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ORIGINAL ARTICLE

Effective Use of *Enzyme Linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR)* for Detection of Herpes Simplex Virus Infection in Pregnant Females at Sohag University Hospital

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ABSTRACT

Background: *Infection with herpes simplex virus is one of the most common sexually transmitted infections. Because the infection is common in women of reproductive age it can be contracted and transmitted to the fetus during pregnancy and the newborn, which can lead to death or long-term disabilities.* ***Objectives:*** *The aim of our study was to detect the seroprevalence of Herpes Simplex Virus infection in pregnant females at Sohag University Hospital using Enzyme Linked Immunosorbent Assay (ELISA) confirmed by Polymerase Chain Reaction (PCR) as well as the fetal outcome of infected women in comparison with healthy.* ***Methodology:*** *The study was carried out on 60 pregnant women attending the Obestetric and Gynacology department of Sohag University Hospital fulfilled the inclusion criteria. They were screened for type specific HSV antibodies (HSV-1 IgG and IgM, HSV-2 IgG and IgM) using Enzyme Linked Immunosorbent Assay (ELISA). For IgM positive cases, HSV DNA was detected by Polymerase Chain Reaction (PCR). Blood samples were collected from all the participants after written informed consent. Univariate and multivariate analysis were performed to identify the risk factors associated with HSV positivity.* ***Results:*** *ELISA test for HSV-1 antibodies revealed 14 (23.33%) cases positive for IgM and 44 (73.33%) cases positive for IgG antibodies. For HSV-2 antibodies there were 4 (6.67%) cases positive for IgM and 38 (63.33%) cases positive for IgG antibodies. By PCR HSV-DNA was detected in 14 (77.77%) of the 18 ELISA positive cases representing (23.33%) of the studied populations.* ***Conclusion:*** *According to our study overall prevalence of HSV infection among pregnant women in Sohag University Hospital is about 23%, mostly due to HSV-1.*

INTRODUCTION

HSV-1 and HSV-2 are DNA viruses that belong to Alphaherpesvirinae, a subfamily of the Herpesviridae family. Both viruses, transmitted across epithelial mucosal cells, as well as through skin interruptions, migrate to nerve tissues, where they persist in a latent state. HSV-1 predominates in oro-facial lesions and it is typically found in the trigeminal ganglia, whereas HSV-2 is most commonly found in the lumbosacral ganglia. Nevertheless these viruses can infect both oro-facial areas and the genital tract [1].

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Herpes simplex virus (HSV) infection is one of the most common viral sexually transmitted diseases (STD) worldwide [2]. Herpes simplex virus type 2 (HSV-2) is the cause of most genital herpes and is almost always sexually transmitted. Herpes simplex virus type 1 (HSV-1) is usually transmitted during childhood via non-sexual contacts; however, it has emerged as a principle causative agent of genital herpes in some developed countries [3].

The greatest incidence of HSV infections occurs in women of reproductive age, the risk of maternal transmission of the virus to the fetus or neonate has become a major health concern[4].

Recent findings reveal that first-time infection of the mother is the most important factor for the transmission of genital herpes from mother to fetus/newborn. In fact, the pregnant woman who acquires genital herpes as a primary infection in the latter half of pregnancy, rather than prior to pregnancy,

is at greatest risk of transmitting these viruses to her newborn [5].

HSV infection of the newborn can be acquired in utero, intra-partum and post-natally, while intrauterine HSV infection is a rare disorder and accounts for 5% of HSV infections in neonates. The highest risk of intrauterine infection has been observed in pregnant women (about 50%) who develop disseminated HSV infections. Intrauterine viral transmission is highest during the first 20 weeks of gestation leading to abortion, stillbirth and congenital anomalies in infants who survive [6].

In 85–90% of neonatal HSV infections, HSV is acquired at the time of delivery and 5–10% of cases are caused by early postnatal viral acquisition. 70–85% of neonatal HSV infections are caused by HSV-2, whereas the remaining cases are due to HSV-1. Usually, an infection with HSV-2 carries a graver prognosis than that caused by HSV-1 [7].

METHODOLOGY

Study population:

This study was conducted on a total of 60 pregnant women including those who were having manifestations of HSV infection, had history suggestive of the infection, as well as history of preterm labor, abortion, fetal loss or neonatal death. Patients were recruited from the outpatient clinic of gynecology and obstetrics department in Sohag University Hospital during the period from June 2013 to December 2013. Blood samples were collected from all selected women. *Written informed consent was taken from all females included in our study.*

History and physical examination

A detailed history was taken including vesicular eruption around the mouth, chin and the lips suggesting infection with HSV-1. Concerning infection with HSV-2, history was taken for genital and peri-genital skin infection and ulceration in addition to history of preterm labor, neonatal death, abortion and familial history of vesicular eruption or u**l**ceration. General examination of fascial skin lesion and vaginal examination for ulceration was done.

Sample Collection

10 ml of venous blood was obtained by venipuncture from each patient, using disposable syringes. 7 ml of the blood was left to coagulate at 37° C for 15 min. and blood was then centrifuged at 300 rpm for 10 min. The collected serum was then placed in 1.5 ml micro-centrifuge tubes and stored at -20° C until examined.

Enzyme linked immunosorbent assay (ELISA):

For Detection of anti HSV-1, HSV-2 antibodies (IgG and IgM) by Enzyme Linked Immuno-sorbent Assay (ELISA) we used kits from *(DRG International, USA)* following the manufacturer instructions. Purified HSV-1, HSV-2 antigen was coated on the surface of microwells. Diluted serum was added to wells, and the HSV-1, HSV-2 IgG, IgM-specific antibody, if present, bound to the antigen. All unbound materials were washed away. Horse radish peroxidase (HRP)-conjugate was added, which bound to the antibody-antigen complex. Excess enzyme conjugate was washed off and a solution of TMB Reagent was added. The enzyme conjugate catalytic reaction was stopped at a specific time. The intensity of the color generated was proportional to the amount of HSV-1, HSV-2 IgG, IgM - specific antibody in the sample. The results were read by a microwell reader compared in a parallel manner with calibrator and controls.

Polymerase chain reaction (PCR):

PCR was performed to amplify and to detect HSV DNA in IgM antibodies positive serum samples taken from the pregnant women who fulfilled the mentioned inclusion criteria**.**

DNA extraction:

HSV-DNA was extracted from the collected serum samples by *(QIAamp DNA Mini and Blood Mini kit QIAGEN*) following the manufacturer instructions**.**

Amplification:

PCR reaction was setup in a final volume of 25 ul volume in which 12.5 ul of Taq PCR Master Mix *(QIAGEN cat .No.2011443)* was used . The primers used bracketed a 142 base pair segment of the DNA polymerase gene of the HSV genome (5'-ATCCGAACGCAGCCCCGCTG, 3'-CTCCGTCCAGTCGTTTATCTTC) adding 1.25 ul of each primer and Template DNA in a volume of (1 ul) with RN ase free water provided in a volume of (9 ul). The mixture was placed in a thermo - cycler *(T-Gradient Biometra, USA*) and heated for 1 minute at 94°C for denaturation, cooled for 1 minute at 55°C for annealing, and incubated for 1 minute at 72°C for extension. This cycle was repeated 30 times.

Detection:

Seven µL of the amplified PCR product of each sample and 3µL of the loading dye were electrophoresed on a 2% agarose gel containing 0.5µg/ml ethidium bromide. Negative controls (PCR mix without DNA extract) were run with each PCR assay and one well was kept for 5 µL of the PCR DNA marker *(EzWayTM DNA Ladder 100bp, 6 bands)* from *(KOMA BIOTECH INC.),* and photographed under ultraviolet light by gel documentation instrument. PCR was interpreted as positive when the HSV-DNA band with molecular weight (142 bp) was detected.

Statistical analysis

Data was analyzed using STATA inter cooled version 9.2. Quantitative data was analyzed using student t-test to compare means of two groups. Qualitative data was compared using Chi-square test.

P value was considered significant if it was less than 0.05.

RESULTS

Medical history and examination of studied population

As presented in table (1) of the 60 pregnant women included in the study 4(6.67%) had history of diabetes ,12(20.00%) had history of hypertention, 38(63.33%)had history of fascial vesicular eruption, 14(23.33%) had history of genital ulceration, 10(16.67%) had history of neonatal death , and 36(60.00%) had familial history of vesicular eruption as regards history of abortion 28 (46.67%) had no history of abortion, 16 (26.67%) had history of one abortion, 12(20.00%) had history of 2 times abortion, 2(3.33%) and 3(3.33%) had 3 and 4 times abortion respectively, by examination 40(66.67%) women had fascial skin lesion while there was no ulcers by vaginal examination in any of the studied females.

Table 1: Demographic data, medical history and medical examination of the studied population.

Variable	Summary statistics
Age	
Mean (SD)	27.27 (3.83)
Median (range)	27.5 (20-36)
History of diabetes	
No	56 (93.33%)
Yes	4 (6.67%)
History of Hypertension	
No	48 (80.00%)
Yes	12 (20.00%)
History of facial vesicular eruption	
No	22 (36.67%)
Yes	38 (63.33%)
History of genital ulceration	
No	46 (76.67%)
Yes	14 (23.33%)
History of abortion	
0	28 (46.67%)
1	16 (26.67%)
2	12 (20.00%)
3	2 (3.33%)
4	2 (3.33%)
History of preterm labour	
No	60 (100 %)
History of neonatal death	
No	50 (83.33%)
Yes	10 (16.67%)
Family history of vesicular eruption or ulceration	
No	24 (40.00%)
Yes	36 (60.00%)
General examination of facial skin lesion	
No	20 (33.33%)
Yes	40 (66.67%)
Ulcer in vaginal examination	
No	60 (100%)

- **HSV lab. Investigations of the studied population.**

HSV lab. Investigations of the studied population is outlined in table (2) among the 60 pregnant women included in the study 14(23.33%) were positive for anti –HSV1 IgM antibodies, 44 (73.33%) were positive for anti-HSV1 IgG antibodies, while 4 (6.67%) were positive for anti HSV2 IgM antibodies and 38 (63.33%) were positive for anti –HSV2 IgG antibodies. As regards PCR results HSV DNA was detected in 14 (23.33%) women.

Table 2: HSV lab investigations of the studied populations.

Variable	Summary statistics
ELISA test for HSV1 antibodies	
IgM	
Negative	46 (76.67%)
Positive	14 (23.33%)
IgG	
Negative	16 (26.67%)
Positive	44 (73.33%)
ELISA test for HSV-2 antibodies	
IgM	
Negative	56 (93.33%)
Positive	4 (6.67%)
IgG	
Negative	22 (36.67%)
Positive	38 (63.33%)
PCR(HSV -DNA)	
Negative	46 (76.67%)
Positive	14 (23.33%)

- **ELISA results of HSV1 IgM compared to medical history and examination:**

Comparison of tested pregnant women diagnosed as positive IgM by ELISA test for HSV1 and those diagnosed as negative with medical history and examination is outlined in table (3) showing that there is association between HSV1 IgM antibodies presence and younger age (p value<0.0001), history of fascial vesicular eruption(p value<0.0001), fascial skin lesion in their general examination(p value 0.003)and family history of vesicular eruption or ulceration(p value <0.0001).

Table 3: Comparison of pregnant females diagnosed as positive for HSV-1 IgM by ELISA test and those diagnosed as negative as regards their medical history and examination.

Variable	Results of IgM by ELISA test for HSV -1		P value
	Negative (n=46)	Positive (n=14)	
Age			
Mean (SD)	28.65 (2.91)	22.71 (2.81)	<0.0001
History of facial vesicular eruption			
No	22 (47.83%)	0	0.001
Yes	24 (52.17%)	14 (100%)	
History of genital ulceration			
No	38(82.61%)	8 (57.14%)	0.049
Yes	8 (17.39%)	6 (42.86%)	
History of abortion			
0	18 (39.13%)	10 (71.43%)	0.17
1	14 (30.43%)	2 (14.29%)	
2	10 (21.74%)	2 (14.29%)	
3 or 4	4 (8.70%)	0	
History of neonatal death			
No	40 (86.96%)	10(71.43%)	0.17
Yes	6 (13.04%)	4 (28.57%)	
Family history of vesicular eruption or ulceration			
No	24 (52.17%)	0	
Yes	22 (47.83%)	14 (100%)	<0.0001
General examination of facial skin lesion			
No	20 (43.48%)	0	0.003
Yes	26 (56.52%)	14 (100%)	

- **ELISA results of HSV 2 IgM compared to medical history and examination:**

Comparison of tested pregnant women diagnosed as positive IgM by ELISA test for HSV2 and those diagnosed as negative with medical history and examination is outlined in Table (4) showing that there is association between HSV2 IgM antibodies presence and younger age (p value0.04)**.**

Table 4: Comparison of pregnant females diagnosed as positive for HSV-2 IgM by ELISA test and those diagnosed as negative as regards their medical history and examination.

Variable	Results of IgM by ELISA test for HSV-2		P value
	Negative (n=56)	Positive (n=4)	
Age			
Mean (SD)	27.54 (3.70)	23.5 (4.04)	0.04
History of facial vesicular eruption			
No	22 (39.29%)	0	0.12
Yes	34 (60.71%)	4 (100%)	
History of genital ulceration			
No	44 (78.57%)	2 (50.00%)	0.11
Yes	12 (21.43%)	2 (50.00%)	
History of abortion			
0	26 (46.43%)	2 (50.00%)	0.57
1	14 (25.00%)	2 (50.00%)	
2	12 (21.43%)	0	
3 or 4	4 (7.14%)	0	
History of neonatal death			
No	48 (85.71%)	2 (50.00%)	0.06
Yes	8 (14.29%)	2 (50.00%)	
Family history of vesicular eruption or ulceration			
No			
Yes	24 (42.86%)	0	
	32 (57.14%)	4 (100%)	0.09
General examination of facial skin lesion			
No	20 (35.71%)	0	0.14
Yes	36 (64.29%)	4 (100%)	

- **Comparison of pregnant females diagnosed as positive with PCR and those diagnosed as negative as regards medical history and examination**:

Comparison of patient diagnosed as positive with PCR and those diagnosed as negative is outlined in table (5) showing that Among 14 tested positive 12(85.71%) had history for fascial vesiculation (P value 0.001), 14 (100%)had fascial vesiclation in their general examination(P value 0.003). 14 (100%) had familial history of vesiculation or ulceration (P value<0.0001).

Table 5: Comparison of pregnant females diagnosed as positive for HSV infection by PCR and those diagnosed as negative as regards medical history, examination.

Variable	Results of PCR		P value
	Negative (n=46)	Positive (n=14)	
Age			
Mean (SD)	28.35 (3.41)	23.71 (2.92)	<0.0001
History of facial vesicular eruption			
No	22 (47.83%)	0	
Yes	24 (52.17%)	14 (100%)	0.001
History of genital ulceration			
No	36 (78.26%)	10 (71.43%)	0.60
Yes	10 (21.74%)	4 (28.57%)	
History of abortion			
0	16 (34.78%)	12 (85.71%)	
1	16 (34.78%)	0	0.006
2	10 (21.74%)	2 (14.29%)	
3 or 4	4 (8.70%)	0	
History of neonatal death			
No	38 (82.61%)	12 (85.29%)	
Yes	8 (17.39%)	2 (14.29%)	0.79
Family history of vesicular eruption or ulceration			
No			
Yes	24 (52.17%)	0	
	22 (47.83%)	14 (100%)	<0.0001
General examination of facial skin lesion			
No	20 (43.48%)	0	
Yes	26 (56.52%)	14 (100%)	0.003

- **ELISA and PCR results compared to foetal outcome:**

Comparison between the studied population foetal outcome and their lab results is outlined in table (6)**.** among the 60 tested pregnant women 16 lost their babies, 10 (62.50%)of them carried HSV DNA detected by PCR (P value 0.001), also 10 (62.50%) of them had positive anti HSV1 IgM antibodies(P value 0.004), and 12 of them had positive HSV2 IgM (P value 0.02), this agree with the suggested relation between recent HSV infection and bad foetal outcome .

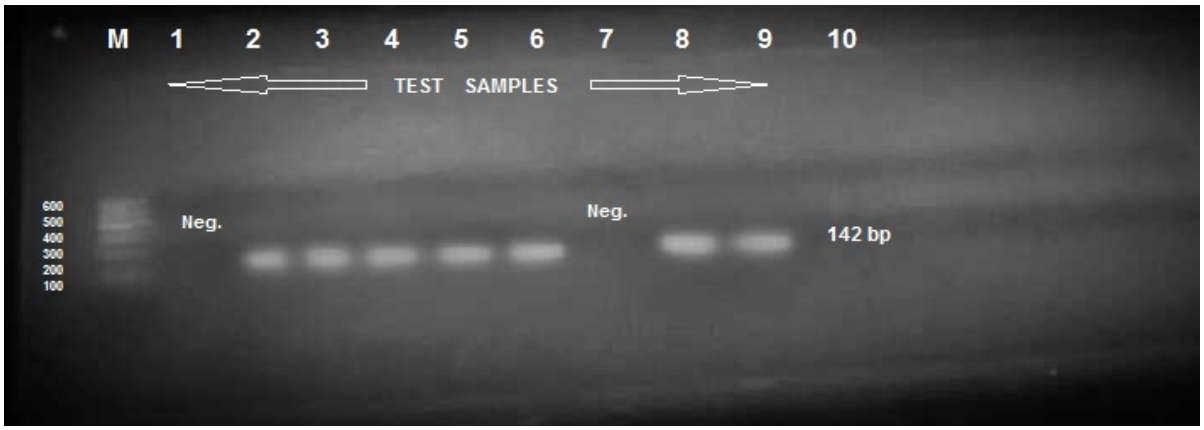
Table 6: Comparison of studied population by outcome according to lab results

Variable	Outcome		P value
	Baby loss(n=16)	Normal (n=44)	
IgM Eliza test for HSV(1)			
Negative	6 (37.50%)	40 (90.00%)	
Positive	10 (62.50%)	4(10.00%)	0.004
IgG Eliza test for HSV(1)			
Negative	10 (62.50%)	18 (40.9%)	0.11
Positive	6 (37.50%)	26 (59.1%)	
IgM by Eliza test for HSV(2)			
Negative	12 (75.00%)	44 (100.00%)	0.02
Positive	4 (25.00%)	0(0.00%)	
IgG Eliza test for HSV(2)			
Negative	8 (50.00%)	14 (31.82%)	0.20
Positive	8 (50.00%)	30 (68.18%)	
PCR			
Negative	6 (37.50%)	40 (90.00%)	0.001
Positive	10 (62.50%)	4 (10.00%)	

Accuracy of the used ELISA kits:
It was found that out of 14 HSV1 IgM positive 12 were PCR positive while 2 out of 4 in HSV 2
According to these results the accuracy of HSV 1 IgM detection kits found to be 90.68% and that of HSV 2 IgM 54.97% this is presented in Table 7.

Table 7: Accuracy of ELISA kit result in detection of HSV acute infection in comparison with PCR result.

ELISA result	Sensitivity	specificity	PPV	NPV	accuracy
HSV (1) IgM	85.71%	95.65%	85.71%	95.65%	90.68%
HSV (2) IgM	14.29%	95.65%	50.00%	78.57%	54.97%



Detection of *Herpes simplex* virus DNA in serum samples.Agarose gel electrophoresis of PCR products. Lanes 1-9 represent serum from 9 pregnant women positive for anti HSV-IgM antibody by ELISA. Samples No. 2, 3, 4, 5, 6, 8 and 9 show the 142 bp amplified bands of HSV gene.

DISCUSSION

Some maternal infections, especially during the early gestation, 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 [8].

HSV genital infection has been rising in prevalence in the developing world. 22% of pregnant women are sero- positive for herpes simplex virus (HSV)-2, and more than 2% of women acquire genital herpes during pregnancy. Given the high prevalence of HSV infection in women of reproductive age, obstetricians need to be able to diagnose and manage HSV infection during pregnancy and are in the unique position to prevent HSV transmission to the neonate[9].

According to our study younger females are more risky for HSV infection which agree with many studies including a Sudanese study concluded that females age group 15-25 are risky for HSV infection[10]

HSV-2 sero-prevalence studies show variation in infection by geographic location. Some of the highest prevalence of HSV-2 has been found in Africa and the Americas. Lower prevalence has been found in Western and Southern Europe than in Northern Europe and North America. Although there have been few studies, the lowest prevalence has been seen in Asia [7].

In our study Serological evaluation for HSV infections was carried out by ELISA test through detecting anti HSV IgG and IgM antibodies in serum of pregnant women confirmed by PCR test for positive herpes simplex virus IgM-Ab by detection of HSV DNA in the samples.

The present study found that the anti-herpes simplex type-2 IgM present in 4 (6.67%) of the study group. In comparison to other studies this percentage range from 0.5% in a study carried out in Saudia Arabia [11] to 58.7% in a study carried out in South Africa [12] which reflect the cultural religious considerations as regards sexual relations.

In our study there was a significant relation between sero-positivity for anti HSV-2, HSV-1 IgM and bad obstetric history (history of neonatal death, history of previous abortion) which agree with ***(Rajendra et al.***[13], Haider et al.[8]**,** and Aljumaili et al.[14] and disagree with Abul-Razak et al.[15], Basim,[16]**,** and Hala et al .[17].

Also we found a significant relation skin lesions and HSV IgMseropositivity which agree with El-Amin et al.[10]

PCR test for positive anti HSV IgM antibodies was done to detect HSV DNA in 14 **(23.33%)** of the total cases.

Delivery data were extracted from participants' charts. At 10-14 days post-partum, participants were contacted by phone to assess the clinical status of their neonates. Out of the 60 tested pregnant women; 16 lost their babies, 10 (62.50%) of them their samples were positive for HSV DNA by PCR (P value 0.001). This finding agrees with Rajendra et al.[12] whom documented that bad foetal outcome is strongly related to HSV infection but disagree with ***El-Amin et al.***[10] whom did not document a real risk for newborns of HSV infected mothers.

CONCLUSIONS

According to our study overall prevalence of HSV infection among pregnant women in Sohag University Hospital is about 23%, mostly due to HSV-1. Also the study confirmed the association of HSV infection and bad obstetric history as will as bad fetal outcome.

REFERENCES

1. Kriebs JM. Understanding herpes simplex virus: transmission, diagnosis, and considerations in pregnancy management. J Midwifery Womens Health, 2008 ; 53: 202-208
2. Xu F, Sternberg MR, Kottiri BJ, McQuillan GM, Lee FK, NahmiasAj. et al .eg. Trends in herpes simplex virus type 1 and type 2 seroprevalence in the United States. JAMA. 2006; 296:964-973
3. Gupta R, Warren T, Wald A. Genital herpes. Lancet, 2007; 22:2127-2137
4. Kriebs JM. Understanding herpes simplex virus: transmission, diagnosis, and considerations in pregnancy management. J Midwifery Womens Health, 2008;53:202-208
5. Baker DA. Consequences of herpes simplex virus in pregnancy and their prevention. CurrOpin Infect Dis, 2007;20:73-76
6. Sauerbrei A, Wutzler P. Herpes simplex and varicella-zoster virus infections during pregnancy: current concepts of prevention, diagnosis and therapy. Med MicrobiolImmunol, 2007;196:89-94
7. Levett PN.seroprevalence of HSV- 1 and HSV-2 in Barbados. Medical Microbiology and Immunology, 2005 ;194: 105 -7
8. Haider M, Rizvi M, Khan N, Malik A. serological study of herpes virus infection in female patients with bad obstetric history, biology and medicine 2011; 3(2) :284-290
9. Brown MD, Carolyn Gardella MPH, Anna Wald, Rhoda Ashley Morrow, Genital Herpes Complicating pregnancy, ACOG, 2005; 106(4): 105-109
10. Elamin OE, Ahmed RAM, Abdulla AK, Elamin SE.Sero-Prevalence of Herpes Virus Infection in Sudanese Pregnant Women. Trop Med Surg. 2013;1: 138
11. Alzahrani, A J, Obeid, O E, Almulhim A A, Analysis of herpes simplex 1and 2 IgG and IgM antibodies in pregnant women and their neonates. Saudi JObstetGynaecol. 2005; 5: 53
12. Anna W, AmaliaM,Lisa M., Frequent Genital HSV-2 Shedding Among Women During Labor in Soweto, J ObstetGynecol India 2013;63(1):113-45
13. Rajendra B. Surpam, Usha PK, Amlakar RK, Khadse MS, Qazi Suresh, Serological study for TORCH infections in women with bad obstetric history. J ObstetGynecol India, 2006; 56 (1): 200-20
14. Aljumaili ZK, Alsamarai AGM, Najem WS., Seroprevalence of herpes simplex type 2 (HSV-2) in women with bad obstetric history. Am J DermVener 2013; 2, 31
15. Abul-Razak S, Abbas A, Asmaa H,Wasan A.Seroprevalence of Anti- Herpes Simplex Virus Type2 IgG, IgM Antibodies Among Pregnant Women in Diyala. ProvinceDiyala Journal of Medicine , 2013; 5, 1- 36
16. Basim M, Study the prevelance of ACL, APL, CMV, HSV, Rubella and Toxoplasma Gondii in aborted women in Baghdad. Medical Journal of Babylon, 2013; 10(2): 112-50
17. Hala M, Abdulghani M, Zainab K, association between herpes simplex virus type 2 (HSV2)and bad obstetric outcomes, our Dermatol online, 2014; 5(1) :19-28