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DETECTION OF BETA-2 MICROGLOBULIN GENE AND SOME BIOCHEMICAL PARAMETERS IN WOMEN WITH INFERTILE IN DIYALA PROVINCE

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Abstract
Female infertility is a disorder that occurs in women for many known or unknown reasons. The present study aimed to find out Beta-2 microglobulin gene variants and their association with infertility using conventional PCR and electrophoresis techniques. Furthermore, it showed the differences in some variables such as age, WBC, Hb, Duration of infertility. Forty-five participants were selected for this study, 25 were identified as infertile women, and the rest were considered control (healthy women). Blood samples were collected from the participants on the day (12-13) of the menstrual cycle and sent to the laboratory; all blood samples were preserved. Plasma was isolated from blood cells, ready for immediate DNA extraction, and kept at -20°C. DNA was extracted for detection of the Beta-2 microglobulin gene by PCR. The results of the study are included that higher value of infertile women percentage was (44%) at the age (36-40) year while the lower value of infertile women percentage was (4%) at the age (15-20) year at a level significant (P value<0.05). Moreover, the results demonstrated that the average of WBC, B2M, Hb, Duration of infertility (year), and Duration of the period (day) were (6.8), (1), (12.7), (1.5), and (5) in the control group, while an average of WBC, B2M, Hb, Duration of infertility (year), and Duration of the period (day) were (6.7), (1), (12.6), (5), and (5) in the infertile group at a significant level (p>0.05). In the end, there are no significant differences between the infertile women group and the control group in WBC count, B2M, Hb, Duration of infertility (year), and Duration of the period (day) at a significant level (p>0.05).

Keywords: Beta-2 microglobulin, women, reproductive disorders, Diyala, PCR

Introduction

Infertility is the inability to have children after two years of regular sexual life without any contraceptive for both spouses. Infertility in women is divided as primary sterility that affects a woman since the beginning of her sexual life or marriage, usually due to glandular or hormonal diseases or the immaturity of the reproductive organs for its formation (1). The rate of primary infertility is high in cold countries. Secondary infertility affects a woman after giving birth to one or two children or after having an abortion. It is caused by childbirth complications, miscarriage and all infections that may affect the uterus and nevus. Secondary relative infertility is high in developing countries (2).

The World Health Organization (WHO) has documented infertility as a public health issue worldwide by the World Health Organization (WHO) (3). Based on WHO criteria, infertility is defined as failure to achieve conception after 12 months or more of regular unprotected sexual intercourse. Infertility is primary for a couple with no children or secondary after having one child (4).

The global estimate of infertility for non-surgically sterilized fertile women revealed a regional variation in infertility, with a global estimation of 72.4 million infertile women worldwide, of whom 40.5 million are seeking fertility treatment (5). Moreover, estimation of infertility based on 277 surveillances representing 190 different regions revealed that 48.5 million couples worldwide could not have a child after five years (6). An epidemiological study of infertility in Scotland revealed that approximately one in five women had experienced infertility (7). The authors concluded that the main factors associated with infertility were endometriosis, Chlamydia trachomatis infection and pelvic surgery (8).

Moreover, the prevalence of infertility was estimated at 2.5% in the U.K. (9). A subsequent study in 2009 estimated annual infertility at 0.9 among 1000 couples. The latest estimated infertility rate in the U.K. is one in seven couples, but there is considerable variation in the prevalence of infertility among different studies. This is probably because of differences in the definition of infertility and the measurements used. For instance, one study was conducted on infertile couples who had experienced 5 years of infertility (10).

In comparison, the inclusion criteria stipulated that any infertile couple had to attempt conception for 24–48 months in another study. The epidemiology of infertility is based on those infertile couples who seek help. The leading overall causes of female infertility are ovulatory disorders, with an incidence ranging between 21–32%, and tubal disorders, which vary between 14–26% (11).

Our study aims to determine whether there is a role for WBC, Hb, or the period of infertility has a differential role between healthy women and women with infertility

Material and Working Methods

Sample Collection

Blood samples were collected from among (45) females volunteers who attended Al-Batool Teaching Hospital, Al-Naba Laboratory and Al-Shams Laboratory in Baquba, Diyala Governorate Center from November 20, 2020, to January 20, 2021, for 25 women (18 to 39 years

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old): consisting of twenty healthy subjects who considered as control and the rest are infertile. The period of sample collection at (day 12 or 13 of the menstrual cycle) and delivery to the laboratory using test tubes, the plasma was isolated from the rest of the blood components using a centrifuge and then kept in the freezer.

Laboratory Equipment and Supplies

They have used the laboratory equipment, and supplies are (BSC-ll) Kimo, 24700, Oven (Memmert), Centrifuge cooling (Sigma 1-13), Harz, High-speed cold centrifuge (Memmert), Vortex mixer (Sigma), P.H. meter (Biomax), Sensitive balance (Sartorius), Refrigerator (Diora), Nanodrop spectrophotometer (Thermo), Thermal Cycle (Alpha DNA), Electrophoresis (Helena), UV-transillminatotou (Herolab), Gel documentation (Herolab), Micropipettes (Brand), Magnetic stirrer, Screw capped (Schott),Flasks (Schott).

The used chemicals:

The use of the chemicals shown in table (2) during the study period Agarose Ethidium Bromide, TBE _ buffer (10x), Loading dye, DNA Ladder 100bp/1000bp (Promega, USA(.

WBC:

Blood samples were collected in tubes containing anticoagulants (EDTA) to prevent normal blood clotting. Blood was drawn from a vein for an automated analyzer. The examination results indicate that a decrease or increase in the number of WBC leads to a disorder in the Blood or the presence of severe infections or other viral infections, which may be one of the causes of infertility.

Hb measurements:

A low level of H.B. leads to anemia, while a high percentage of RBC leads to a high level of H.B., which can be one of the causes of infertility. It was found that all percentages fall within the normal range for normal and infertile women.

DNA extraction:

All blood samples were preserved. Plasma was isolated from blood cells, ready for immediate DNA extraction, and kept at -20°C. DNA was extracted by DNAMini®QIAamp kit.

B2M Gene detection:

The concentrations of DNA extracted from serum samples (45 samples), purity was measured based on absorbance values 260/280 as it ranged between 1.43- 0.79. All samples are consecutively numbered by electrophoresis by detecting special β2M sequences of the selected gene characteristic. The interaction of its size is 105 base pairs, and it has been proven through the results that all samples used in this study belong to β2M sequence amplification.

PCR for Beta-2-Microglobulin

DNA extracting from Blood the b2microglubulin sequences were downloaded from the Gen Bank sequence database of the National Center for Bioinformatics NCBI, using the Primer3 program. The primers were manufactured by Koma Biotech Inc. (Korea), as shown in table (1).

Table (1): showed sequence of the forward and reverse primers

B2m (forward)	B2m(reverse)
5_TCCAACATCAACATCTTGT_3F	5_ TCCCCCAAATTCTAAGCAGA _3R

Preparation of PCR Reaction

The primers were processed with nuclease-free water according to the instructions prescribed by the manufacturer for making a stock of (100) picomoles/µl and stored at -20℃ in a deep freezer. The processed primer was diluted accordingly to prepare a stock of 10 Pico moles / µl by PCR reaction. The reaction mixture for PCR was prepared with the primers mentioned in the present study as listed in the table**.** The components of the polymerase chain reaction and the mixed quantities are shown in Table (2).

Table (2): Protocol of PCR reaction mixture volumes used in the current study

PCR reaction	Volume (µl)
PCR Master mix	10µl
DNA template	5µl
Forward primer (10 p mol)	1µl
Reverse primer (10 p mol)	1µl
ddH20	3µl
Total volume	20 µl

The reaction components mentioned above are placed in the tubes in the standard kit, which contains all the other components needed for the reaction (Tag DNA Polymerase, Tris-Hcl PH 9.0, dNNTPs, KCL, Mgcl2, Stabilizer, tracing dye PCR). All the reaction tubes were

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mixed by Vortex At a speed of 3000 rpm for 3 minutes. It was placed in a PCR thermocycler using the Conventional PCR thermocycler system for each gene, as shown in table (3).

Table (3): Protocol of PCR reaction mixture volumes used in the current study

PCR program	Temperature (°C)	Time	Number of cycles
Initial Denaturation	**94**	**5min**	**35**
denaturation	**94**	**30 second**	
Annealing	**55**	**30 second**	
extension	**72**	**30 second**	
Final extension	**72**	**10 minutes**	
Hold	**4**	**5 minutes**	

Preparation of agarose gel and electrophoresis:

An agarose gel was prepared at a concentration of 2% by dissolving 1 g of agarose powder in 50 ml of 1X TBE buffer. The solution was heated to boiling. The solution was homogenized to a clear form and then allowed to cool at 50°C; 1 microliter of 0.3mg/ml ethidium bromide dye was added to and mixed well; gently tower the solution. The mixture is poured into a tray (to which the Combo is pre-installed) for the holes needed to load the DNA samples, left at room temperature to solidify for 45 minutes, the hardened gel is transferred to a gel tank, and the comb is raised quietly, and placed Under the transfer basin is an opaque black plate so that the pits are visible. The transfer basin is filled with 1X TBE buffer until it covers the gel. DNA samples are placed in the holes using a micropipette with a volume of 10 microliters from each sample, taking into account that the sample does not come out from the hole's surface. The DNA ladder is placed in the hole designated for it on one side of the gel with a volume of 7 microliters mixed with 3 microliters of buffer loading. Then, electrophoresis is carried out by connecting the electrodes and preparing it with a capacity of 65 volts, and the relay is carried out towards the positive electrode. After 45 minutes, when the blue dye reaches the end of the gel, the relay is stopped, and the gel is transferred to the UV Trans-illuminator (Figure 6). At a wavelength of 320 nm, to visualize the DNA bundles and estimate their molecular size compared to the volume guide, photograph the gel using an imaging device (Sambrook and Russel, 2001).

The Results:

Our results divided the age categories of the tested individuals for five categories (15-20), (21-25), (26-30), (31-35), and (36-40). The age distribution of control group were 1(5%), 3(15%), 6(30%), 6(30%), 4(20%) at ($P > 0.05$) respectively, while the age distribution of Infertile were 1(4%), 5(20%), 3(12%), 5(20%), 11(44%) at ($P > 0.05$) respectively.

However, higher value of infertile women percentage was (44%) at the age (36-40) year while the lower value of infertile women percentage was (4%) at the age (15-20) year at a level significant (*P value*<0.05) as shown in table (4) and figure (1) A and B.

Table (4): The age distribution of participants

Age period of study groups				
Age periods	**Age group**		**Groups of Participants**	
			Control	**Infertile**
	15-20	**N**	**1**	**1**
		%	**5%**	**4%**
	21-25	**N**	**3**	***5**
		%	**15%**	**20%**
	26-30	**N**	**6**	**3**
		%	**30%**	***12%**
	31-35	**N**	**6**	**5**
		%	**30%**	***20%**
	36-40	**N**	**4**	**11**
		%	**20%**	***44%**
Total		**N**	**20**	**25**
		%	**100%**	**100%**

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P-values	*P value*>0.05	**P value* ≤0.05

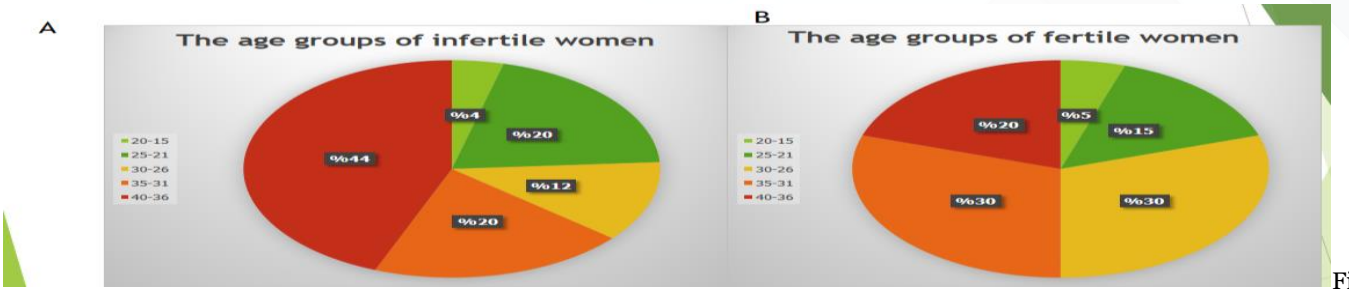

Figure 7(A, B) shows no significant difference between the number of infertile women and control.

Figure (1) A: The infertile women age groups: B the fertile women age group

The results of the study demonstrated that the mean value of WBC, B2M, Hb, Duration of infertility (year), and Duration of the period (day) were 6.8, 1, 12.7, 1.5, and 5 in the control group, while the mean value of WBC, B2M, Hb, Duration of infertility (year), and Duration of the period (day) was 6.7, 1, 12.6, 5, and 5 in Infertile group.

The results showed no significant differences in blood variables at a significant level (p>0.05) between the infertile women and controls women. Table (5) showed average values for all blood variables in the infertile and control for all the participants.

Table (5): The comparison between infertile women and controls by using some variables

Variables	Control			Infertile			T-test	P-value
	Mean	SD	SEM	Mean	SD	SEM		
WBC	6.8	1.5	0.3	6.7	1.3	0.2	0.2525	P > 0.05
B2M	1	0	0	1	0	0	0	P > 0.05
Hb	12.7	1.4	0.2	12.6	1.2	0.2	0.1894	P > 0.05
Duration of infertility (year)	1.5	3	0.6	5	3	0.6	2.209	*P < 0.05
Duration of period (day)	5	1.2	0.2	5	0.9	0.1	0.2525	P > 0.05

The extraction of genomic DNA from (45) samples were confirmed as bands by gel electrophoresis. DNA concentration was determined. All samples showed positive bands for B2M for both infertile and controls. DNA was extracted from the plasma, and PCR was performed to amplify B2M gene (105 bp), then electrophoresis was performed to detect the DNA bunds using the specific primers. Gel electrophoresis was used to confirm the PCR results, as shown in figure (2).

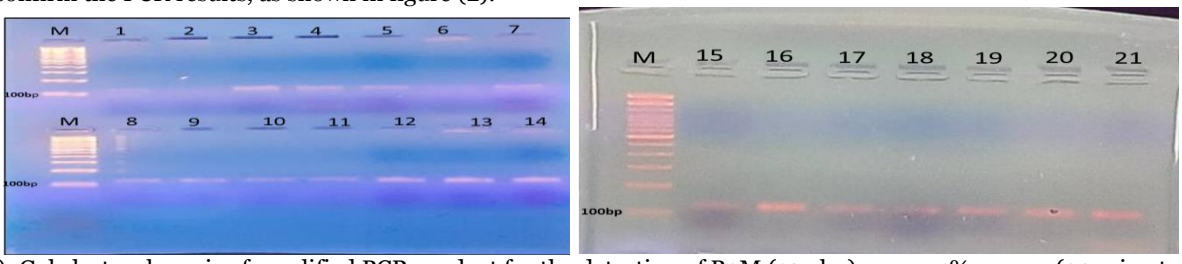

Figure (2): Gel electrophoresis of amplified PCR product for the detection of B2M (105 bp) run on 1%agarose (90 min at 70 volts), stained with ethidium bromide, lane 1-21; M: Marker DNA ladder (100-1000bp); all lanes are positive

Discussion

Our results reveal that a higher rate of infertile women percentage was (44%) at the age (36-40) year while the lower rate was (4%) at the age (15-20) year. The results conducted in this study showed no significant differences in blood variables (WBC and Hb) between the infertile women and controls. Also, the Duration of the period and differences between the Duration of infertility did not show a significant difference at (p>0.05).

Oocyte quality and number are decreased with age, especially after the mid-30s. Patients with 35 years or more should regularly evaluate infertility (12) (13) and agree with our findings.

The rate of fertility begins to decline at age 35 (14). Infertility is a condition of the reproductive system characterized by the inability to conceive after one year or more of frequent unprotected sexual contact with a male partner. Infertility affects large numbers of individuals

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of reproductive age all over the globe, and it is a common problem (15). Infertility may be caused by various conditions affecting the reproductive system, including uterus abnormalities, ovaries, fallopian, and hormones (16).
In practice, infertility is unable to pregnant after a year of trying (or longer). When it comes to fertility, the most prevalent causes are issues with ovulation or hormones (17). Specific issues prohibit an egg from being released at all, whilst others prevent an egg from being produced sometimes during cycles but not others, depending on the issue. Ovulation issues may occur due to the following conditions: polycystic ovarian syndrome (PCOS), hypothyroidism, and hypothyroidism (18).
According to the studies (15-18), there is no relationship between infertility and some haematological markers such as WBC count and haemoglobin level in th females. There are no reports talk about this relationship
Based on the results of our study, all the samples have shown the Beta-2 microglobulin gene by using PCR techniques (100%); B2 microglobulin (B2M) is a component of MHC class I molecules. MHC class I molecules include the proteins 1, 2, and 3 found on all nucleated cells. The B2M gene (19) in humans is responsible for encoding the 2 microglobulin proteins (20).

Conclusion

WBC count and haemoglobin concentration are not related to infertility in females. Furthermore, a higher rate of infertile women occurred at (36-40) years, while the lower rate occurred at (15-20) years old. WBC, B2M, Hb, Duration of infertility, and Duration of the period does not show an association between them with infertility of the females.

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