

Association of ANGPTL8 gene polymorphism with the occurrence of coronary artery disease in Iraqi patients

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Abstract

OBJECTIVE: It has been shown that ANGPTL8 controls the metabolism of fatty acids and triglycerides (TG). Reduced adipogenesis, which was defined by decreased TG, was linked to decreased ANGPTL8. The aim of the present study was to evaluate the association of ANGPTL8 gene polymorphism with the occurrence of coronary artery disease in Iraqi population. **RESEARCH DESIGN AND METHODS:** two hundred coronary artery disease patients with >70 blockage coronary artery as well as to 200 apparently individuals who were diagnosed as negative findings by coronary angiography (Control group) were investigated for several biochemical parameters as well as genotyping for rs892066 and rs2278426 of ANGPTL8 gene SNPs by ARM-PCR technique. **RESULTS:** The examinations of rs892066 approved significant variation after the adjustment for age and sex under the codominant model in heterozygous genotype, and under the dominant model the GG+CG genotype in patients group was be higher than of control group. The assessment of the minor allele frequency (G) approved the association of rs892066 in the occurrence of CAD. Along with the most recent outcomes, Multinomial logistic regression analysis was used to analyze the genotype and allele frequencies of the rs2278426 ANGPTL8 gene polymorphism in CAD and control individuals under the codominant, dominant, recessive, and overdominant models. The examinations failed to explore significant variation after the adjustment for age, sex and BMI and smoking status. The assessment of the minor allele frequency (T) indicated that no association between presence minor allele frequency (T) in CAD patients and the control group. **CONCLUSIONS:** SNP rs892066 may play unique role in the occurrence of CAD while rs2278426 of ANGPTL8 genes are not associated with CAD in Iraqi patients.

Keywords: Coronary artery disease, ANGPTL8, single nucleotide polymorphisms, ARM-PCR.

INTRODUCTION

It has been shown that ANGPTL8 affects beta-cell proliferation and has been proposed as a potential target for beta-cell regeneration (Yi et al 2013). It has been shown to control the metabolism of fatty acids and triglycerides (TG). Reduction in ANGPTL8 was linked to adipogenesis that was marked by a decline in TG. Likewise, animals missing betatrophin showed identical fasting TG levels to wild type mice and somewhat lower TG levels after eating (Ren et al 2012). While ANGPTL8 is only found in the liver of humans, it is abundant in the liver and adipose tissue of mice, including both brown and white adipose tissue. (Zhang R,2012).

It seems that a number of processes had a role in controlling the expression of ANGPTL8. The expression of ANGPTL8 is greatly influenced by structured diet. While the fasting treatment seemed to decrease ANGPTL8 expression to roughly 80% in WAT and BAT, feeding mice a high-fat meal showed noticeably elevated ANGPTL8 expression in both the BAT and liver. (R. Zhang, 2012)

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The rate-determining step in the postprandial clearance of circulating triglyceride-rich lipoproteins is the enzymatic activity of lipoprotein lipase (LPL). The essential enzyme for lipid metabolism is LPL. Chylomicron and very-low-density lipoprotein (VLDL) triglycerides may be hydrolyzed to release free fatty acids for storage in adipose tissue as well as for oxidation and use in the heart and other tissues (He et al 2018). Variations in the LPL locus' activity, serum lipid levels, and risk of CAD are all influenced by many polymorphisms (Ahmadi et al 2015). The genes ANGPTL8 and ANGPTL6 were examined for their relationship to coronary artery disease. (Guo et al 2015, Quagliarini et al 2012 and Locke 2015 et 2015).The results were controversial. However, for our knowledge, no genotyping was studied in relevance to the lipoprotein lipase.

In the current study the association of two common SNPs (rs892066 & rs2278426) of ANGPTL8 gene with lipid profile will be studied in coronary artery disease patients. The outcome of the study will improve the plan of the management of the disease.

Materials and Methods

Study subjects

The patient group included 200 coronary artery subjects who attended the Cardiac Center in AL-Sader Medical City, Najaf, Iraq, as well as 200 individuals (Control group) were enrolled, randomly selected from September to December 2020. The inclusion criteria were those patients more than 30 years old who were diagnosed as having ≥ 70 blockage of the coronary artery as well as 200 individuals who were diagnosed as negative findings by coronary angiography (Control group) were enrolled based on AHA guidelines. Patients with non-atherosclerotic vascular diseases ,Renal disease , Hepatic disease , Cancer, Those treated with antihyperlipidaemic medicines were excluded from the study.200 presumably individuals from a cardiology facility who were determined to have had negative results from coronary angiography made comprised the control group. The inclusion criteria were those over the age of 30. Age, sex, family history, drug history, medical history, and other relevant information were collected from each subject. We also assessed each person's weight, height, and BMI.The research was authorized by the ethical committee of Kufa Medical College.

Phenotyping analysis

All of the subjects had five milliliters (ml) of blood drawn from them, which was then collected in two tubes: three milliliters were placed in a plain tube that did not contain any anticoagulants in order to estimate fasting atherogenic lipid concentrations and lipoprotein lipase level, and two milliliters were placed in an EDTA tube in order to extract DNA from the samples.

DNA extraction and SNPs Genotyping

The kit of G-spin™ complete DNA was used in the process of extracting DNA from whole blood samples. 2017 publication by INTRON Biotechnology. The EasyTaq® PCR Super Mix is a solution that is ready to use and contains EasyTaq® DNA polymerase, dNTPs, and optimized buffer all in one convenient package. The SuperMix PCR is delivered at a concentration of 2x, but it is adjusted to a concentration of 1x by the addition of the template, primers, and water. The extension rate is around 1-2 kb per minute. At the 3' end of the PCR product, it is possible to produce "A" without using a template. (www.transgenbiotech.com), after which the PCR product was inspected on an agarose gel containing 2%, and finally photo documentation was used to see the results.

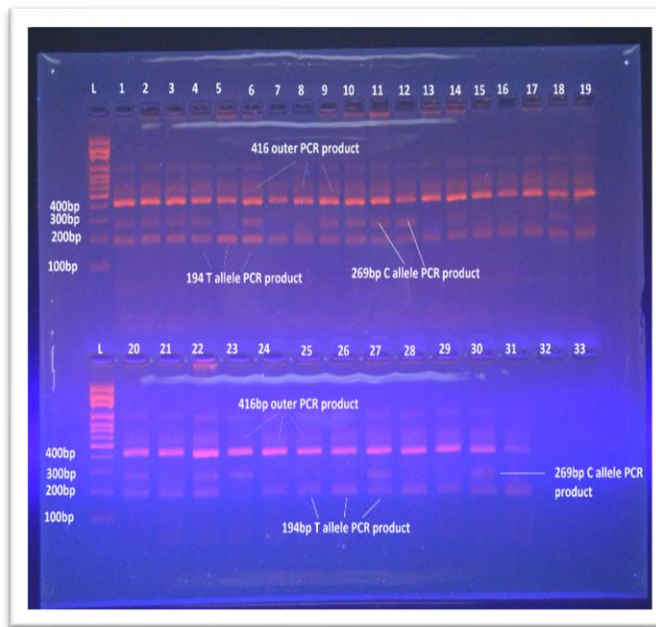
Statistical analysis

The numerical variables were represented using mean and standard deviation. To analyze the differences in mean scores between the control group and the T2DM group, a Student's t-test was carried out. The Student's t-test, implemented in SPSS version 20.0, was used in order to do genotype-based comparisons of the mean values of continuous variables (SPSS Inc., Chicago, IL). Data that was classified into categories, such as genotypes and alleles, were given a frequency value. In every single statistical test, the threshold of significance was set at less than 0.05. The Hardy–Weinberg equilibrium, sometimes known as HWE for short, is a mathematical relationship that links genotypes and allele frequencies (Thompson et al.1991). The genetic power of the population was calculated with the help of the web program OSSE (online sample size estimator) (osse.bii.a-star.edu.sg). This type of statistical analysis and these models were used according to Hashemi et al. (Mohammad Hashemi et al. 2013) and Matharoo et al. The output data included as odds ratio (OR), confidence interval (CI 95%), and P value with and without adjustment for age,sex, and BMI. This type of statistical analysis and these models were used (Matharoo et al. 2013).

Results

The genotype and allele frequencies

The PCR product was electrophoresed on 2% agarose and directly visualized with ethidium bromide under UV light. The amplification product of rs2278426 of ANGPTL8 gene polymorphism was done by T- ARM PCR. The products of amplification were analyzed by agarose gel electrophoresis. Results demonstrated two bands of 416 bp and 269 bp wild type(CC), three bands of 416, 269 and 194 bp heterozygous(CT), two bands of 416, 194 bp homozygous (TT)(figure 1.1)



Multinomial logistic regression analysis was used to analyze the genotype and allele frequencies of the rs2278426 ANGPTL8 gene polymorphism in CAD and control individuals under the codominant, dominant, recessive, and overdominant models. After adjusting for age and sex status, these tests fell short in their attempts to uncover considerable variation. Minor allele frequency (T) evaluation revealed no correlation between the existence of minor allele frequency (T) in CAD patients and the control group (OR=1.07, CI 95%= 0.77-1.48, P value = 0.67) (Table 1.1).

Figure 1.1: PCR product of ANGPTL8 gene polymorphism (rs2278426) analyzed by agarose gel electrophoresis.

Table 1.1: Distribution of the genotype and allele frequency of rs2278426 of ANGPTL8 gene polymorphism in patient and control groups

	CAD n=200	Control n=200	adjusted OR (95% CI)	P value	AIC
Codominant					
CC	1 (3%)	6(0.5%)			578
CT	90 (45%)	85 (42.5%)	5.66 (0.66-48)	0.111	
TT	109 (54.5%)	109 (54.5%)	6 (0.71-50)	0.099	
Dominant					
TT	109 (54.5%)	109 (54.5%)			579.9
CC-CT	91 (45.5%)	91 (45.5%)	1.0 (0.67-1.48)	1.0	
Recessive					
TT-CT	194 (97%)	199 (99.5%)			576.2
CC	6 (0.5%)	1(3%)	5.84 (0.69-49)	0.103	
Overdominant					

CC-TT	110 (55%)	115 (57%)			
CT	90 (45%)	85 (42.5%)	0.90(0.60-1.34)	0.614	579.5
T allele Frequency	92(23%)	97(24%)	1.07 (0.77-1.48)	0.67	

T-ARM PCR was used to amplify the rs 892066 ANGPTL8 gene polymorphism product. Agar gel electrophoresis was used to evaluate the amplified products. The results showed two wild-type bands of 351 bp and 178 bp (CC), three heterozygous bands of 351, 216, and 178 bp (CG), and two homozygous bands of 351 bp and 178 bp (GG) Figuring 1.2

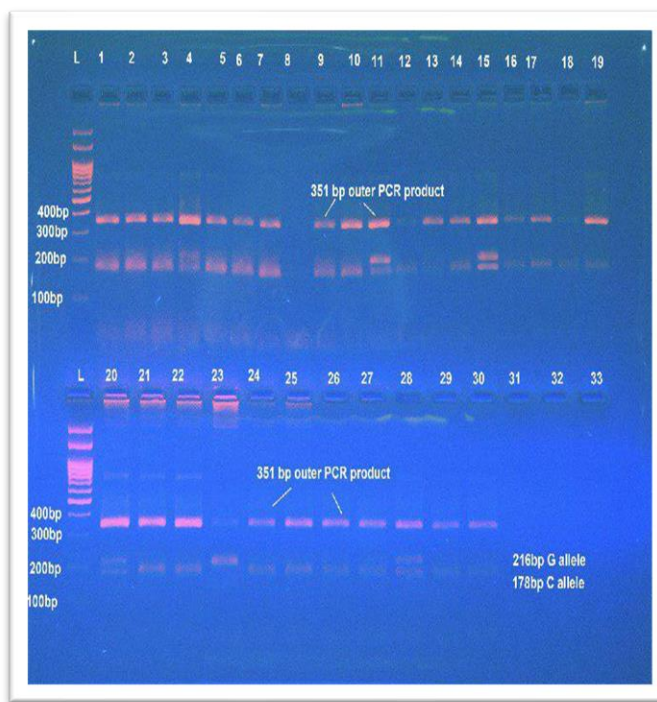


Figure 1.2: product of ANGPTL8 gene

polymorphism(rs892066) analyzed by agarose gel electrophoresis.

Using multinomial logistic regression analysis, the genotype and allele frequencies of the rs892066 ANGPTL8 gene polymorphism in CAD and control individuals were calculated under several models (Table 1.2). Patients were clearly more likely to have the heterozygous genotype (CG) under the codominant model (OR= 1.7, CI 95%= 1.12-2.85, P=0.011) than those in the control group. Additionally, according to the dominant model, CAD patients with the GG+CG genotype were predicted to have substantially greater odds of developing the disease (OR=1.71, CI 95% =1.13-2.58, P=0.01) than those in the control group. The examination of the minor allele frequency (G) revealed that the proportion of CAD patients with the minor allele A was substantially greater than that of the control group (OR=1.52, CI 95%=1.06-2.17, P=0.020) (Table 1.2).

Table 1.2: Distribution of the genotype and allele frequency of rs892066 of ANGPTL8 gene polymorphism in patient and control groups

	CAD n=200	Control n=200	adjusted OR (95% CI)	P value	AIC
Codominant					
CC	113 (56.5%)	138 (69%)			
CG	84 (42%)	60 (30%)	1.7 (1.12-2.85)	0.011	575.4

GG	3 (1.5%)	2 (1%)	1.44(0.92-2.65)	0.085	
Dominant					
CC	113 (56.5%)	138 (69%)			
GG-CG	87 (43.5%)	62 (31%)	1.71 (1.13-2.58)	0.01	573.4
Recessive					
CC-CG	197 (98.5%)	198 (99%)			
GG	3 (1.5%)	2 (1%)	1.05 (0.75-1.85)	0.84	576.2
Overdominant					
CC-GG	116 (58%)	140 (70%)			
CG	84 (42%)	60 (30%)	1.16 (0.76-1.75)	0.480	573.9
G allele Frequency	90(22%)	64(16%)	1.52 (1.06-2.17)	0.020	

Genotypes of the rs892066 of ANGPTL8 gene were considered only due to the significant changes of this SNP with respect to the occurrence of CAD that were obtained and to less value of AIC factor. The analyses were carried out by using the T test of data among the various groups.

approximately comparable results were obtained. There are significant increases in the levels of triglycerides(P=0.000) and VLDL-cholesterol (P=0.000) in the group of patients with the CC+CG genotypes when they were compared with those of the GG genotype (Table 1.3).

When the data were analyzed under the dominant model,

Table 1.3: Biochemical characteristics of CAD patients in relevance to the genotypes of rs892066 of ANGPTL8 gene polymorphism analyzed under dominant model

	GG	CG-CC	P value
LPL	6.62 ± 1.85	7.08 ± 1.41	0.615
Total Cholesterol (mg/dl)	156 ± 25.17	155 ± 38.53	0.939
Triglycerides (mg/dl)	70 ± 8.77	115 ± 64.72	0.000
HDL-C (mg/dl)	47.40 ± 21.52	42.56 ± 19.32	0.643
LDL-C (mg/dl)	95 ± 27.05	90 ± 42.49	0.688

VLDL-C (mg/dl)	14 ± 1.75	23 ± 12.94	0.000
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Discussion

The genetic involvement of several genes related coronary artery disease are considered to be a field of continues researches. Basically, the goals of these studies are to understand the etiology of the disease, improve the therapeutic strategies and to identify individuals that are at high risk to develop coronary artery disease. Lipoprotein lipase considers as key enzyme in the metabolism of lipid profile, it can be hydrolyzed triglyceride in the circulation either in the form of chylomicron after fatty meal or in the form of VLDL that store in adipose tissue to release free fatty acid for energy(Bogari, N. M., et al. 2020) As one of the risk factors for CAD, dyslipidemia has been linked in a number of studies to genetic variants in the LPL gene and its levels. Horton, J. D. 2019, Gerodias et al. . Incomplete digestion of lipoproteins due to partial or total lipoprotein lipase impairment can increase the risk of coronary artery disease (CAD) (Horton, J. D. 2019). By interfering with ANGPTL3 and ANGPTL4, ANGPTL8 has a significant role in reducing the activity of lipoprotein lipase (Wang Y, et al. 2013) According to recent research by Huang et al. using microarray data, the GNMT/PTEN/PI3K/Akt signaling pathway in the liver adversely regulates insulin-induced expression of ANGPTL8 after HFD eating (J.W. Huang, et al. 2019). Additionally, it has been shown that circulating ANGPTL8 protein levels are much greater in anorexic patients than in normal-weight individuals, but lower in morbidly obese women (S. Barja-Fernandez, et al. 2015). Additionally, those who consume less protein have been observed to have greater plasma ANGPTL8 protein levels. (< 67.1 g/day) compared with those consuming higher amounts of protein (> 67.1 g/ day) (A.B. Crujeiras, et al. 2016).

In the current study the SNP rs2278426 that represent a SNP (CGG to TGG) where amino acid arginine (R) is replaced by tryptophan (W)(Consortium, G. P.2012). The study of genotype and allele frequencies for rs2278426 of the ANGPTL8 gene polymorphism in CAD patients and controls found no evidence of a link between ANGPTL8 polymorphism and lipoprotein lipase in either group. There have been no additional research that have looked at SNP rs2278426 (C/T) in coronary artery disease, but rather in other diseases such as Diabetes Mellitus and metabolic syndromes. Another research indicated that rs 2278426 C/T was connected with the risk of Metabolic Syndrome MS and that the variations are associated with reducing the risk of high cholesterol and hyperglycemia, both of which are Metabolic Syndrome components (Alenad, A., et al. 2020), This finding is consistent with recent research, which found that the ANGPTL8 mutation rs 2278426 (C/T) is associated

with higher fasting blood glucose levels in non-diabetic subjects in the Arab community as compared to the wild type (Abu-Farha et al. 2016) Similar findings were seen in the African American and Hispanic populations (Quagliarini et al.2012). Guo et al. discovered that ANGPTL8 rs 227826 C/T is connected with serum lipid profile in 2015, however in 2020 they found that people who possessed the (T) allele in rs2278426 were more likely to develop T2DM and hence increase their risk of Coronary Heart Disease (Guo, et al. 2020). Another research found that the SNP rs2278426 variations are associated with an increased risk of type 2 diabetes and may induce lipid problems that result in a drop in HDLC levels (Ghasemi, H., et al. 2018). One research described the gene's significance in lipid metabolism, focusing on the ANGPTL8 role in adipose cell differentiation and suggesting that insulin induces ANGPTL8 expression (Ren.G, et al. 2012). ANGPTL8 was discovered as a regulated protein that controlled lipid metabolism by influencing LPL activity (R. Zhang and A. B. Abou-Samra., 2013) and regulated plasma triglyceride levels by inhibiting lipoprotein lipase. (Quagliarini F., et al. 2012) (Abo Farha, et al. 2016). The other SNP of ANGPTL8 in the present study suggests that the codominant model of rs892066 in heterozygous genotype patients was evident to be significantly higher than those of the control group and suggested a risk factor of about 1.7 folds for the occurrence of the disease in the allele carriers. In addition, in the dominant model, the group of CAD with the GG+CG genotypes were be higher than of control group, suggesting a risk factor of about 1.71 folds for the occurrence of the disease in the allele carriers. In addition to results above we found the minor allele G of rs892066 was considered as risk factor that increased the occurrence of CAD for about 1.5 fold. On the other side the results were obvious and showed a significant association of triglycerides with VLDL in patients with CC- CG, GG genotypes. A study on the Kuwaiti population showed that there is no association between rs892066 (C/G) variant and level of HDL-C and LDL-C(Abu-Farha et al. 2016), while there is no association for rs892066 with LPL in coronary artery disease patients the IUI attempts failed. After through the relatives the couple got to know about the wardha test tube baby center. There after they moved to wardha test Tube Baby Center where they underwent IVF ICSI treatment in 2020.

Female- haemoglobin12.4 mm/deciliter.Total white blood cells count is 8900.Total red blood cells is 5.08 millions/cu.mm. platelets are 3.13 lakhs/cu.mm.E2 [Estradiol]level is 257.08 pg/ml. Kidney Function Test And Liver Function Test isnormal.Thyroid stimulating hormone level is 1.91 uIU/ML.HIV ,HBsAg, HCG-VDRL is Non-Reactive .Anti-Mullerian Hormone [AMH]level is 3.24

.Follicular Stimulating Hormone (FSH) level is 10.46 and Luteinizing Hormone (LH) level is 7.29 .Prolactin level is 32.59. Hysteroscopy reports are Normal. Laparoscopy shows two follicular cyst on right ovary.left ovary is absent.left tube hydrosalpinx and torsion + adhesions. RTPCR is Negative.The diagnosis of female shows The female had 2 follicular cyst on right ovary.left ovary is absent. Left tube hydrosalpinx and torsion dissected.primary infertility with absence of left ovary [oophorectomy].

Male-haemoglobin 15.4 mm/deciliter. Total white blood cells count is 10850.Total red blood cells is 6.02 millions/cu.mm. platelets are 4.15 lakhs/cu.mm Kidney Function Test And Liver Function Test is normal.Thyroid stimulating hormone level is 2.41uIU/ML.HIV ,HBsAg, HCG-VDRL is Non-Reactive. Follicular Stimulating Hormone (FSH) level is 10.46 and Luteinizing Hormone (LH) level is 7.29 . RTPCR is Negative. Semen analysis report shows Sperm count is 35 mil/ml. Motility Of total Sperm is 60% motile.Sperm morphology is 34%.

Prognosis- Treatment on follicular cyst showed positive results. The quality of the oocyte from the right ovary is good.all the hormonal levels are normal and the semen analysis report of male was good. The IVF, ICSI showed the positive result with husband sperm sample.

Therapeutic Intervention-Treatment has been taken for hydrosalpinx which contain DOXY 100 MG tablet BD for 14 days. Metronidazole Tablet TDS for 14 days.Medication was recommended for 15 days following the embryo transfer [03 day 3 embryos and GRADE A] in which the dose of tab.estradiol was 2 mg QID ['quarter in die'] for 5 days and 2 mg TDS ['ter die'] for 10 days, increasing endometrial thickness.

FOLLOW-UP AND OUTCOMES- This case is of partial ovariectomy in female.The couple of 31 years female and 36 years of male facing primary infertility from last 8 years. They underwent the twice iui treatment which eventually failed due to ovulation was not occurred during iui process. Later In wardha test tube baby Center,IVF[ICSI] is suggested due to partial oophorectomy on left side, the left tube hydrosalpinx and torsion is there. With right ovarian cysts.The semen analysis of male partner was done. The count was 35 mil/ml.with the short antagonist protocol 04 MII ,03MI were retrieved on 21/11/20.04 Day 3 embryos are formed. On 17/02/21 the 03 Day 03 embryos were thawed and transferred. The β -HCG test was done of the Patient, on the 14th day of the embryo transfer she came positive which displays that the implantation is successful [B-HCG =220.75 mIU/ml]four days after the value of B- HCG was raised to 967.6 mIU/ml. Atlast she gave birth to the baby girl in just single cycle of ART.

Follow up is necessaryafter the process of embryo transfer a regular follow up is necessary up to the 14 days. During the each follow up the thickness of the endometrium is checked and observation of the gradually increase in the thickness of endometrium is done which helps to the proper and

successful implantation of the Embryo in the uterus. On 14th day the B-HCG test was done which had shown positive result.Out of the 3 transferred embryos only one embryo of good quality was implanted.

Even after of having unprotected intercourse of 1 year,still unable to reproduce is called as infertility. Itcan occur in male or female partner[27]. According to WHO, normally there are 15% couples facing infertility all over the world from which the 3.9-16.8% couples are infertile in India. 14% female facing infertility all over the world. Generally, most of the studies show that the women who underwent ovariectomy have 53.9% chances of increased risk of infertility [28].

Some studies suggest that the ovulation from right side ovary is more important for pregnancy. Absence or removal of one ovary causes reduction in estrogen level in blood, progesterone level may causes difficulty to get conceive also leads to bone disorders. Diminished ovarian reserve is one of the cause of removal of ovary due to AMH value gets affected. Various factors causes the oophorectomy like endometriosis,tumor, torsion,PCOS etc. Removal of ovary can cause the infection or fallopian tube cancer[29-40]. Despite having this if the remaining ovary has proper morphology and in function will not occur any complications for pregnancy. Still in some cases the ovariectomy has may have alternative like increased screening for cancer of ovaries, so that the doctors get early stage diagnosis by regular check- up and tests. Taking frequent birth control pills also affect the ovaries can cause cancer. Avoiding its use may reduce cancer.patients with ovariectomy undergo ART treatments for having own child. ART is procedure of complex series which used to help with fertility or prevent genetic problems. Through the IVF/ICSI procedure chances of successful outcome increases [41-50].

CONCLUSION

The popularity and the use of ART treatment is increasing day by day. It gives the lifetime happiness and satisfaction to the Patients who suffering from infertility. Especially to the women's who underwent removal of the ovary [ovariectomy] due to the various diseases. Having single ovary already reduces the chances of pregnancy by some percent. The ovarian reserve gets affected. Removal of ovary causes the reduction in the various levels of hormones like estrogen, AMH, progesterone in blood which affect the proper uterine lining during menstruation for implantation due to which the it affects the outcome of getting conceive. Generally the positive outcome depends on the how much functional and morphologically normal the intact ovary is.if any pathology is there to the present ovary via medicines it can be curable. Also, ART also helps to get conceive. Due to oophorectomy in some cases the fallopian tube passage gets blocked due to adhesions, scar tissues, which causes infection, accumulation of water that is hydrosalpinx leads to infertility.it block the way of an egg to travel up to the uterus.This is referred as unexplained infertility. Females who underwent surgery of

ovary removal, and having other ovary in proper function [proper ovulation, no blockage in tube] won't affect much the positive outcome of successful pregnancy. To guarantee confidentiality, the Patients information was identified. For ART related process such as what is ivf treatment and how doctors will treat patients. [Therapy plan], risk factors associated with advanced Cancer, pregnancy age, procedure risk. Proper informed consent of patient in taken in local and English language. The author has gathered and saved written ethical approval in accordance with international or university standards[s]. There are no competing interests stated by the author [50].

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