European Journal of Molecular & Clinical Medicine

ISSN 2515-8260 Volume 07, Issue 8 , 2020

Evaluation of MicroRNA-22 and MicroRNA-184 levels in aborted women having Herpes simplex virus type 2 infection

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Abstract

Spontaneous abortion is one of the most pregnancy problems that happen in early pregnancy when the uterus empties the output of conception. Several factors are associated to causing abortion implicating genetic, endocrinological, immunological and anatomical anomalies as well as infectious agent as infection with herpes simplex virus (HSV-2) is most common virus that widely spread in the world.

Objective: the objective of this study to detect the rate of MicroRNA-22 and MicroRNA-184 in the serum of aborted women and compared with pregnant controls.

Patients and methods: The current study done on 120 women who include 60 aborted women and 60 nonaborted pregnant women as a control group, the age of both groups are ranged from 15-43 years. The blood samples are obtained from both groups and then centrifuged to obtain the serum that used for detection of HSV-2IgM and other part used for RNA extraction. After extraction of RNA, the relative expression of microRNAs was measured using real-time quantitative reverse transcription-PCR method.

Results: The high expression of miRNA-184 was observed in 8(34.8%) aborted women have two or three abortion but only 3(8.1%) of aborted women with one abortion have high miRNA-184. About 23(38.3%) of aborted women with HSV-2 have elevated MicroRNA-22 .Statistically, there was a significant relation between prevalence of MicroRNA-22 and studied groupd (p value=0.005).

Conclusion: miR-184 is overexpressed in recurrent spontaneous abortion.

Keywords: Spontaneous abortion, Herpes simplex virus-2 and Polymerase chain reaction.

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Introduction

Abortion refers to ending of gestation prior 20 weeks of pregnancy period or an embryo with less than 500g weight (Ulsafitri *et al.*,2018). There are different causative agents of spontaneous abortion such as paternal anomalies; presence the history of previous abortion, the mothers age during gestation; autoimmunity as well as environmental factors like cigarette; alcohol and infectious agents (Hosseini *et al.*,2018). During pregnancy period; a number of viral agents may transmitted through placenta and cause pregnancy loss(Mouillet *et al.*,2014).TORCH agents are consider the causative agents of spontaneous abortion and the herpes simplex virus is related with elevating the frequency of spontaneous abortion(lamichhane *et al.*,2016). MicroRNA have a critical roles in pathological and biological process; therefore the detection of MicroRNA in the serum can inverts physiological states so they acts as an important markers of gestational diseases and act as impact factor on biology of trophoblast and immune regulation(LaresgoitiServitje,2015). MicroRNAs are single stranded RNA(ssRNA), short with 19-25 nucleotide in long, noncoding genes that are capable realize mRNAs and suppressing mRNAs translation or mRNAs dissolution (Cretoiu *et al.*,2016).

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More than 1000 MicroRNA encoded in human genome and are able to control more than 60% of our genes (Piedade and Azevedo-Pereira,2016).Utmost of the MicroRNA are located in antisense sequence or intergenic regions(Parveen and Agrawal,2015). MicroRNA is included in each cellular process adjusting regulatory pathways that control the growth, differentiation and functioning of organ in health and disorders (Mouillet *et al.*,2015). Several MicroRNAs such as miRNA-22(Tian *et al.*,2019) and miRNA-184 causing spontaneous abortion(Wang *et al.*, 2017).

Materials and methods

In this case-control study, 60 aborted women and 60 pregnant as a control was included in this study, their age ranged from 15-43 years. 5 ml of blood sample was taken from both studied groups and then centrifuges to obtain serum. Part used for detection of HSV-2IgM bu utilize ELISA kit for the detection of Herpes simplex virus 2 IgM(bioactiva diagnostic, Germany). all samples should be diluted 1+100 with IgM Sample Diluent and then the added 100µL of each diluted serum sample and standard/controls to the appropriate wells. Leaved one well A1 for the blank and incubated for 1 hour at 37C' then washed each well 3 times for 30 seconds with 300µL washing solution , blot and dry by inverting plate on absorbance paper and then added 100 µl Conjugate into all wells except for the substrate Blank well A1and incubated for 30 minutes at 37C°and then aspirated ,washed each well 4 times for 30 seconds with 300µL washing solution using an automatic microplate washer , blot and dry by inverting plate on absorbance paper and then added 100µL of TMB chromogen solution to each well, incubated for 15 minutes at 20-25C' and avoided exposure to direct sunlight and then added 100µL of stopping solution to each well using dispenser. Read the absorbance of the solution in the wells within 30 minutes by using a microplate reader set to 450nm.

Interpretation of results: The Cut-off is the mean absorbance value of the Cut-off Control determinations Sample (mean) absorbance value x 10 = [Bioactiva Units = U]

$$\frac{}{\text{Cut-off}}$$

Cut-off= 0.287, Positive > 11 U , Negative < 9 U

For molecular method, use 400 microliter(µl) of serum mixed with 600µl Trizol and total RNA was extracted from samples according to the manufacturer's instructions from the Trizol protocol(Thermo Scientific, USA). The extracted RNA was used in cDNA synthesis by using the GoScript™ Reverse Transcription System (Promega, USA).The GoScript™ Reverse Transcription System is a convenient kit that includes a reverse transcriptase and an optimized set of reagents for efficient synthesis of first-strand cDNA optimized in preparation for PCR amplification. The components of the GoScript™ Reverse Transcription System can be used to reverse transcribe RNA templates starting with either total RNA, poly(A)+ mRNA or synthetic transcript RNA. Added 4µl of extracted RNA in 0.2 ml PCR tube and then added to it 1 µl of RT primer. The total volume must be 5µL.Thermal cycler program for first reaction is: annealing (70C° for 5 minute), Hold(4C°for 10minute). The Second reaction include 4µl Goscript 5x reaction Buffer, 2 µl MgCl2, 1 µl DNTPs, 0.5 µl Rnasin, 0.5 µl Goscript Reverse Transcriptase, 7 µl Nuclease Free Water , 15 µl of second reaction mix/tube and added 5 µl of template the program of thermal cycler for second reaction include annealing(25 C' for 5 minute), Extension(42 f C' for 60 minutes), Enzyme inactivation(70 C' for 15 minutes) , Hold (4 C' for 10minutes). At the end of the run, the RNA converted to cDNA. Quantus Fluorometer was used to detect the concentration of extracted cDNA. GoTaq qPCR master mix components which includes MgCl2, Thermos aquaticus polymerase, dNTPs, reaction buffer& sybr green.

The primer of MicroRNA-22 (Macrogen, South Korea):
Forward (5`-CTTCAACTGGCAGCTT-3`)

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Reverse (5`-GTGCAGGGTCCGAGGT-3`)
Reverse transcriptase (GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAA CACAGTT)
Primers for MicroRNA-184(Macrogen, South Korea)
Forward (5`-GTGTGGACGGAGAACTGAT-3`) Reverse
(5`-GTGCAGGGTCCGAGGT-3`)
Reverse:GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACACCCTT.

For each MicroRNA detection, in the real time PCR step uses Master mix components which includes 5µl qPCR Master Mix, 0.25 µl MgCl2, 0.5 µl Forward primer, 0.5 µl Reverse primer, 2.75 µl Nuclease Free Water, 1 µl cDNA. Aliquot per single rxn is 9µl of Master mix per tube and add 1µl of Template(Total volume is 10 µl.). The real time program are initial denaturation (95 C° for 5minutes, denaturation (95 C° for 15sec), annealing (55 C° for 15sec.), extension (72 C° for 15sec.).

Relative quantification
Folding =2-ΔΔCT
ΔΔCT =ΔCT Treated - ΔCT Control
ΔCT =CT gene - CT House Keeping gene.

Statistical Analysis

Statistical analyses were performed using SPSS Statistical Package for Social Sciences (version 20.0 for windows, SPSS, Chicago, IL, USA). **Results**

Table (1) showed the incidence of Herpes Simplex virus-2 IgM antibody in studied groups according to age groups. among aborted women most HSV-2 IgM antibody occur in age groups 21-30 &more than 30 years with rate 26(100.0%)&28(100.0%). There was a significant relation between IgM and cases in all ages (P=0.005).

Table (1): Distribution of HSV-2 IgM in studied groups according to age

Cases					
		Abortion		Normal	
Age	HSV-2 IgM	Count	%	Count	%
≤20	-ve	0	0.0%	16	100.0%
	+ve	6	100.0%	0	0.0%
21-30	-ve	0	0.0%	24	100.0%
	+ve	26	100.0%	0	0.0%
>31	-ve	0	0.0%	20	100.0%
	+ve	28	100.0%	0	0.0%

Among aborted women, most of the positive HSV-2 IgM localized in rural residence with a rate 33/60(55.0%) and about 27(45%) localized in urban area. There was a significant relation between incidence of HSV-2 IgM antibody and residence (table2).

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Table (2) prevalence of HSV-2 according to residence

Risk factor	Variable	Cases				P_{value}
		Aborted women with HSV-2 IgM (n=60)		Non-aborted women (n=60)		
		No.	%	No.	%	
Residence	Urban	27	45.0%	16	26.7%	0.005
	Rural	33	55.0%	44	73.3%	

The prevalence of MicroRNA-22 are reviewed in table (3), 23(38.3%) of aborted women with HSV-2 have elevated MicroRNA-22 and 37(61.7%) have low MicroRNA-22 expression. Statistically, there was a significant relation between prevalence of MicroRNA-22 and studied group (p value=0.005). **Table (3): Prevalence of MicroRNA-22 in studied cases**

miRNA-22	Groups			
	Aborted women with HSV-2		Pregnant women	
	No.	%	No.	%
Low	37	61.7%	60	100.0%
High	23	38.3%	0	0.0%
Total	60	100.0%	60	100.0%
P value	0.005			

Table (4) showed the distribution of MicroRNA-184 in aborted women according to the number of abortion. The high expression of miRNA-184 was observed in 8(34.8%) aborted women have two or three abortion but only 3(8.1%) of aborted women with one abortion has high miRNA-184. Statistically, there was no significant relation between distribution of MicroRNA-184 and number of abortion (P value=0.776).

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Table (4): Prevalence of MicroRNA-184 according to number of abortion

		Number of abortion			
		One		Two or more	
		No.	%	No.	%
miRNA-184	Low	34	91.9%	15	65.2%
	High	3	8.1%	8	34.8%
P value	0.776				

Discussion

The current study may be agreed with study done in Al-Hilla that found the percent of HSV-2 was about 25/60 (41.6 %)(at age 20–29 years and showed the aborted women carry IgM-HSV-2 with a rate of 112(70%) (Almamory,2016). Other study may be disagreed with current outcomes that found HSV-2 seroprevalence were elevated in urban regions compared to rural areas (Biškup *et al.,* 2015). Other study found the HSV IgM was detected in 73.9% of patients (lamichhane *et al.,* 2016 .The MicroRNAs control the gene expression in the uterus that related to anti-inflammatory responses and also have a role in maternal-fetal immune tolerance(Barchitta *et al.*,2017) so that the dysfunction in the expression of MicroRNA consider as a factor in causing spontaneous abortion by effecting the angiogenesis, invasion, proliferation and apoptosis(Zhou *et al.*,2018). MicroRNAs of Herpes viruses are capable to target the viral and host cellular mRNA. Cellular MicroRNAs are mainly associated to cell regulation, proliferation, apoptosis and host immunity (Piedade and Azevedo-Pereira, 2016). Several MicroRNAs such as miRNA22 that suppress the growth of endothelial cell and invasion by decrease the expression of HIF-α and inhibiting it (Xiong,2012)as well as inhibit angiogenesis by targeting the VEGF and causing spontaneous abortion(Tian *et al*,2019). The microRNA184 boosts the trophoblast cell apoptosis by targets the WIG1 and causing early spontaneous pregnancy loss (Zhang *et al*, 2019). Other result may be agreed with current outcomes that found the MicroRNA-184 was over-regulated in the villus and decidua of recurrent spontaneous aborted women (Qin *et al.,* 2016). Also agreed with other study that found the MicroRNA-22 was highly expressed among women with spontaneous abortion (Tian *at el.,* 2019).

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