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The Polymorphism Function of Protamine 1 Gene and Some Immunological Factors in Sterile Couples

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***Abstract*—Background:Infertility is a global problematic that impacts nearly 15% of all couples, and is definite as, incapability to conceive following 12 months of free-contraceptive un-protected interaction.**
Material and methodology:serum OD of anti-follicle hormone as stimulation antibody and anti-sperm antibody was identified through assay method of enzyme–linked immune-sorbent. One Protamine 1gene polymorphic sites was genotyped on 72 couple's Sterility. Other 20 couples group was obviously healthy ones. Genotypes were assessed through (PCR-RFLP) method.
Results: Regarding the Serum positivity of anti-sperm anti-body and anti-follicle stimulation hormone antibody in peripheral blood of sterile couples.The results observed significant association between anti-sperm antibody, anti-follicle stimulation hormone antibody and sterile women (P value 0.18, 0.32 respectively), while non-significant for anti-sperm antibody in sterile males P value (0.129), the reported SNP frequency was of no significance difference in comparison to males of normal fertility, that propose that the SNP might not aid as a good molecular genetic diagnosis indicator for males sterility (MI).
Conclusions: The SNP G197T are absent totally and are not related to MI with above-mentioned, thus such SNPs might not signify as a molecular indicator for the MI genetic cause diagnosis in the current population of the study.
Keywords: Genetic; Sterility, anti-FSH anti-bodies, Anti-sperm antibody, Protamine 1 gene. Allele polymorphism and Genotype.

I. INTRODUCTION

No agreed fe-MI definition is there, but good guidelines mention that: A woman age of reproduction, which is not conceiving after 12 months of un-protected vaginal sex, in the absenteeism of whatever recognized sterility reason, and must be gotten additional clinical investigation and assessment besides her husband (Mascarenhas*et al.*, 2012). Such community health problematic involves all world regions. Its occurrence differs from 10-30 %, with the male being of responsibility for approximately ½ the cases (Wolomby, 2012). Sterility could be resulting from physiological, anatomical, pathological, endocrinological, psychosexual and immunological character; immune-sterility is one of the main sterility reasons in humans (Khatoon *et al.*, 2012). Several sterility cases etiology persists unwell understood. It is clear that causes of ssterility are heterogeneous due to many factors lead to reproductive success; couples around 30% with reproduction difficulty are detected with un-explained sterility (Quaas and Dokras, 2008). Recent proof proposes that Genetic factors (GF) lead to the feMI etiology (Matzuk and Lamb, 2008). GF affect all periods of the reproductive system operational and development. GF that might disturb reproductive function include abnormalities of chromosomes (structural and/or numerical), genes mutations controlling reproductive function (X-linked, Y-linked, mitochondrial, autosomal), mosaicism, diverse of polymorphisms DNAs and chromosome besides epiGF (Chernykh, 2004).

The male infertility majority genetic causes remains un-known, protamines are minor elementary proteins conserved widely among all species, all mammals are of 1 protamine, PRM1, whereas some species along with human are of a 2nd protamine, PRM2. Owing to the crucial functions, the protamines have a role in sperms differentiation, protamine expression aberrations or protein structure alterations might lead to definite idiopathic infertilities of male (Iguchi *et al.*, 2006).

Damage of DNA minimizes fertility in male's sperm and females ovocytes, as reasoned to smoking (Zenzes, 2000). Another xenobiotic agents of damaging of DNA i.e., chemotherapy or radiation (Mark-Kappeler*et al.*, 2011) or oxidative DNA damage 8-hydroxy-deoxyguanosine accumulation (Seino *et al.*, 2002) are causes as well besides agents of DNA damaging including reactive oxygen species, high testicular temperature or fever (Shamsi *et al.*, 2011). Toxins; i.e., glues, silicones or organic volatile solvents , chemical dusts, physical causes, and pesticides elevated sterility hazard (Mendiola *et al.*, 2008). Smokers of tobacco are 60% expected to be sterile more compared to non-smokers (GOV.UK, 2009). German experts have stated that Adeno- associated virus may has a part in MI (Young, 2013). Anti-sperm anti-bodies (ASAs) are existing in < 2% of fertile and 10% of sterile males and affect sperm task otherwise as follows: sperm penetration impairment into cervical mucus, sperm capacitation inhibition, incomplete reaction as acrosomal, sperm-egg binding disruption, and egg fertilization disorder (Bonyadi*et al.*, 2013). Hormone of Follicular Stimulation (FSH) is 1 of the 2 pituitary gonadotrophins involving in regulating ovarian function; it

has some effect on the follicles progress (Thomas and Vanderhyden, 2006). Sterile females with anti-ovarian anti-bodies frequently show anti-bodies versus FSH (anti-FSH) (Shatavi*et al*., 2006). Previously, it has been confirmed that anti-FSH anti-bodies are increased in sterile females (Haller *et al*., 2005).

The present study's objective is to determine whether sterile males with the G197T PRM1 polymorphism, sterile males and females with anti-sperm antibodies, and sterile females with anti-AFSH antibodies, have any significance.

II. MATERIAL AND METHODOLOGY

Cases. The study covered 72 couples (72 females and 72 males) whom Sterility-suffering for more than 12 months duration. Another 20 couples group obviously healthy ones (20 males and 20 females) with no any systemic disease history were considered clinically being healthy besides a control group. The current work was in settlement with Al-Nasiriya Teaching Hospital/Thi-Qar/Iraq ethics and informed verbal consent was achieved from all members. The participants were clinically identified by specific specialist of sterility.

A. Blood samples for ASA and Anti FSH Antibody.

Venous blood samples of 3 ml were taken from both controls and couples subjects are distress from primary and secondary ssterility. A sum of 184 blood samples were taken in order to detect the anti-sperm anti-bodies and anti FSH anti-bodies by utilizing ELISA. Collection of blood samples was done by utilizing a sterile syringe and replace in a glass test tube being clean sterile with no anticoagulant. The blood put aside for clotting for 30 min and centrifuged then for 5 min at 3000 rpm. Sample of serum was replaced in plane tube and labeled for every participant. All samples of serum were frozen at -20˚C till assayed via kit of ELISA.

B. Extraction of DNA and Genotyping.

Extraction of Genomic DNA was done based on the protocol of the manufacturer; from the frozen whole blood, 5 ml were taken utilizing a Kit of DNA Extraction (Geneaid / USA). The region as polymorphic was amplified via PCR. Amplification reaction was done in Accu Power PCR Premix tube (0.2 ml) based on the Bioneer Corporation, the forward primer 5'-CCCCTGGCATCTATAACAGGCCGC-3' and revers primer 5'-TCAAGAACAAGGAGAGAAGAGTGG_3'. Then, for this reaction, the thermo-cycling conditioninitial denaturation of 94°C for 5 min, then 35 cycles of denaturation at 94°C for 30 sec., annealing at 52°C for 45 sec., extension at 72°C for 1 min. Final extension of 8 minutes at 72°C, was performed and analyzing for the products was done via gel electrophoresis (1% agarose) (Tanaka *et al.*, 2003). Products of PCR were overnight digested along with enzymes of restriction (BSeRI), according to the protocol of the manufacturer, and analyzed via gel electrophoresis (2% agarose).

C. Statistical analysis.

Analyses statistically were done by SPSS computer software "version 20 along with Microsoft Excel 2010. Ratio being odd was utilized for measuring the association strength between 2 variables as categorical and the statistical odd measured ratio significance is evaluated via a special $\chi 2$ formulation. Hardy-Weinberg equilibrium deviations were inspected for all polymorphisms utilizing the $\chi 2$ statistic, with frequencies being expected derivative from frequencies of allele. Estimation was statistically regarded significant where its P value was < an α level of 0.05 significance.

III. RESULTS

A. Demographic characteristics of patients.

The current work performed on 72 couples (72 males and 72 females) at Al-Nasiriya Teaching Hospital, the sterile males age differed from 18-52 years with a 30.85 years mean age (SD ± 7.17), in comparison to 20 healthy males with 19-51 years age range, and 31.25 years (SD ± 7.85) mean age as a control group (Table 1).

TABLE (1): STERILE AND CONTROL MALES DISTRIBUTION BASED ON AGE.

	Groups	
	Controls	Sterile subjects
Age (males)	N (%)	N (%)
< 20 years	1 (5%)	2 (3%)
20-29 years	9 (45%)	33 (46%)
30-39 years	7 (35%)	29 (40%)
40-49 years	2 (10%)	6 (8%)
≥ 50 years	1(5%)	2 (3%)
Total	20 (100%)	72 (100%)

B. Serum ASA among control and sterile males.

Out of 72 sterile males partitioned for serum IgG Anti-sperm anti-bodies, 8 (11%) were perceived +ve, but with no statistical significant when compare it with control group, (P = 0.129) (Table 2).

TABLE(2): SERUM ASA FREQUENCY DISTRIBUTION AMONGSTERILE AND CONTROL MALES.

		Groups		Exact Test of Fisher
		Control	Sterile	
		NO. (%)	NO. (%)	
Serum ASA	-ve	20 (100%)	64 (89%)	0.129 (NS)
	+ve	0 (0%)	8 (11%)	
Total		20 (100%)	72 (100%)	

NS=Not-significant (p>0.05)

C. Serum ASA Among Sterile and Control Females.

In this study, out of 72 sterile female,there was 15 (21%) were carrying circulating ASA, with significant difference, (p=0.018) when compared with 20 fertile females, table (3).

TABLE (3): FREQUENCY DISTRIBUTION OF SERUM ASA AMONG STERILE AND CONTROL FEMALES.

	Study Groups		Fisher's Exact Test
Serum ASA	Control	Sterile Patients	
	NO. (%)	NO. (%)	
-ve	20 (100%)	57 (79%)	0.018*
+ve	0 (0%)	15 (21%)	
Total	20 (100%)	72 (100%)	

* = Difference of significance (P ≤0.05)

D. AFSH serum among control and sterile females.

The +ve % cases of 72 females (sterile) for (IgG) AFSH Antibody among serum was (18%), with (0%) among females (fertile) nonetheless with significant (P = 0.032) (Table 4).

TABLE (4): AFSH SERUM LEVEL COMPARISON IN FEMALES PATIENTS AND CONTROLS.

	Study groups		Exact Test of Fisher
Serum AFSH	Control	Sterile subjects	
	N (%)	N (%)	
-ve	20 (100%)	59 (82%)	0.032*
+ve	0 (0%)	13 (18%)	
Total	20 (100%)	72 (100%)	

* = Difference of significance (P≤0.05).

E. BseRI polymorphism recognition:

Image of gel electrophoresis as Agarose elucidate protamine 1 gene REFLP PCR product in healthy and patient samples, BseRI as enzyme of restriction was cut the protamine 1 gene 557bp resulting in 2 products 238bp and 319bp that indicate no mutation of allele was perceived. The stated SNP was not recognized in the screened people in the current work. In all 92 samples (20 fertile and 72 sterile ones) both of fragments hat were products following digestion of BSeRI, signifying that there is no G197T SNP of PRM1 in the undertaken populace (Figure 1).

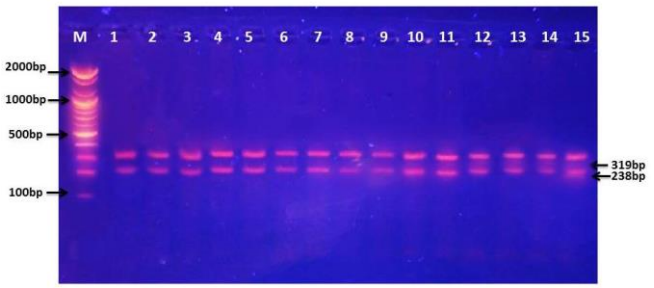

Figure (1): PCR – RFLP Ethidium bromide-stained agarose gel amplified 319bp and 238bp of protamin 1 gene for undertaken groups. Lane (M): DNA molecular size indicator (KAPA Universal Ladder) , Lane 1-10 for sterile patients, Lane 11-15 for control group.

IV. DISCUSSION

The current work perceived the uppermost males occurrence with sterility was among 20-29 years representing (46%), then by 30-39 years representing (40%), such finding in agreement with those of Mostafa (2011) stated that the uppermost sterile prevalence of males at <30 years, then by 30-34 years and declined with age (Mostafa, 2011). Alike findings were gained in Iran from a study that found that the patient's majority were within 20-29 years (Moghadam *et al*., 2011). Such results might be attributed to early marriage if they married at young age and unsuccessful to conceive they look for treatment early even before 12 months. Other reported data mentioned upsurge of sterile males at 26-35 years (63.50%)" since the groups of young ages are looking for additional medical assistances and they are directed further to have a child, furthermore they are facing additional psychological stress and pressure regarding their families, it was measured that semen quality, ejaculation frequency, and tasks of sperm decline steadily with highly advanced age and begins to decline afterward 35 years (Omran*et al*., 2013). The stress, pollutions, and economic problems faced may be the chief issues resulting in sterility that distress such active group of age (Fakhrildin and Rasheed, 2013). Anti-sperm anti-bodies are perceived in males Sterility-suspected in 8% -21% of the situations (Haller *et al.,* 2012). As that 11%, ASA were perceived in sterile males of the studied cases. The present findings is nearby to the gained ones by Alam (2012) that shown that out of 132 sterile screened males for ASAs, 8 were observed to be +ve. Such mentions that 6% of the presented subjects with +ve ASA among sterile males studied cases, with proof that ASA are one of the un-explained Sterility reasons (Presti*et al.*, 2009). They do not just decline motility of sperm but as well decline counts of sperm and normal morphology of sperm. In India, Gul-ArNavi*et al.*, 2011 showed a significant ASAs level in sterile males serum compared to fertile males that indicated 6 (25%) from a total of 24 sterile subjects whose serum presented +ve ASAs with P value of (0.039) (Alam, 2012). Regarding the positive percentage of ASA among studied groups, study revealed significant difference among fertile and sterile females this result agree with Sima and Hefzy (2011), they reported that the prevalence of ASA in the sera from sterile females was 18/90 (20%), as compared with 4/80 (5%) in control females, there was a variance of significant between both groups (P=0.04) (Al-Daghistani and Fram, 2009). In other study

conducted by Al-Daghistani and Fram (2009) in Jordan on 73sterile women, the ASAs was 6(8.2%) and 0/41 (0.0%) in control females with highly significant for ASAs and Sterility cases ($p<0.05$). Another study reported that among 73 of sterile females and 41helthy fertile control, ASA was existing in 6 (8.2%) sterile females and 0 (0.0%) fertile women and was associated significantly with Sterility ($P < 0.05$) (Restrepo and Cardona-Maya, 2012). Thus, the different studies indicate that anti-sperm anti-bodies might have important functions in the sterility causation through diverse ways (Fakhrildin and EL-Nahi, 2009). In women, the causing factors including: mechanical i.e., surgery of uterine cervix or the genital tract mucosal layer chemical disruption, foreign anti-gens accessing the women genital tract, semen lymphocytes, sperm with surface bound anti-bodies, senescent being abnormal or sperm damage, gastro-intestinal exposure to sperm and sperm within the peritoneal cavity following trans-tubal passage (Salamian*et al*., 2008). Although the mucosa is extra exposed heavily, dissimilar to males, reproductive tract of females is of no immunological barrier (Kichine*et al*., 2008). The variances in values of anti-body between the control and patients groups were relevant statistically nonetheless quiet rather small, mentioning that such anti-bodies were as well mutual among healthy females and might consequently simply signify natural examples of occurring anti-bodies. Such information supports our results Haller *et al*., (2012) observed ($P <0.05$) of groups were compared with the control ones and deliberated as of difference of significance (Haller *et al*., 2012). The system of immunity should be changed to produce constantly anti-FSH anti-bodies, if the antigenic FSH from the circulating hormone from the organism of female, or produced from fluid of semen; the idea of system of immunity changes besides the greater production of numerous kinds of auto-anti-bodies in polycystic ovary syndrome and endometriosis (Shatavi*et al*., 2006). There is particular indirect sign that anti-FSH IgG anti-bodies might, though, extra worsen fecundity of females via declining the functionality of FSH; such data made us investigating the anti-FSH anti-bodies influence on - and developing sterility in females (Fakhrildin and Rasheed, 2013). Furthermore, to reflecting just alterations of immunity system, FSH-anti-bodies might as well have an influence on the Sterility development, the serum anti-FSH anti-bodies were engaged versus the human FSH molecule β -chain immune-dominant epitope, the 78–93 amino acid region, in such specific region, there is a loop named determinant loop or cysteine noose, the residues which have a function in assessing the FSH receptor binding specificity (Shatavi*et al*., 2006). Such information together specifies that Sterility itself, rather than the sterility reason, might be an element of productivity for the anti-FSH anti-bodies emergence (Fakhrildin and Rasheed, 2013).

Such finding was in accordance with (Presti*et al*., 2009) in Iran, detected in 308 samples (35 fertile and 273 sterile cases) all PRM1 genomic fragments (557 bp) PCR products were utilized for digestion with BSeRI, the mentioned SNP absence results in full enzymatic amplified fragment digestion that yields 2 fragments with various lengths (319 bp and 238 bp) and there is no PRM1G197T SNP. Such finding differ from Iguchi *et al.*(2006) results in USA (7), detected in 40 samples (10 control and 30 sterile cases) 1 heterozygous single nucleotide polymorphism (SNP) at nucleotide 197 was recognized in the DNA of 3 of the 30 sterile examined males, were observed in the 3 un-related sterile males mentioning 1 *PRM1* allele polymorphism. According to this SNP absence in the current study, it seems to be as un-common SNP (Tanaka *et al*., 2003). Besides, arginine core disruption necessary for binding of DNA, since SNP lead to AA conversion to serine from arginine in an extremely conserved cluster of arginine (Iguchi *et al*., 2006). Certainly, evaluating 2 SNPs in PRM1 in 1195 individuals, where one was identical as the SNP evaluated in the current study and they determined that such SNP has of no significant influence in MI (Kichine, E.; Msaidie and Bokilo, 2008).

V. CONCLUSIONS

1. The circulating anti-sperm anti-bodies presence can be a reason for idiopathic Sterility among couples, and it can be directly correlated with Sterility.
2. Fertility of females can be influenced by the system of immunity. Anti-ovarian auto-anti-bodies are directed mostly aversus follicle stimulating hormone (anti-FSH).
3. The SNP G197T are absent totally and are not linked with MI above-mentioned, thus such SNPs might not signify as a molecular indicator for the MI genetic cause diagnosis in the undertaken population.
4. GF Analysis which influences male's factor Sterility will offer appreciated insights into the targeted treatments creation for subjects and the idiopathic Sterility causes determination.

VI. RECOMMENDATIONS

For additional exact outcomes other further developed investigative exams i.e., assay of IF and PCR for ASA recognition and Anti-FSH antibody are suggested.

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