrapartum period in pregnant women.

Objectives: Estimate the prevalence of HSV-2 antibodies among pregnant women in Kirkuk city.
Patients and Methods: A cross sectional study (M.Sc. Thesis) was conducted in Kirkuk city and included 176 pregnant women, and 134 non-pregnant married women (control group) who attended at Azadi Teaching Hospital and Al Ta'akhi Health Care Center from the 20th of November 2012 to the 23rd of April 2013.

Results: The study revealed that the 62.48 % of pregnant women were infected with HSV-2. The highest rate of IgM antibodies was found in 50% of pregnant women aged 18-23; this was also true for both IgM and IgG antibodies together that were found in 41.17% of women. The relation of seropositive HSV-2 antibodies with the total white blood cells (W.B.Cs) count showed a non-significant result with the probability (P) value >0.05. This was also true for the relation with absolute lymphocyte count (ALC), while its relation with absolute eosinophil count (AEC) showed a significant result, P <0.05. In regards to the relation of HSV-2 antibodies with serum interleukin-2 (IL-2), the result was non-significant. The relation with abortion number was significant. There was significant relation of abortion with gestational time of pregnancy in seropositive pregnant women.

Conclusion: The seroprevalence of HSV-2 was relatively high in pregnant women in Kirkuk city. Primary and re-infection of latency occurred at the highest rate in age group 18-23 years old. Primary HSV-2 infection increases the AEC and IL-2 during pregnancy. The highest rate of abortion occurred during the first trimester of pregnancy in women with HSV-2.

Key words: HSV-2, Iraq.

Introduction

The HSV-2, is a widespread viral pathogen. It has been described as an important etiological agent in uterus and during the intrapartum period in pregnant women. The HSV-2 infection has been found to be a sexually transmitted disease affecting most commonly, individuals who are in their adolescence or young adulthood[1,2].

The HSV-2 belongs to the Herpesviridae family. The virion particle is spherical 150-200 nanometer (nm) in diameter [3,4], with four structural elements; an electron opaque core, a protein capsid, surrounding the virus core comprising 162 capsomeres, an amorphous tegument surrounding the capsid, and an outer envelope with spikes on its surface. The core is composed of linear dsDNA [5,6].

The primary route of acquisition of HSV-2 infections is through genital sexual contact with an infected partner who is shedding the virus symptomatically or asymptomatically [7]. The HSV-2 infection is more common in women than men[8].

Neonatal HSV-2 infection is acquired from the mother during vaginal delivery [9]. The chances of the baby becoming infected increase if there is an outbreak at the time she delivers the infant[10]. The risk of transmission of HSV-2 during primary infection in the third trimester of pregnancy to the infant is estimated to be 30%-50%[11]. Intrauterine and postnatal transmissions are rare[12]. The HSV-2 virus is also associated with a higher rate of miscarriages than normal[13]. Latency of the virus is in sacral nerve ganglia[14]. The HSV-2 can reactivate upon stress[15].

Direct detection of viral DNA by liquid or in situ hybridization, and after, by the polymerase chain reaction (PCR), are considerably more sensitive[16]. Enzyme-linked immunosorbent assay (ELISA) can be used to detect immunoglobulin M and G (IgM and IgG respectively) in serum[17].

Acyclovir is selectively effective against HSV-2. Other drugs effective in treating HSV-2 infection include famciclovir and topical Penciclovir[16].

Objectives: Estimate the prevalence of HSV-2 antibodies among pregnant women in Kirkuk city, and its relation to some blood cells and IL-2.

Materials and Methods

Study Population:

A cross sectional study [M.Sc. thesis] conducted in Kirkuk city included 176 pregnant women, and 134 non-pregnant married women (control group) attending Azadi Teaching Hospital and Al Ta'akhi Health Care Center from the 20th of November 2012 to the 23rd of April 2013 and aged (18-40) years old. A blood sample of 7.5 ml was drawn from each patient and separated into two parts; one part 5 ml was with no Ethylene Diamine Tetraacetic Acid (EDTA) anticoagulant used for detection of anti-HSV-2 IgM, IgG antibodies, and serum IL-2 using Enzyme Linked-Immunosorbant assay (ELISA) technique, and the other part 2.5 ml was with EDTA for detection of blood cells using specialized fully automated hematological analyzer machine (CELL-DYN RUBY).

Detection of anti-HSV-2 IgM and IgG antibodies

Enzyme Immunoassay for Detection of IgM antibodies to HSV-2 in Human serum From BioCheck, Inc 323 Vintage Park Dr. Foster City, CA 94404.

Purified HSV-2 antigen is coated on the surface of microwells. Diluted patient serum is added to the wells, and the HSV-2 IgM-specific antibody, if present, binds to the antigen. All unbound materials are washed away. Horse radish peroxidase (HRP-conjugate) is added, which binds to the antibody-antigen complex. Excess HRP-Conjugate is washed off and a solution of Tetramethyl Benzidine (TMB) reagent is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of HSV-2 IgM-specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and control.

Samples: Serum (stored at -20 °C).

Enzyme Immunoassay for Detection of IgG Antibodies to HSV-2 in Human Serum. From BioCheck, Inc 323 Vintage Park Dr. Foster City, CA 94404.

Purified HSV-2 antigen is coated on the surface of microwells. Diluted patient serum is added to the wells, and the HSV-2 IgG-specific antibody, if present, binds to the antigen. All unbound materials are washed away. HRP-conjugate is added, which binds to the antibody-antigen complex. Excess HRP-conjugate is washed off and a solution of TMB reagent is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of HSV-2 IgG-specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

Samples: Serum (stored at -20 °C).

Detection of Human IL-2 in Human Serum

Enzyme Immunoassay for Detection of Human IL-2 in Human Serum. From Biolegend Inc. Pacific Heights Blvd. San diego, CA 92121

Human IL-2 EIA Kit is a sandwich enzyme immunoassay (EIA) with a 96-well strip plate that is pre-coated with a capture antibody. This kit is specifically designed for the accurate quantization of human IL-2 from cell culture supernatant, serum, plasma, and other biological fluids. This kit is analytically validated with ready-to-use reagents.

Samples: Serum (stored at -20 °C).

Detection of Blood Cells

The CELL-DYN Ruby uses flow cytometric techniques to analyze the RBC/PLA, WBC, and nuclear optical count (NOC) populations. Flow cytometry is a process in which individual cells or other biological particles in a single file produced by a fluid stream are passed through a beam of light. A sensor or sensors measure, by the loss or scattering of light, the physical or chemical characteristics of the cells or particles.

Samples: EDTA anticoagulant treated vein's whole blood.

Statistical Analysis

Computerized statistical analysis was performed using Mintab version 11 statistic program. Comparison was carried out using; Chi-square ($X2$), and probability (P value). The P value < 0.05 was considered statistically significant, and for results where its P value was less than 0.01 was considered highly significant, while for those which its P value was greater than 0.05 was considered non-significant statistically.

Results

Detection of anti-HSV-2 IgM and IgG antibodies

The current findings revealed that anti-HSV-2-IgM was found in 7.95 % of pregnant women, anti-HSV-2-IgG in 35.22 % and both IgM and IgG at the same time in 19.31 %, while 37.3 % of them had neither IgM nor IgG against HSV-2. Regarding the control group, the rate of IgM, IgG, and both IgM and IgG (at the same time) was 11.19%, 22.38 % and 8.2 % respectively. However 58.2 % were negative for both IgM and IgG. The result was highly significant (Table 1).

The highest rate (50 %) of HSV-2- IgM antibodies was found in pregnant women aged 18-23 years, while the highest rate (33.87 %) of HSV-2- IgG antibodies was found in those aged 24-29 years. Also the highest rate (41.17 %) of HSV-2- IgM &IgG together was found in the age group 18-23 years. The result was non-significant (Table 2).

Detection of Blood Cells

Detection of total W.B.Cs

The highest rate (35.71%) of increased W.B.Cs counts was seen with seropositive HSV-2-IgM antibodies. The result was non-significant (Table 3).

Detection of ALC

The highest rate (29.41 %) of increased to ALC was found with HSV-2 (IgM & IgG) antibodies. The result was non-significant (Table 4)

Table 1: Summary of the HSV-2 Antibodies Seroprevalence in Pregnant Women and Control Group 1 (Non-Pregnant Married Women)

Seroprevalence of HSV-2 Antibodies	Seroprevalence of HSV-2 Antibodies			
	Pregnant Women		Control Group[1]	
	No.	%	No.	%
IgM (+) and IgG (-)	14	7.95	15	11.19
IgM (+) and IgG (+)	34	19.31	11	8.20
IgM (-) and IgG (+)	62	35.22	30	22.38
IgM (-) and IgG (-)	66	37.5	78	58.20
Total	176	100	134	100
X^2=18.571	P= 0.0002	P < 0.01	Highly significant (Hs)	

Table 2: Relation of Seropositive HSV-2 Antibodies to Age Groups of pregnant Women

Age Groups (Years)	Seropositive HSV-2 Antibodies					
	HSV-2- IgM		HSV-2 - IgG		HSV-2 (IgM & IgG)	
	No.	%	No.	%	No.	%
18-23	7	50	18	29.03	14	41.17
24-29	3	21.42	21	33.87	10	29.41
30-35	3	21.42	16	25.80	7	20.59
36-40	1	7.14	7	11.29	3	8.82
Total	14	100	62	100	34	100
X^2 = 3.029	P = 0.992	P > 0.05	Non significant(Ns)			

Table 3: Relation of Seropositive HSV-2 Antibodies with the Total W.B.C.s Counts

Total W.B.C.s Count	Seropositive HSV-2 Antibodies					
	HSV-2- IgM		HSV-2- IgG		HSV(IgM & IgG)	
	No.	%	No.	%	No.	%
Normal *	8	57.14	52	83.87	22	64.71
Increased**	5	35.71	8	12.90	11	32.35
Decreased***	1	7.14	2	3.23	1	2.94
Total	14	100	62	100	34	100
X^2 = 7.508	P = 0.512	P>0.05	Ns			

Table 4: Relation of Seropositive HSV-2 Antibodies with Peripheral ALC

Peripheral ALC	Seropositive HSV-2 Antibodies					
	HSV-2- IgM		HSV-2- IgG		HSV-2 (IgM & IgG)	
	No.	%	No.	%	No.	%
Normal*	8	57.14	46	74.19	23	67.65
Increased**	4	28.57	12	19.35	10	29.41
Decreased***	2	14.29	4	6.45	1	2.94
Total	14	100	62	100	34	100
X^2 = 3.625		P = 0.471		P>0.05		Ns

Table 5: Relation of Seropositive HSV-2 Antibodies with Peripheral AEC

Peripheral AEC	Seropositive HSV-2 Antibodies					
	HSV-2- IgM		HSV-2- IgG		HSV-2(IgM & IgG)	
	No.	%	No.	%	No.	%
Normal *	8	57.15	57	91.94	32	94.12
Increased**	4	28.58	5	8.07	1	2.95
Decreased***	2	14.29	0	0	1	2.95
Total	14	100	62	100	34	100
X^2 = 17.650		P = 0.014		P <0.05		Significant

Table 6: Relation of Seropositive HSV-2 Antibodies with Serum IL-2 Levels

IL-2 Levels	Seropositive HSV-2 Antibodies					
	HSV-2- IgM		HSV-2- IgG		HSV-2 (IgM & IgG)	
	No.	%	No.	%	No.	%
Normal *	2	18.19	6	23.08	3	23.08
Increased**	9	81.82	20	76.93	10	76.93
Total	11	100	26	100	13	100
X^2 = 0.120		P= 0.942		P>0.05		NS

Table 7: Relation of Seropositive HSV-2 Antibodies in Pregnancy with History of Abortion and Frequency of Abortion

Total Number of HSV-2 Antibodies Seropositive Pregnant Women	History of Abortion							
	No abortion		One abortion		Two abortions		Three abortions or more	
	No.	%	No.	%	No.	%	No.	%
110	83	75.45	19	17.27	4	3.64	4	3.64
			27 (24.55 %)					
Abortion	$X^2 = 9.444$		P= 0.031	P<0.5	Significant			
Abortion freq.	$X^2 = 5.840$		P= 0.082	P>0.05	Ns			

Table 8: Relation of Abortions with Gestational Time in Pregnant Women with Seropositive HSV-2 Antibodies

Total Number of Aborted Pregnant Women	Gestational Time of Pregnancy					
	1st trimester		2nd trimester		3rd trimester	
	No.	%	No.	%	No.	%
27	21	77.78	5	18.52	1	3.70
$X^2 = 8.424$	P = 0.026		P<0.05		S	

Detection of AEC

The highest rate (28.58%) of increased AEC was found with HSV-2-IgM antibodies. The result was significant (Table 5).

Detection of serum IL-2

The highest rate of increased IL-2 level was found in all types of the HSV-2 antibodies and as following: 81.82 %, 76.93 %, and 76.93 % for HSV-2-IgM, HSV-2-IgG, and HSV-2 (IgM & IgG) respectively. The result was non-significant (Table 6).

Relation of Anti-HSV-2 Antibodies to History of Abortion, and Frequency of Abortion

The total rate of abortion was (24.55 %) out of a total 110 seropositive pregnant women. The rate of abortion number was 17.27 % for one abortion and 3.64 % for each of two and three abortions or more. The results were significant for abortion, and non-significant for frequency of abortion (Table 7).

Relation of Abortion with Gestational Time of Pregnancy in Pregnant Women with Seropositive HSV-2 Antibodies

The highest rate (77.78 %) of abortion was found in the 1st trimester, while the lowest rate was found in the 3rd trimester. The result was significant (Table 8).

Discussion

The HSV-2 is the leading cause of genital ulcer disease worldwide. The virus can be transmitted to neonates[18]. Maternal-fetal transmission of HSV-2, which is frequently asymptomatic, can cause severe and permanent neurological damage to the neonate[19]. The prenatal form of the infection in newborns can be observed when, a neonate passes through the infected birth canal. Contamination of the neonate with this condition may cause meningitis with a serious complication[20]. Furthermore and according to our information, no such study of similarity has been published regarding pregnant women with HSV-2 infection in Iraq.

In the present study, the HSV-2 infection was relatively common among pregnant women. ELISA method was used as a serological method for detection of seropositive HSV-2 antibodies, and then the results were classified according to seropositive HSV-2 antibodies type to: HSV-2-IgM represented the acute state of (primary) infection, HSV-2-IgG represented the past (chronic) infection, and both HSV-2-IgM & HSV-2-IgG at the same time represented the re-infection or reactivation of latent infection[21].

The rate of seropositive anti-HSV-2-IgM antibodies obtained by the current study was similar to that obtained from other Iraqi cities like Baghdad (8.1 %) and Waset province (7.7 %), but slightly lower than that recorded in Mosul (10 %)[22,23,24]. In Turkey, it was 8.2% which is close to our

findings too, but in another study in Turkey, it was 11.2 % which is slightly higher than that recorded by the current findings[25]. These variations in results may be attributed to the fact that different ELISA kits used in the other studies from different companies may be with different reagents qualities and properties. Other factors which may also be attributed to the differences are steps, and techniques used by the investigators. While our finding was higher than that recorded in Saudi Arabia 0.5 %[26], this may be due to the lack of a nationwide screening program in our country to control the infection, which maybe Saudi Arabia has. Results obtained by the current study were largely lower than that reported in northern India (33.5 %), [27], in which most people from this area are known to have a very low living standard, this high rate may be an indication that the HSV-2 infection may be endemic in this area.

Regarding the anti-HSV-2-IgG antibodies rates; the present study revealed that, the rate of anti-HSV-2-IgG antibodies was 35.22 % of the pregnant women. This result was similar to those reported in Waset province (31.3 %), Tanzania (33 %) and Sweden (34 %)[23][28,29]. While the rate was lower than that recorded in Turkey (63.1 %), Iran (43.75 %), and Uganda (86 %)[25][29,30]. This may be related to different cultural factors and different socioeconomic factors too. In addition it may be associated with co-infection of HSV-2 with other viruses infections that enhance the transmission and increase the prevalence of HSV-2, especially HIV which has the same route of transmission and is present at high rates in these areas and may be endemic. The current findings were higher than that recorded In Japan (7 %), Italy (7.6 %), USA (22 %), and Germany (18 %)[28][31,32]. This may be attributed to the fact that these countries are considered as developed countries, and may have good nationwide surveillance programs to control the infection. The HSV-2 infection has a high prevalence rate in pregnant women in developing countries, especially those with a high rate of HIV prevalence[33].

The rate of both anti-HSV-2 (IgM & IgG at the same time) antibodies in pregnant women in the present study was 19.31 %. This was higher than that recorded in India (2.9 %)[34,35]. This may be due to the fact that in India, a safety program may have been developed for pregnant women to protect them from HSV-2 infection by following some special criteria like examining those mothers who got primary infection in the past and were at great risk of reactivation during pregnancy. Primary infection with HSV-2 acquired by women during pregnancy accounts for a half of the morbidity and mortality from HSV-2 among neonates, and the other half results from reactivation of old infection. (24) Since there are physiological changes during pregnancy that might affect the hormone levels; hormones like progesterone for instance may increase the susceptibility and decrease the immune response to genital herpes infection[36].

In the current study the highest rate (50 %) of seropositive anti-HSV-2-IgM antibodies was found in pregnant women aged 18-23 years. This was also true for the seropositive anti-HSV-2 (IgM &IgG) antibodies which were 41.17 % in pregnant women aged 18-23 years (as shown in Table 2). Age is one of the determinant factors associated with the prevalence of HSV-2[37]. Ashley, et al, [38], said that the acquisition of primary infection of HSV-2 increases in earlier ages, less than the third decade of life. This also agrees with Sen, et al [27]. The reason may be due to the fact that most pregnancies occur at this age. In addition to that, this age group may have more contact with infected persons.

Data obtained by the current work revealed that the re-infection and reactivation had also occurred at a highest rate in age group 18-23 years. This may be associated with some factors like stress, hormonal changes, especially most of these women have married recently, so once they got married and pregnant a lot of physiological changes may be happening in their bodies which make them more vulnerable to the infection. The highest rate of anti-HSV-2-IgG antibodies was 33.87 %, which was lower than that recorded in Colombia (64.3 %), and in Thailand (36.8 %)[39]. This may be due to socio-demographic reasons, and most women in these areas might have been infected with the virus at younger ages. Although the age was the determinant factor influencing HSV-2 seroprevalence, the results were non-significant (P > 0.05), in correlation with age groups which was disagreed with by Smith, et al [40]. This is may be due to low differences in demographic distribution of the virus in our society compared to the other countries.

Regarding the total W.B.Cs count; the current study agrees with Lakhan, et al, [41] who found normal W.B.Cs count in HSV-2 positive patients. On the other hand, Navaneethan, et al, [42] recorded a higher rate of decreased W.B.Cs in seropositive HSV-2 pregnant women. These differences may be due to the fact that pregnant women are particularly susceptible as immunological changes during pregnancy suppress T-cell mediated immunity promoting disseminated infection like HSV-2 hepatitis.

The current study showed that the highest rate of normal ALC was found with all types of the anti-HSV-2 antibodies, while the highest rate of increased ALC was found with seropositive anti-HSV-2 (IgM & IgG together) antibodies. The HSV-2 is considered one of the infectious agents that lead to lymphocytosis and increase in the peripheral ALC[43,44]. Although lymphocytes increased during HSV-2 infections in pregnant women, the result was non-significant (P > 0.05) (as shown in Table 4). This may be due to the differences in kinetic responses of the lymphocyte cells in these pregnant women. In addition, these pregnant women may have other viral infections or hematological conditions that affect the response of the lymphocytes. These findings agree with Koelle, et al, [45] who recorded non-significant (P > 0.05) results regarding the relation of HSV-2 infection with lymphocytes count.

The present study showed that the highest rate of normal AEC was found with all types of the anti-HSV-2 antibodies, while the highest rate of increased AEC was found in pregnant women who were seropositive for anti-HSV-2-IgM antibodies. The result was significant (P < 0.05) (as shown in Table 5).

The HSV-2 infection is associated with eosinophilia[46]. This may be attributed to the fact that HSV-2 causes severe itching when infecting immune-compromised individuals such as pregnant women leading to increase in the eosinophils count. This finding agrees with Tarkkanen, et al[47], who recorded high eosinophil count in relation with seropositive anti-HSV-2 antibodies. Eosinophilia may be associated with HSV-2 infections because of its effects on the skin causing rashes, on the eyes, on the genitalia and so on[48].

The IL-2 is a cytokine secreted by Th1 cells[49]. Although the current study showed that the highest rate of increased IL-2 was found with all types of the anti-HSV-2 antibodies, there were non-significant ($P > 0.05$) differences in the results in regards to the relation of IL-2 levels with the seropositive anti-HSV-2 antibodies in the pregnant women (as shown in Table 6). This agrees with Rushbrook, et al[50], who said that there was no relation between IL-2 levels and HSV-2 severity. In other studies using whole HSV antigen, adults who had a better IFN- response during genital HSV infection had a longer interval to recurrence, and recurrences have been associated with decreased IL-2 production induced by HSV antigen[51]. These differences in the results may be due to the differences in the ability of HSV-2 to switch the Th2 cells to Th1, the latter which are responsible for secreting of IL-2, in pregnant women. Besides, these women may have had other infections that led to the changes in IL-2 secretion, and as a result these infections led to the differences in the results that have been noted above.

The acquisition of genital herpes during pregnancy has been associated with spontaneous abortion, prematurity, and congenital and neonatal herpes[52]. The present study showed that the highest rate of seropositive anti-HSV-2 antibodies was found in pregnant women who have had no history of abortion, followed by those who had a history of one abortion and showed significant ($P < 0.05$) results in regards to the history of abortion with seropositive anti-HSV-2 antibodies (as shown in Table 7). This agrees with Kim, et al [53], who recorded significant difference in regards to HSV-2 infections with a history of abortion.

Regarding the frequency and recurrent abortion due to HSV-2 infection in pregnant women; the current study showed non-significant ($P > 0.05$) difference in number of abortions among single, twice, and three times or more of abortion frequency (as shown in Table 7). This agrees with Jasim, et al[23] who observed a non-significant relation in regards to abortion frequency and seropositive anti-HSV-2 antibodies. These findings point to that acute infection or reactivation of latent infection of HSV-2 that may occur as a result of immune suppression or certain physiological changes in the body during pregnancy.

Furthermore to have a safe pregnancy there has to be a switch from Th1 to Th2 and not the other way around, and this is due to the fact that Th1 cytokines are considered to be detrimental to pregnancy, via direct embryo toxic activity, or via damage to the placental trophoblast, or possibly by activating cells that are deleterious to the conceptus, whereas Th-2 cytokines

may directly or indirectly contribute to the success of pregnancy by down regulating potential Th-1 reactivity[54].

The present study showed that the highest rate (77.78 %) of abortion was found in the first trimester of pregnancy, and the result was significant ($P < 0.05$) (as shown in Table 8). This agrees with Borhani, et al [55] who said; the danger of intrauterine HSV transmission is highest during the first trimester of gestation and it can lead to abortion, stillbirth and congenital anomalies. The differences in results may be due to some maternal infections, such as CMV, especially during the early gestation, which can result in fetal loss or malformations because the ability of the fetus to resist infectious organisms is limited and the fetal immune system is unable to prevent the dissemination of infectious organisms to various tissues. The fetus and/or neonate are infected predominantly by viral and also by bacterial and protozoal pathogens. Infections with various pathogens cause miscarriage or may lead to congenital anomalies in the fetus while others are associated with neonatal infectious morbidity[56].

Conclusions

The seroprevalence of HSV-2 was relatively high in pregnant women in Kirkuk city. Primary and re-infection of latency occurred at highest rate in age group 18-23 years old. Primary HSV-2 infection increases the AEC and IL-2 during pregnancy. The highest rate of abortion occurred during the first trimester of pregnancy in women with HSV-2.

References

1- Kelika A, Konda B, Jeffrey D, et al. The Epidemiology of herpes simplex virus type 2 infection in low-income urban populations in coastal Peru. Sex Trans Dis 2005; 32: 534-541.

2- Mahy's Virology dictionary. 3rd ed. Atlanta, Georgia: Acadmic press 2001: 186-187.

3- Drannik A. Characterization of antiviral properties of trappin-2 and elafin against HIV-1 and HSV-2 in the female genital mucosa [Ph.D. thesis]. Hamilton, Ontario: University of McMaster 2012.

4- Orosz L. The effect of vesicular stomatitis virus and herpes simplex virus infection on the expression patterns of p63 and Bax in different epithelial cell lines [Ph.D. thesis]. Szeged, Hungary: University of Szseged, Faculty of Medicine 2010.

5- Zuckerman A, Banatvala J, Schoub B, Griffiths P, Mortimer P. Principles and practice of clinical Virology. 6th ed. Oxford, UK: John Wiley and Sons Ltd; 2009: 95-96.

6- Zuckerman A, Banatvala J, Schoub B, Griffiths P, Pattison J. Principles and practice of clinical Virology. 5th ed. Oxford, UK: John Wiley and Sons Ltd; 2004: 27-29.

7- Kimberlin D, Rouse D. Genital herpes. N Eng J Med 2004 350:1970-1971.

8- American AIDS education and training center. Herpes simplex cold sores and genital herpes. New Mexico, USA: National Library of Medicine; 2012.

9- Tidy C. Congenital, perinatal and neonatal infections. London, UK: Egton Medical Information Systems Limited; 2013: 3-7.

10- Counseling messages for herpes simplex type 2 genital herpes. Centers for Disease Control and Prevention; 2002: 3-8.

11- Herpes simplex virus type 2 programmatic and research priorities in developing countries. London: World Health Organization and the Joint United Nations Programme on HIV/AIDS; 2001: 8-19.

12- LiHsu W. Herpes simplex virus type 1 infection and ND10 characteristics in cultured fibroblast and neuronal-like cells [Ph.D. thesis]. Scotland, UK: University of Glasgow 2002.

13- Prescott L, Harley J, Klein D. Microbiology. 5th ed. Boston, U.S.A: The McGraw-Hill Companies; 2002: 886-887.

14- Todd P, Yumi I, Li Y, Vallas V, Krause P. Herpes simplex virus type 2 establishes latent infection in a different population of ganglionic neurons than HSV-1, role of latency associated transcripts. J Virol 2007; 81: 1872-1878.

15- Goldman E, Green L. Practical Handbook of Microbiology. 2nd ed. London, UK: Chemical Rubber Company Press; 2009: 807-808.

16- Harvery R, Champe P, Fisher B. Lippincott's illustrated reviews: Microbiology. 2nd ed. Baltimore, Md: Lippincott Williams and Wikins; 2007: 261-262.

17- Nick S, Metzger B, Muller S, Falke D. Virus specific IgM and IgG antibody production by B cells during herpes simplex virus type 2 induced immunosuppression as analysed by an immunospot assay. J Gen Virol 1987; 68: 1951-1959.

18- Munjoma M, Kurewa E. The prevalence, incidence and risk factors of herpes simplex virus type 2 infection among pregnant Zimbabwean women followed up nine months after childbirth. Wom heal 2010; 10: 2-4.

19- Leylan B, Kennedy M, Wimberly Y, Levine B, Cherpes T. Serologic detection of herpes simplex virus type 2 antibodies among pregnant women using a point-of-care test from Focus diagnostics. J Clin Virol 2009; 44: 125-128.

20- Ziyaeyan M, Japoni A, Roostaee M, Slehi S. Soleimanjahi H. A serological survey of herpes simplex virus type 1 and 2 immunity in pregnant women at labor stage in Tehran, Ira Pak J Bio Sci 2007; 10: 148-151.

21- Butel S, Morse A. Herpes viruses. In: Jawetz, Melnick and Adelberg's (eds.). Medical Microbiology. 23rd ed. McGraw-Hill company 2004: 440-446.

22- Abdul Mohymen N, Hussein A, Hassan F. Association between TORCH agents and recurrent spontaneous abortion. Iraqi J Med Sci 2009; 7 40-46.

23- Jasim M, Majeed H, Ali A. Performance of serological diagnosis of TORCH agents in aborted versus non aborted women of Waset province in Iraq. Tik Med J 2011; 17: 141-147.

24- Al-Taie A. Serological study for TORCH infections in women with high delivery risk factors in Mosul. Tik J Pur Sci 2010; 15: 193-196.

25- Duran N, Yarkin F, Evruke C, Koksal F. Asymptomatic herpes simplex virus type 2 infection among pregnant women in Turkey. Ind J Med Res 2004; 120: 106-110.

26- Alzahrani A, Obeid O, ALmulhim A, et al. Analysis of herpes simplex 1 and 2 IgG and IgM antibodies in pregnant women and their neonates. J Fam Com Med 2007; 14: 3-7.

27- Sen M, Shukla B, Banerjee T. Prevalence of serum antibodies to TORCH infection in and around Varanasi, Northern India. J Clin Diag Res 2012; 6: 1483-1485.

28- Kasubi M, Nilsen A, Marsden H, Bergstrom T, Langeland N, Haarr L. Prevalence of antibodies against herpes simplex virus type 1 and 2 in children and young people in an urban region in Tanzania. J Clin Microbiol 2006; 44: 2801-2807.

29- Msuya S, Mbizvo E, Hussain A, et al. Seroprevalence and correlates of herpes simplex virus type 2 among urban Tanzanian women. Sex Trans Dis 2003; 30: 588-592.

30- Shahraki A, Moghim S, Akbari P. A survey on herpes simplex virus type 2 antibody among pregnant women in Isfahan, Iran. J Res Med Sci 2010; 15: 243-244.

31- Anzivino E, Fioriti D, Mischitelli M, et al. Herpes simplex virus infection in pregnancy and in neonate: status of art of epidemiology, diagnosis, therapy and prevention. J Virol 2009; 6: 1-4.

32- Sauerbrei A, Schmitt S, Scheper T, et al. Seroprevalence of herpes simplex virus type 1 and 2 in Thuringia, Germany, 1999 to 2006. Euro Surveill J 2011; 16: 6-7.

33- Nakubulwa S, Mirembe F, Kaye D, Kaddu-Mulindwa D. Association between HSV-2 and HIV serostatus in pregnant women of known HIV serostatus attending Mulago hospital antenatal clinic, Kampala, Uganda. J Infet Dev Ctries 2009; 3: 803-806.

34- Mahalakshmi B, Therese K, Devipriya U, Pushpalatha V, Margrita S, Madhavan H. Infectious aetiology of congenital cataract based TORCHES screening in a tertiary eye hospital in Chennai, Tamil Nadu, and India. Ind J Med Res 2010; 131: 559-564.

35- Kropp R, Wong T, Cormier L, et al. Neonatal herpes simplex virus infections in Canada, results of a 3 years national prospective study. Ped J 2006; 117: 1955-1956.

36- Kaushic C, Ashkar A, Reid L, Rosenthal K. Progesterone increases susceptibility and decreases immune responses to genital herpes infection. J Virol 2003; 77: 4558-4565.

37- Margan M, Chicin G, Moldovan R. Clinical and epidemiological considerations on herpes simplex genital infections. J Hyg Pub Heal 2009; 59: 7-8.

38- Ashley R, Wald A. Genital herpes: Review of the epidemic and potential use of type-specific serology. Clin Microbiol Rev 1999; 12: 1-8.

39- Patnaik P, Herrero R, Morrow R, et al. Type-specific seroprevalence of herpes simplex virus type 2 and associated risk factors in middle-aged women from 6 countries: The IARC multicentric study. Sex Trans Dis 2007; 34: 2-3.

40- Smith J, Rosinska M, Trzcinska A, Pimenta J, Litwinska B, Siennicka J. Type specific seroprevalence of HSV-1 and HSV-2 in four geographical regions of Poland. Sex Trans Inf 2006; 82: 159-163.

2: 356.

42- Navaneethan U, Lancaster E, Venkatesh P, Wang J, **Neff G. Herpes simplex virus hepatitis- it's high time we**

41- Lakhan S, Harle L. Fatal fulminant herpes simplex hepatitis secondary to tongue piercing in an immunocompetent adult: a case report. J Med Cas Rep 2008; 2: 356.

42- Navaneethan U, Lancaster E, Venkatesh P, Wang J, Neff G. Herpes simplex virus hepatitis- it's high time we consider empiric treatment. J GIT Liv Dis 2011; 20: 93-96.

43- Hoffbrand A, Moss P, Pettit J. Essential Hematology. 5th ed. Massachusetts, USA: Blackwell; 2006: 117-119.

44- America lab tests. Complete blood count. Washington, USA: American association for clinical chemistry; 2012: 2-3.

45- Koelle D, Posavad C, Barnum G, Johnson M, Frank J, Corey L. Clearance of HSV-2 from recurrent genital lesions correlates with infiltration of HSV-specific cytotoxic T lymphocyte. J Clin Inves 1998; 101: 1500-1508.

46- England Affinity and society. Assessment of eosinophilia; London, UK: Brit Med J publishing group limited; 2011.

47- Tarkkanen A, Laatikainen L. Late ocular manifestations in neonatal herpes simplex virus infection. Brit J Ophth1977; 61: 608-616.

48- Pettay O, Leinikki P, Donner M, Lapinleimu K. Herpes simplex virus infection in the newborn. Arch Dis Child 1972; 47: 97.

49- Toliver-Kinsky T, Lin C, Herndon D, Sherwood E. Stimulation of hematopoiesis by the Fms-like tyrosine kinase 3 ligand restores bacterial induction of Th1 cytokines in thermally injured mice. Inf Immun 2003; 71: 3058- 3067.

50- Rushbrook S, Ward S, Unitt E, et al. Regulatory T cells suppress in vitro proliferation of virus specific CD8 T cells during persistent Hepatitis C Virus infection. J Virol 2005; 79: 7852-7859.

51- Carmack M, Yasukawa L, Chang S. T cell recognition and cytokine production elicited by common and type-specific glycoproteins of herpes simplex virus type 1 and 2. J Inf Dis 1996; 174: 899-906.

52- Apurba S, Sandhya B, Senthamarai S, et al. Serological evaluation of herpes simplex virus type 1 and 2 infections in pregnant women with bad obstetric history in a tertiary care hospital, Kanchipuram. Inter J Adv Res 2013; 1: 123-128.

53- Kim D, Chang H, Hwang K. Herpes simplex virus type 2 infection rate and necessity of screening during pregnancy: A clinical and seroepidemiologic study. Yons Med J 2012; 53: 401-407.

54- Ahmed D. Effects of Interleukin-2 (IL-2) and Interleukin-6 (IL-6) in recurrent spontaneous abortion. J Virol 2008; 17: 74-76.

55- Borhani M, Hosseini S, Chamani-Tabrizi L, et al. PCR detection of herpes simplex virus in human placenta and aborted material with spontaneous abortion. Iran J Clin Inf Dis 2011; 6: 18-20.

56- Haider M, Rizvi M, Khan N, Malik A. Serological study of herpes virus infection in female patients with bad obstetric history. Bio Med 2011; 3: 284-290.