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Research Article

STUDY ON THE IGG AND IGM ANTIBODIES RATE OF VIRUS HSV, CMV AND RUBELLA IN THE WOMEN WITH RECURRENT PREGNANCY LOSS HISTORY

***Sharareh Ariani and Leila Modiri Arash Chaichi**
Department of Microbiology, Lahijan Branch, Islamic Azad University, Lahijan, Iran
**Author for Correspondence*

ABSTRACT
Abortion is one of the most common complexities of gestation. Abortion is referred to the cases where due to various maternal – fetal reasons, gestation terminates until the week 20 of the gestation. If this event occurred 3 subsequent times in s single patient, it is called recurrent pregnancy loss. Several infectious factors are involved in the occurrence of abortion, most important of them is TORCH (toxa – rubella – CMV – HSV), leading to spontaneous abortion. Genital herpes simplex which is a chronic viral disease is the most common reason of genital zone ulcer. Cytomegalovirus of the family herpes virus. May be transferred to the fetus through the placenta. In addition, infection to rubella during the gestation is very hazardous. Thus given the importance of these viruses, we are tended to study their titers in Mehr Institute of Infertility. Present study is analytic – descriptive and was performed on 150 women with recurrent pregnancy loss which were randomly selected. In present study, prevalence of infections due to Rubella – HSV – CMV was performed with evaluation of antibodies IgG and IgM using Elisa and EUROIMMUN kit. Results indicated that frequencies of immunoglobulin's M and G for virus CMV was 2 individuals (1.3%) and 90 (60%) for virus HSV, and 4 (2.7%) and 130 (86.6%) for Rubella virus, respectively. Consequently, frequency of IgG positive cases in all 3 studied viruses was more than IgM, this fact indicates that these women had previous contact to the mentioned viruses. It's worth mentioning that some viruses such as CMV are of recurring nature, that is, they will persist in the body for a long time even after recovery of disease and then under specific conditions, the will appear again.

Keywords: *Abortion, Recurrent Pregnancy Loss, TORCH, Rubella, Cytomegalo Virus, Genital Herpes Simplex, IgM – IgG.*

INTRODUCTION
Since long ago, abortion was considered as one of major medical challenges in societies. This problem is more significantly observed in low income societies and is considered as one of family failure factors (Alimagham and Motvasli, 2001). Abortion is referred to the cases of pregnancy termination in which fetus is passed off up to the week 24 or even 28 of pregnancy. It is worth mentioning that even though abortion may a potential useful event o avoid development of abnormal. But if it is repeated 2 or 3 times, it will find a pathologic aspect and s called Recurrent Pregnancy Loss (RPL), and requires examination and suitable treatment (Anonymous, 2004). TORCH infections in pregnant women may be transferred to the fetus inside the womb or during the birth, leading to spontaneous abortion or occurrence of disorders accompanied with symptom's in the neonate.

Frequently, such infections occure in pregnant woman in a hidden and creeping manner, and don't involve any clinical representations or signs, due to this fact, in many cases, while pregnant women are suffering the mentioned infections, but clinical detection is very difficult, and in some cases it is impossible. Occurrence of spontaneous abortion sin the first months of pregnancy due to asymptomatic infections relating to TORCH organisms and hazardous consequences of outbreak of these infections in the fetus or neonate resulted that in various parts across the world, wide spread research are performed on different areas of these infections and on high risk groups (pregnant women, fetus or neonate) (Anonymous, 2004).

Spontaneous abortion of fetus, with prevalence of 15% - 20% of overall known pregnancies, is the most common complexity of pregnancy. On the other hand, there are evidences that this unwelcomed disorder is more that what is imagined. Since, spontaneous abortions are frequently occur around the menstruation

time period and mostly are not detected. Spontaneous abortions by developing undesirable complexities not only may produce dangerous problems both in mother and fetus but also in some cases may create important mental, psychological or even behavioral consequences in mother (Anonymous, 2004). Between 50% to 70% of abortions in the first three months of pregnancy are due to accidental events as follows:

Abortion may occur due to chromosome disorders in the fertile ovum. In most cases, this types of abortion indicates that ovum or sperm had incorrect chromosome numbers and as a result, fertile ovum was not able to grow normally (Kenneth and Kistner, 1995). In other cases, abortion occurs due to the problems happened during the initial complicated growth. For example, when ovum is not correctly infused in the womb or when fetus has structural defect not allowing to continue the growth. Since most health care workers don't perform detailed examinations after abortion, it is mostly not possible to explain the reason of pregnancy loss. Even when detailed evaluation is performed, for example after occurrence of two or 3 subsequent abortion, in half the abortions, its reason remains unknown (Kenneth and Kistner, 1995). When fertile ovum has chromosome defects, presumably it is suffering a condition called disappointed sperm (Now, in the medical communities it is called as initial fertility defect). In such cases, fertile ovum is infused in the womb and placenta and embryo sac begin to grow but the fetus resulting from it will terminate the growth early or it is not formed at all. Since, the placenta begins to secrete the hormone, pregnancy test will be positive and initial signs of pregnancy is produced, but ultrasound test will display the embryo sac as empty. In other cases, embryo will grow but it suffers some disorders which makes the life continuation impossible. Then, before beginning the heart beating, the growth is terminated (Kenneth and Kistner, 1995).

Abortion occurs due to the causes such as production of toxic materials, and metabolic, immunologic reasons, placenta infection and endometric reasons (Kenneth and Kistner, 1995). Viral infections result to spontaneous abortion. Among viral infections are cytomegalovirus, Herpes simplex and rubella and a virus on which great studies has performed recently i.e. para virus B19. Since, treatment for viral infections, it is associated with more problems (Karim and Tahery, 1379).

CMV virus is an infections virus which as most common cause of inters uterus death of fetus; CNV is the abbreviation of cytomegalovirus. This virus infects high percentage of humans during their life and after seemingly recovery of disease; it will hide in a group of leukocyte.

This virus, however, is not considered as a hazardous agent for health, but in pregnant women, it is a major factor to threaten the health of neonate, transfer of the virus to neonate occurs while child birth as well during breast feeding by infected mother and causes some problems such as hepatitis, pneumonia, blood disorders, mental retardation and heaving loss. 15% of the infected neonates display the evidence of involvement of CMV, these signs are including: cutaneous representations such as porphyria, cerebral atrophy, hepato splenomegaly, choriretinitis pneumonia, blood disorders, microcephaly, loss of sense – nervous hearing. Almost all of these infants have some degrees of mental retardation and brain involvement.

Herpes simplex (HSV) is a large linear virus with double stranded DNA. Its external cover has glycoprotein's which is shared among simplex types 1 and 2. But each virus has also specific glycoprotein's based on them it is possible o distinguish the type 1 and type 2. During infection phase, herps virus will finally kill the cell in which it proliferates (Alimagham and Motvasli, 2001). Genital herpes is greatly due to Human Heroes virus 2 (HHV – 2) or Herpes simplex virus 2 HSV – 2) and is spread through sexual contact. While Herpes simplex virus type 1 (HSV – 1) causes Labial herpes, and is transferred through kissing.

HSV – 2 belongs to family Herpes viride and subfamily a – herpes virine. Its genum is a big linear double – strand DNA of 125 – 229 kbp. Measles is produced by a virus with RNA from toga virus group. This virus is susceptible to the fat solvents, very acidic and very basic mediums as well to uv, and it is inactive in such media. In addition, direct sun light and heat significantly is effective in killing this virus.

The major establishment place of these viruses and their transfer from person to person in most patients is through nasopharyngeal aspirates (Anne, 2010). Accordingly, present study was performed aiming to study the effect of 3 viruses HSV, CMV and rubella on spontaneous abortion.

MATERIALS AND METHODS

Present study was performed on women suffering the spontaneous abortion having 3 previous abortion history and admitted to Mehr infertility center of Rasht during the time period of Farvardin to Khordad 1392. With patient's agreement and after completing the questionnaire, 5 ml blood was taken from each patient and its serum was isolated. Then the serum obtained by centrifuge was poured in plastic tubes (gamma). Then tubes containing serum were firmly sealed using parafilm. The tubes were kept for 2 weeks in refrigerator or fir more than 2 weeks in refrigerator or for than 2 weeks to 1 month in the freezer. After collecting adequate number of sample using special kits, IgM and IgG rates of each rubella, CMV and HSV virus were measured separately using ELISA.

Firstly 10 µl of sample was diluted by 1 ml buffer sample (we poured 10 µl serum of the patient and 1 cc of buffer sample in the gamma tubes using dispenser, then resulted mixture was shaked to homogenize). Then, 100 µl of calibrators, negative and positive control, standards and diluted samples of patient were added to each well using sampler number 100 then it was incubated for 30 minutes in the room temperature (18 – 25 ºC).

For manual technique, wells were washed using 300 ml washing solution three times. For washing, buffer washing solution was mixed with distilled water in 1: 10 ratio (i. e. for preparing 200 ml wash solution, 180 ml distilled water and 20 ml wash solution was taken and then were mixed). Flowingly, 100 µl of enzyme (antihuman) including antihuman IgM – labeled peroxidase was poured in each well and then incubated for 30 minutes in the room temperature (+180 ºC to +25 ºC). Then it was washed as step 2.

Then 100 µl chromogen was added to each well and incubated for 15 minutes in the room temp (+180 ºC to +25 ºC) (Distant from direct sun light). In addition, 100 µl of stop solution was added to each well to stop the chromogen speed. Photometric measurement was performed in 450 nm or 620 to 650 nm wavelength. Readings is possible 30 minutes after addition of stop solution. Before reading, micro plate was shacked gently to homogenize the solution inside the wells. Calculation of the result was achieved through division of controls and samples over the calibrator.

Finally, results of each one were evaluated using SPSS and t – tests.

RESULTS AND DISCUSSION

Based on the results and analysis of statistics and the tests, following results were achieved. Most studied women were in lower years old age domain (%) while lowest percentage of there were while the lowest percent of studied women were in 41-49 age range. Mean age of studied women was 30.05± 6.95 while youngest women was 16 years old and oldest women was 50 years old (table 1). Most studied women were housekeeper (82.6%), while only 2.7 % of these women had free job (table 2). Most the studied women were living in Guilan province (65.3%), while only 6.7 % of the women were inhabitants of neighbor countries (table 3). Most the studied women reported the history of 3 abortions (78.7 %). While only 21.3 % of studied women reported 4 abortions history (table 4).

Table 1: Age frequency distribution of women with recurrent pregnancy loss

Age range (year)	Number	%
Lower than 30 years old	91	60.6
31-40 years old	43	28.7
41-50 years old	16	10.7
total	150	100

Table 2: Frequency distribution of employment status of women with recurrent abortion

Job	Number	%
Housekeeper	124	82.6
Employee	22	14.7
Free job	4	2.7
Total	150	100

Table 3: Frequency distribution of residence place of women with recurrent pregnancy loss

Residence place	Number	%
Guilan	98	65.3
Other places across Iran	42	28
Other countries	10	6.7
Total	150	109

Table 4: Frequency distribution of the number of abortion in studied women

Number of abortion	Number	%
3	118	78.7
4	32	21.3
Total	195	100

Using chi- square test, it was indicated that there is no significant statistical relationship between studied women's employment status and the number of abortion histories for them with 95 % confidence and low than 5 % error(chi square = 2.17, p= 0.258) (table 5). Using chi- square with 95 % confidence and lower than 5 % error it was indicated that there is no significant statistic relationship between age ranges of studied women and the number of abortions (chi- square, p = 0.615) (table 6). Using chi- square with 95 % confidence and lower than 5 % error it was indicated that there is no significant relationship between the residence place of the studied women and the number of abortion histories (chi- square, 0.214, p= 0.899)(table 7).

Table 5: study on the frequency distribution of previous abortion number in the studied women in respect of employment status

Number of abortions	3 abortions		4 abortions		Total	
Employment status	Number	%	no	%	no	%
House keeper	100	80/6	24	19/4	124	100
Employee	16	72/7	6	27/3	22	100
Free job	2	50	2	50	4	100
Total	118	78/7	32	21/3	150	100

Table 6: Study on the frequency distribution of previous abortion number in the studied women in respect of age range (year)

Number of abortion	3 abortions		4 abortions		Total	
Age range (year)	Number	%	no	%	no	%
Lower than 30 years old	74	81/3	17	18/7	91	100
31-40 years old	32	74/4	11	25/6	43	100
41-50 years old	12	75	4	25	16	100
Total	118	78/7	32	21/3	150	100

Table 7: Study on the frequency distribution of previous abortion number in the studied women in respect of residence place

Number of abortion	3 abortions		4 abortions		Total	
Residence place	Number	%	no	%	no	%
Guilan	78	79/6	20	20/4	98	100
Other places across Iran	32	76/2	10	23/8	42	100
Other countries	8	80	2	20	10	100
Total	118	78/7	32	21/3	150	100

The measure for evaluation of response for IgG in respect of Rubella is: amounts lower than 8 are equal to negative response, amounts between 8 to 11 are equal to Border line and amounts more than 11 are equal to positive response. The measure to evaluate the responses for IgM in respect of Rubella is: amounts lower than 0.8 correspond to negative response, amounts between 0.8 to 1.1 correspond to Border line and amounts more than 1.1 correspond to positive response. Greatest frequencies were obtained for positive IgG and negative IgM in respect to Rubella which were 86.6 % and 96.0 %, respectively. Units are based on $\frac{RU}{ML}$ (table 8).

The measure to evaluate the responses for IgG in respect of CMV is: the amounts lower than 16 correspond to negative response, amounts between 16 to 22 correspond to Border line amounts more than 22 correspond to positive respond. Measure to evaluate the responses for IgM respect of CMU is: the amounts lower than 0.8 correspond to negative response, the amounts between 0.8 to 1.1 correspond to Border line and amounts more than 1.1 correspond to positive response. Greatest frequencies for positive IgG and negative IgM were achieved for CMV which were equal to 80.0 and 94.7 %, respectively. Units were based on $\frac{RU}{ML}$ (table 9). The measure to evaluate the responses for IgG in respect of HSV is: the amounts lower than 16 correspond to negative response, amounts between 16 to 22 correspond to Border line response, amounts more than 22 were correspond as positive response. The measure to evaluate the responses for IgM in respect of HSV is: the amounts lower than 0.8 correspond to negative response, amounts between 0.8 to 1.1 correspond to Border line and amounts more than 1.1 correspond to positive response. Greatest frequencies for positive IgG and negative IgM were related to HSV with 60.0 % and 93.3 %, respectively. Units were based on $\frac{RU}{ML}$ (table 10).

Using chi –square test it was indicated with 95 % confidence and lower than 5 % error that there is no significant statistical relationship between IgG responses in respect of Rubella in the studied women and in the number of abortion histories (p= 0.749, chi square = 0.577). In addition, it was indicated with 95 % confidence and lower than 5 % error that there is no significant statistical relationship between IgG responses in respect of Rubella in the studied women and in the number of abortion cases (p= 0.749, chi square = 0.578). Units are on $\frac{RU}{ML}$ (table 11).

Using chi –square test it was indicated with 95 % confidence and lower than 5 % error that there is no significant statistical relationship between IgG responses in respect of CMV in the studied women and the number of abortions. (p= 0.133, chi square = 2.87). In addition, it was indicated with 95 % confidence and lower than 5 % confidence that there is significant statistical relationship between IgG responses for CMV in the studied women and the number of abortion histories (p= 0.169, chi square = 3.55). Units are on $\frac{RU}{ML}$ (table 12).

Table 8: Frequency distribution of IgG and IgM responses status for Rubella virus in the studied women with recurrent pregnancy loss

		Response status	No	%
Rubella	IgG	negative	19	12/7
		Border line	1	0/7
		Positive	130	86/6
	Total		150	100
	IgM	Positive	4	2/7
		negative	144	96
		Border line	2	1/3
	Total		150	100

Using chi –square test it was indicated with 95 % confidence and lower than 5 % error that there is no statistical relationship between IgG responses for HSV in the studied women and the number of abortions histories (p= 0.84, chi square = 0.106). In addition, it was indicated with 95 % confidence and lower than 5 % confidence that there is no significant statistical relationship between IgM responses for HSV in the studied women and the number of abortions (p= 0.234, chi square = 2.9). Units are on $\frac{RU}{ML}$ (table 13).

Among 150studied women only 2.7 (4)were IgG and IgM positive for Rubella virus,2.7 %(4) were IgG and IgM positive for HSV virus and only 1.3%(2) were IgG and IgM positive for CMV virus (table 14).

Table 9: Frequency distribution of IgG and IgM responses status for CMV in the studied women with recurrent abortion history

		Response status	No	%
CMV	IgG	negative	30	20
		Border line	0	0
		Positive	120	80
	Total		150	100
	IgM	Positive	2	1/3
		negative	142	94/7
		Border line	6	4
	Total		150	100

Table 10: Frequency distribution of IgG and IgM responses status for HSV in the studied women with recurrent pregnancy history

		Response status	No	%
HSV	IgG	negative	60	40
		Border line	0	0
		Positive	90	60
	Total		150	100
	IgM	Positive	6	4
		negative	140	93/3
		Border line	4	2/7
	Total		150	100

Table 11: Frequency distribution of previous abortion number in the studied women based on IgG and IgM responses for Rubella

Rubella	Number of abortions	3 abortions		4 abortions		Total	
		No	%	No	%	No	%
	negative	14	73/7	5	26/3	19	100
IgG	Border line	1	100	0	0	1	100
	Positive	103	79.2	27	20/8	130	100
Total		118	78.7	32	21/3	650	100
	Positive	3	75	1	25	4	100
IgM	negative	113	78.5	31	21/5	144	100
	Border line	2	100	0	0	2	100
Total		118	78/7	32	21/0	150	100

Table 12: Frequency distribution of the number of previous abortions in the studied women based on IgG and IgM responses for CMV

CMV	The	Number abortions	3 abortions		4 abortions		Total	
			No	%	No	%	No	%
		negative	27	90	3	10	30	100
	IgG	Border line	0	0	0	0	0	0
		Positive	91	75/8	29	24/2	120	100
		Total	118	78/7	32	21/3	150	100
		Positive	2	100	0	0	2	100
	IgM	negative	113	79/6	29	20/4	142	100
		Border line	3	50	3	50	6	100
		Total	118	78/7	32	21/3	150	100

Table 13: Frequency distribution of the number of previous abortions in the studied women based on IgG and IgM responses status for HSV

HSV	The Number abortions	3 abortions		4 abortions		Total	
		No	%	No	%	No	%
	negative	48	80	12	20	60	100
IgG	Border line	0	0	0	0	0	0
	Positive	70	77/8	20	22/2	90	100
Total		118	78/7	32	21/3	150	100
	Positive	6	100	0	0	6	100
IgM	negative	108	77/1	32	22/9	140	100
	Border line	4	100	0	0	4	100
Total		118	78/7	32	21/3	150	100

Table 14: Frequency distribution for individuals with positive IgG and IgM for viruses Rubella, HSV, CMV

Virus	No	%
Rubella	4	2.7
HSV	4	2.7
CMV	2	1.3

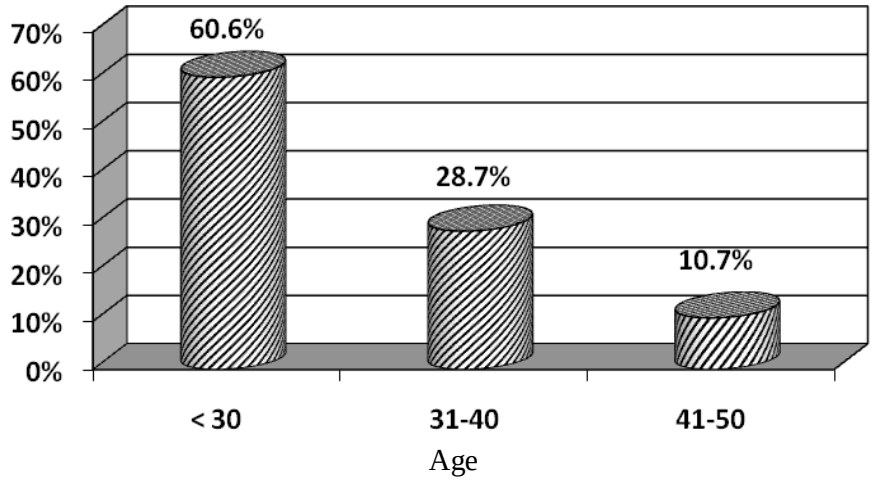

Figure 1: Age frequency distribution for studied women with recurrence pregnancy loss

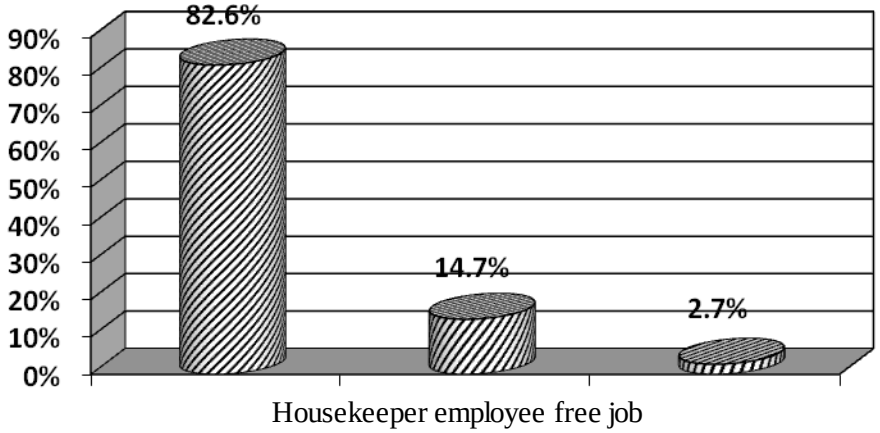

Figure 2: Frequency distribution for job status of studied women with recurrence pregnancy loss

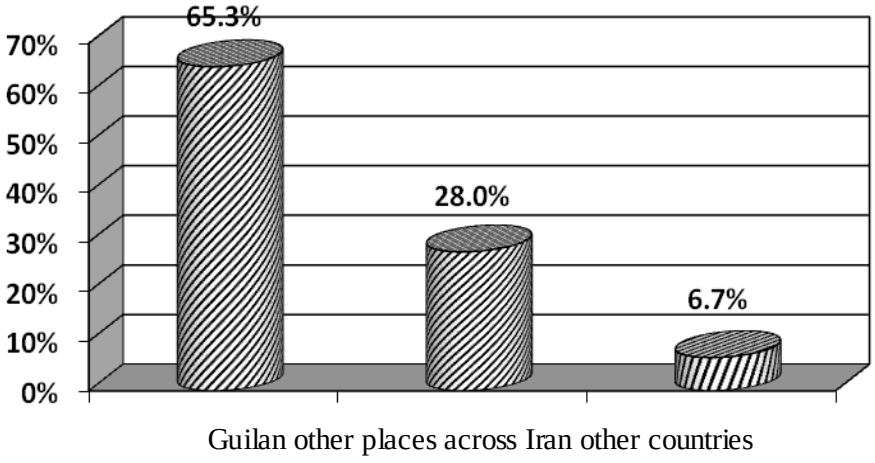

Figure 3: Frequency distribution of residence place of studied women with recurrence pregnancy loss

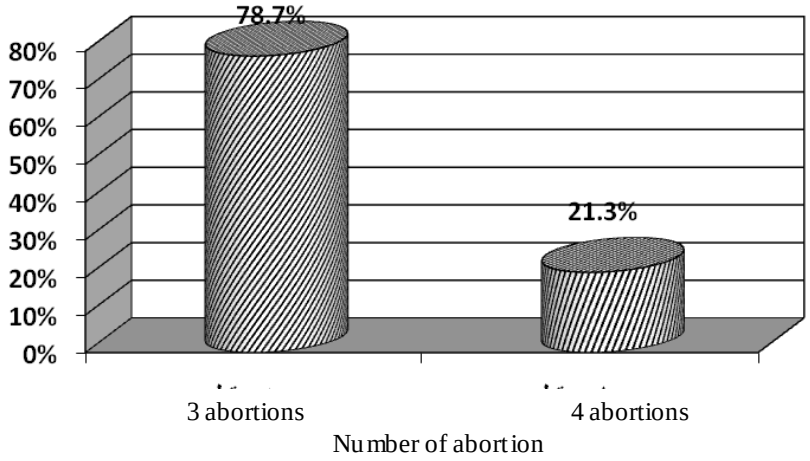

Figure 4: Frequency distribution of the number abortion history in the studied women

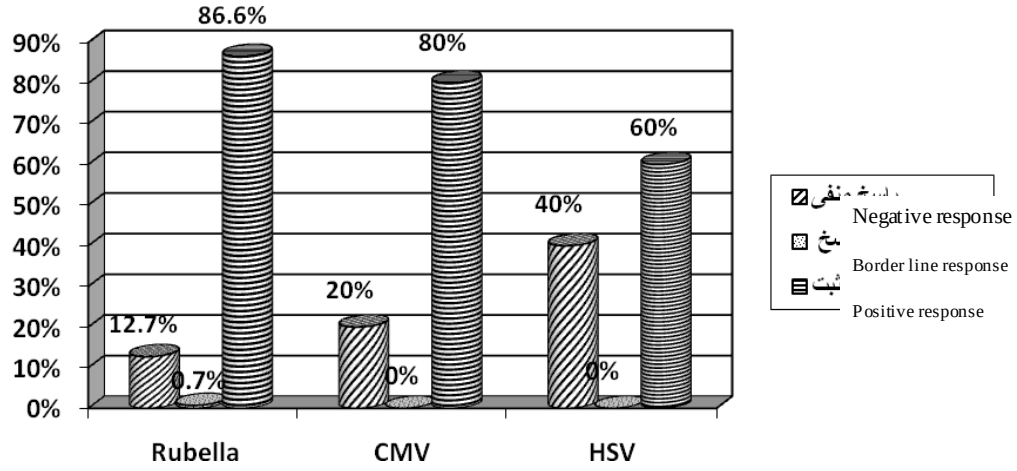

Figure 5: Frequency distribution of IgM response status for Rubella, CMV and HSV in the studied women with recurrent pregnancy

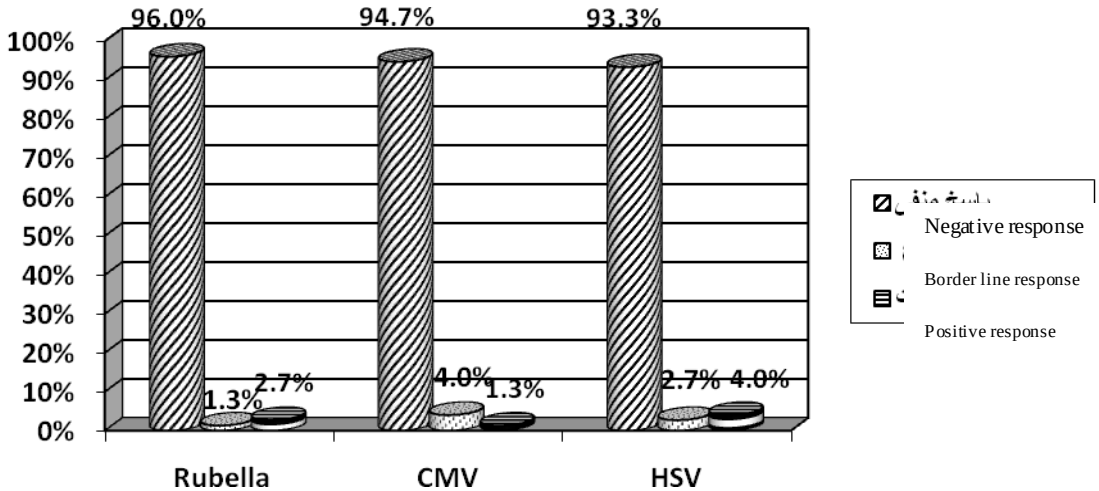

Figure 6: Frequency distribution of IgM positive responses for Rubella, CMV HSV in the studied women with recurrent pregnancy loss

Conclusions

According to the fact that in present study all the studied women had recurrent pregnancy loss history, thus the studied viruses in these women may be the cause the recurrent pregnancy loss in this population. But frequency of these viruses shows any significant relationship to the number of abortion. Thus studied viruses were effective in recurrent pregnancy loss but they didn't affect on the number of abortion, its frequency and intensity. Recommendations are as follows:

1- Since TORCH syndrome is a major factor in recurrent pregnancy loss, to prevent this problem, it is required to study the pregnant women and even those women going to marry in the period before pregnancy in respect of amount of antibodies against these infections agents.

2- Since measles or Rubella is the cause of congenital Rubella and is transferred through mother to the fetus, all the girls must be vaccinated against this virus in the ages before marriage.

3- Some of the mentioned viruses such as CMV persist in the body as hidden viruses and in specific conditions leading to reappearance of virus in the body such as: stress, fear, …, must be avoided.

4- Since there is no specific and it must pass through its natural procedure to recovery and occasionally even after recovery, virus wick persist in the body and recur, thus prevention of disease is better than treatment.

5- In the cases of recurrence of viruses such as HSV and CMV by infection to which IgG of the person will be positive, it is required that infected person should avoid any contact to her child or husband.

6- Since, these viruses transfer through contact, it is favorable that if children are infected to there viruses, they must be avoided to present in the public places to prevent the infection of other people.

7- Informing the publics about these viruses and their transferring paths

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