Cytochrome P450 1A1 gene polymorphism and their haplotypes as a susceptibility factors for oligozoospermia in Iraqi men

Aya A. Abduljabbar[1]*, Ismail A. Abdul-Hassan[2], Saif Abdulhafedh Shihab[3]
[1,2]University of Baghdad/ Institute of Genetic Engineering and Biotechnology for post graduate studies/ Iraq.
[3]Kamal AL.Samraee Hospital for Fertility, Infertility and in Vitro Fertilization/ Baghdad/Iraq
Email: ayaamer201925@gmail.com

Summary
In the present study we investigate the association of CYP1A1*2A (rs4646903) and CYP1A1*2C (rs1048943) SNPs in CYP1A1 gene with oligozoospermia in a sample of Iraqi patients. Blood samples were collected from 50 oligozoospermic patients and 50 apparently healthy subjects (Controls). Genomic DNA was extracted from blood samples by using protocol in EasyPure® blood genomics DNA kit. Genotyping was performed by RT-PCR HRM using rotor gene (Qiagen). No significant differences were noted between study groups as related with semen volume and semen pH. The values of sperm count, normal sperms, sperm motility A, sperm motility B and sperm motility C were in oligozoospermic patients group significantly ($p \leq 0.01$) lower than in apparently healthy subjects group (7.22 versus 37.78 million, 24.3 versus 44.64 %, 0.10 versus 8.0%, 7.5 versus 21% and 17.8 versus 30.5%, respectively). While, the percentage of abnormal sperm and immotile sperm were in oligozoospermic patients group significantly ($p \leq 0.01$) higher than in apparently healthy subjects group (75.7 versus 55.36% and 74.6 versus 40.3%, respectively). As related with rs4646903 SNP of CYP1A1 gene, AA genotype represent a protective factor against the incidence of oligozoospermia, whereas, GG genotype represent a risk factor for oligozoospermia incidence. The percentage of A allele was lower in oligozoospermic patients than in apparently healthy subjects (0.31 versus 0.57, respectively), in contrast, G allele frequency was in oligozoospermic patients higher than in apparently healthy subjects (0.69 versus 0.43, respectively). As related with rs1048943 SNP of CYP1A1 gene, no significant differences were noted between study groups as related with all genotypes and the T allele frequencies were 0.67 and 0.70 and A allele were 0.33 and 0.30 for apparently healthy subjects and oligozoospermic patients, respectively. The results of haplotype defined by rs4646903 and rs1048943 SNPs showed that AT haplotype represent a protective factor against the incidence of oligozoospermia while GT haplotype was represent a risk factor for oligozoospermia incidence in Iraqi patients. Also, both AT/AT and AT/GA haplotype combinations represent as a protective factor against oligozoospermia incidence, whereas, AT/GT and GT/GT haplotype combinations were represent as a risk factor for oligozoospermia incidence in Iraqi patients. In conclusion, the results of the present study support that the CYP1A1 rs4646903 polymorphism (GG genotype) might contribute to individual susceptibility to oligozoospermia in a sample of Iraqi patients.
Keywords: CYP1A1, oligozoospermic , haplotypes, polymorphisms, HRM.

1. Introduction

Infertility is defined as the disability of couples to possess a baby after one year of orderly unprotected intercourse, affecting 10–15 percent of couples [(1)]. Male infertility is a complex multifactorial pathological condition with highly heterogeneous manifestations, from the total absence of spermatozoa in the testicles to distinctive changes in sperm quality .Genetic factors are responsible for at least 15% of male infertility [(2)].

The definition of oligozoospermia is a decrease in the number of sperms in the ejaculate (less than 15 million sperm / ml), which are divided according to the number of spermatozoa into mild (10-15 million sperm / ml), moderate (5-10 million sperm / ml), and severe (<5 million sperm/ml) [(3)] . The causes of male infertility are varied, the most important of which are: hormonal imbalance, physical causes, sexually transmitted problems, environment and lifestyle, and genetic factors [(2)].

2. Materials and Methods

Selection of Patient and Control

The study included one hundred subjects divided into two groups, 50 seemingly healthy cases and 50 cases of oligozoospermia . Patients were selected according to clinical and laboratory examination. The Men with oligozoospermia were already diagnosed by specialist physician in Kamal AL.Samraee Hospital for Fertility, Infertility and in Vitro Fertilization/ Baghdad . The subjects were selected according to the diagnosis of a specialist physician Who sent them to collect blood and semen. Blood and semen samples were obtained during the period from December 16, 2020 to April 15, 2021.

doi.org/10.31838/hiv23.02.85 Received: 15.10.22, Revised: 12.11.22, Accepted: 22.12.22.

3. Methodology

Seminal Fluid Analysis

After liquefaction of semen for 30 minutes at 37°C. Semen samples consist of two parts .The first is the sperm which is assessed by microscopy where one drop of semen sample is placed on the slide and covered with a covering slide and examined by microscope and sperm count is done in 4-5 fields in high power field also. Such as motility, sperm morphology, whether white blood cells and aggregation are found or not. The other part is fluids made by the accessory glands which are evaluated by pH, volume, viscosity and liquefaction time [4] [5], Semen samples were evaluated according to the World Health Organization (WHO) criteria [6].

Blood samples

Each blood sample was taken from a vein (5 ml), and this process was performed under sterile conditions. collected blood sample has been dispensed into EDTA K3 tube to DNA extraction for molecular studies.

DNA Extraction

Genetic material DNA that was kept in eppendorf tube and preserved in -20 C° was extracted from the whole blood samples (frozen blood) by using protocol in EasyPure® Blood Genomic DNA Kit (Catalog No.EE121).

The Estimation of DNA Concentration and Purity

The concentration of DNA samples was evaluated by using nanodrop device by loading 1µl DNA sample into the Nanodrop. The purity was detected by noticing the ratio of optical density (OD) 260/280 nm to discover the contamination of samples with protein. A ratio of ~1.8 is generally accepted as "pure" for DNA and according to [7].

Molecular diagnoses methods

Genomic DNA extraction from blood sample using (EasyPure®Genomic DNA Kit) protocol by genomic DNA purification kit (TransGen biotech ,China).Detecting Primer prepared gene (*CYP1A1*) was assessed for SNPs which included (rs4646903 and rs1048943) primers sequences which supplied by Alpha DNA / Canada, were designed according to their reference sequence (rs) in the database of NCBI (National Center for Biotechnology Information). Designed Primers used in the present study (Table 1).

Table 1: Designed Primers used in the present study.

Primer	Sequence (5'→3' direction)
rs4646903 *(CYP 1A1)* 80 bp	
Forward	GAGGCTGAGGTGGGAGAATC
Reverse	TGGAGTGCACTGGTACCATT
rs1048943 (*CYP 1A1*) 63 bp	
Forward	AGCCAGGAAGAGAAAGACCT
Reverse	AGGCAGAATATTCCCATCAGG
Genotyping of (rs4646903 and rs1048943) was done by HRM technology.	

Statistical analysis

The Statistical Analysis System- [8] program was used to discover the effect of difference factors in study parameters. T-test test was utilized to significant compare between means. Chi-square test was utilized to significant compare between percentage (0.05 and 0.01 probability). assessment of Odd ratio and CI in this study.

4. Results and Discussion

Subjects' data

A total of 100 blood samples were collected from two groups of Iraqi men including 50 oligozoospermia patients and 50 apparently control group.

Semen analysis

In this study seminal fluid was collected from oligozoospermia patients (n=50) and apparently healthy subjects (n=50).

The diagnosis of male infertility is particularly based on the descriptive evaluation of human semen, including the number of spermatozoa that are present in the ejaculate, their motility and their morphology [9]. Some semen parameters of apparently healthy subjects and oligozoospermia patients are display in table 2 .

There were no significant differences between the study groups as linked with semen volume and semen pH. Sperm count was significantly ($p \leq 0.01$) higher in apparently healthy subjects group than in oligozoospermia patients group (37.78 versus 7.22 million per ml, respectively). The cutoff of 20 million spermatozoa per ml has been repeatedly suggested as the lower normal value for sperm concentration in an ejaculate [6].The percentage of normal sperm was equal to approximately two fold in apparently healthy subjects when compared with oligozoospermia patients (44.64 % versus 24.30%, respectively). While, the percentages of abnormal sperm were significantly ($p \leq 0.01$) lower in apparently healthy subjects group than in oligozoospermia patients group (55.36% versus 75.70%, respectively).

The percentages of sperm motility A were significantly ($p \leq 0.01$) higher in apparently healthy subjects group than in oligozoospermia patients (8.0% versus 0.1%, respectively). The percentages of sperm motility B were significantly ($p \leq 0.01$) higher in apparently healthy subjects group than in oligozoospermia patients (21% versus 7.5%, respectively). The percentages of sperm motility C were significantly ($p \leq 0.01$) higher in apparently healthy subjects group than in oligozoospermia patients (30.5% versus 17.8%, respectively). The percentages of immotile sperms D (dead sperm) were significantly ($p \leq 0.01$) higher in oligozoospermia patients group than in apparently healthy subjects group (74.60% versus 40.30%, respectively) as in Table 2 .

Table 2 Semen parameters in oligozoospermic patients versus apparently healthy controls. (mean ± SE)				
Semen parameters	Control	Patients	T-test	*p*- value
Volume (ml)	2.55± 0.13	2.34 ± 0.12	0.356 NS	0.240
*p*H	7.60 ± 0.02	7.56 ± 0.02	0.075 NS	0.248
Sperm count (x10^6)	37.78 ± 1.10	7.22 ± 0.71	2.611 **	0.0001
Normal sperm (%)	44.64 ± 2.06	24.30 ± 1.67	5.276 **	0.0001
Abnormal sperm (%)	55.36 ± 2.06	75.70 ± 1.67	5.276 **	0.0001
Sperm motility, A (%)	8.00 ± 1.56	0.10 ± 0.10	3.098 **	0.0001
Sperm motility, B (%)	21.00 ± 0.76	7.50 ± 0.86	2.290 **	0.0001
Sperm motility, C (%)	30.50 ± 1.09	17.80 ± 1.40	3.522 **	0.0001
Immotile , D (%)	40.30 ± 1.66	74.60± 1.97	5.128 **	0.0001
NS: No significant ; ** means significant at 0.01 level.				

The semen volume results in the present study disagree with the results of [10] who found that the semen volume was equal to more than two fold in control when compared with oligozoospermia patients. Semen volume has no link to pregnancy outcome and it is not an indicative of infertility unless it is far from average according to [11] who studied over 1600 cases in their studies. While agree with [10] as related with semen pH. The seminal pH showed no significant difference among the two groups which is the same results obtained by both of [12] who examined records from 1994 to 1998 that had semen pH measurements and sperm concentration and motility. [10] found that sperm count was significantly higher in control compared with oligozoospermia patients. The presence of progressively motile sperm in the ejaculate is critical to ensure adequate sperm transfer and fertilization [9]. The low sperm motility in the oligozoospermia patients in this study is accompanied with low sperm count. WHO manuals recommended 30% normal forms as the cutoff point for normality [13].

5. Molecular Analysis

Genomic DNA extraction

DNA was extracted from the frozen blood samples for apparently healthy subjects and oligozoospermic patients by EasyPure blood genomic DNA kit. DNA concentration (µg / ml) (40 - 60), purity (1.7 – 1.9). The extracted DNA electrophoresis shown in figure 1 and the result output of HRM for the three genotypes shown in figure 2.

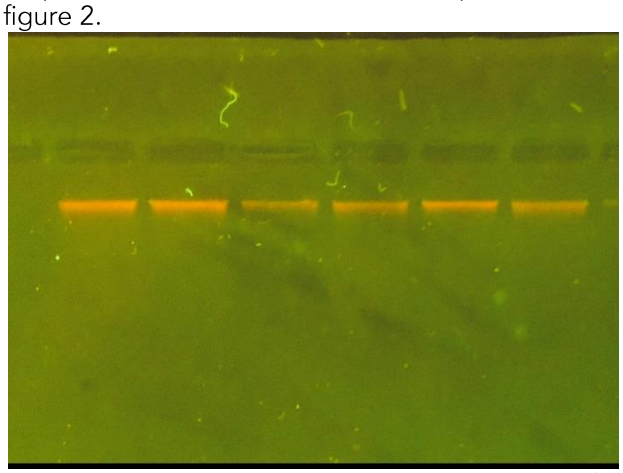
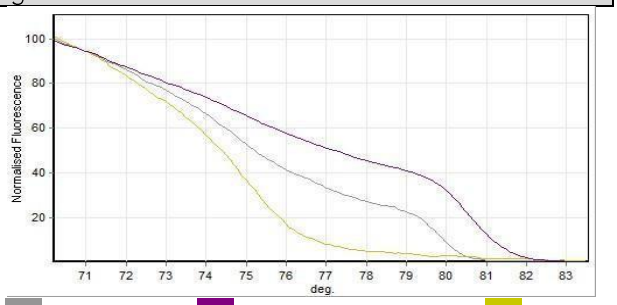


Figure1: DNA bands on 1% agarose gel at 100 volts for 20 min. Lane 1-6 : genomic DNA extracted from blood samples using in this study.



: Hetero CG : Mutant GG CC : Wild

Figure 2.The result output of HRM for the three genotypes.

Position of SNPs studied

rs4646903 SNP

Figure 3 revealed the position of rs4646903 SNP (11229) in 3' UTR of *CYP1A1* gene (NG_061374.1) in chromosome 15.

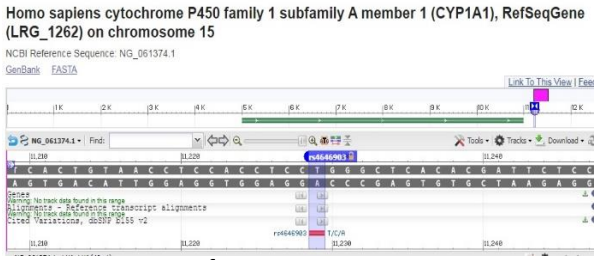


Figure 3. Position of rs4646903 SNP OF CYP1A1 gene in chromosome 15 using ncbi.

rs1048943 SNP

Figure 4 revealed the position of rs1048943 (ile 462 val) SNP (9885) in exon 7 of *CYP1A1* gene (NG_061374.1) in chromosome 15.

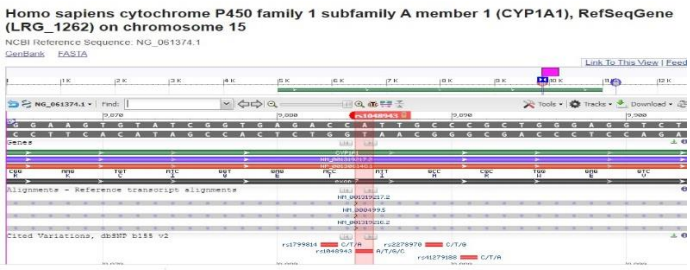


Figure 4. Position of rs1048943 SNP OF CYP1A1 gene in chromosome 15 using ncbi.

6. Genotyping

rs4646903 of *CYP1A1* gene

The results of genotypes and alleles frequencies of rs4646903 SNP in *CYP1A1* gene at 3' UTR region in

apparently healthy subjects versus Iraqi patients with oligozoospermia are presented in table 3.

The percentage of wild-type AA genotype was significantly (p≤0.01) lower in oligozoospermia patients group than in apparently healthy subjects group (10% versus 34%, respectively). Then AA genotype may represent a protective factor against the incidence of oligozoospermia in Iraqi patients. Whereas, the percentage of homozygous mutant GG genotype was significantly (p≤0.01) higher in oligozoospermia patients than in apparently healthy subjects (48% versus 20%, respectively). Then GG genotype may represent a risk factor for the incidence of oligozoospermia in Iraqi patients.

Table 3. Genotypes and alleles frequencies of rs4646903 SNP in *CYP1A1* gene at 3' UTR in apparently healthy subjects *versus* Iraqi oligozoospermic patients.

Rs4646903 SNP	Frequency, n (%)		Chi square X^2	Odd Ratio (CI)
	Control	Patients		
Genotype frequency				
AA	17 (34%)	5 (10%)	8.473 **	1.283 (0.79 – 1.87)
AG	23 (46%)	21 (42%)	1.076 NS	0.449 (0.156 – 0.920)
GG	10 (20%)	24 (48%)	8.907 **	1.472 (0.86 – 2.07)
Allele frequency				
A	0.57	0.31		
G	0.43	0.69		

The most commonly studied is the rs4646903 of *CYP1A1* gene which is characterized by the A to G mutation at nucleotide 3801 in the 3' flanking region of the *CYP1A1* gene. The rs4646903 SNP polymorphism can change the level of gene expression or m RNA stability resulting in a highly inducible activity of the enzyme [14] [15].

As related with *CYP1A1* rs4646903 polymorphism in the present study are in agreement with many genetic studies which investigated the related between the *CYP1A1* rs4646903 polymorphism and the risk of male infertility. [16] concluded that the *CYP1A1* rs4646903 polymorphism is capable of causing male infertility susceptibility in Asians, but not in Caucasians. [17] provided evidence of a significant association between *CYP1A1* rs4646903 polymorphism and idiopathic male infertility risk. [18] reported that GG genotype of rs4646903 SNP of *CYP1A1* gene is linked in the pathogenesis of male infertility in Indian population. Also, [19] precent that the *CYP1A1* rs4646903 polymorphism might contribute to individual susceptibility to male infertility in Asians.

The results of the present study disagree with other studies, [20] and [21] revealed that *CYP1A1* variants had no effect on the genetic susceptibility to the disease in Iranian and Russian populations. [21] revealed that rs4646903 SNP of *CYP1A1* gene polymorphism do not participate to male infertility of Colombian Caribbean men. [22] reported that no significant association was detected between rs4646903 of *CYP1A1* gene polymorphism and male infertility in Chinese population.

In the present study as shown from table 3, the frequency of G allele was 0.69 in oligozoospermic patients. It discovers that individual with the variant G allele may have a higher risk for male infertility than those carrying A homozygote. G allele of rs4646903 in *CYP1A1* gene was highest frequency in Chinese [22] and Iranian [20] with much lower frequency being recorded in Russian [21] and Indian [23]. The sample size was small, thus, studies with larger sample sizes are needed to further investigate the potential relationships of *CYP1A1* rs4646903 polymorphism with male infertility risk.

rs1048943 of *CYP1A1* gene

The genotypes and allele frequencies of rs1048943 SNP (Ile462Val) in exon 7 of *CYP1A1**2C gene in apparently healthy subjects *versus* Iraqi oligozoospermia patients are presented in table 4.

As related with rs1048943 SNP, no significant differences between apparently healthy subjects and oligozoospermia patients in the frequency of TT, TA and AA genotypes of rs1048943 SNP in *CYP1A1**2C gene which were 48% versus 56% ; 38% versus 28% and 14% versus 16%, respectively. The frequency of T allele was 0.67 and 0.70 and A allele was 0.33 and 0.30 for apparently healthy subjects and oligozoospermia patients, respectively.

Table 4. Genotypes and alleles frequency of rs1048943 SNP (Ile462Val) in exon 7 of *CYP1A1**2C gene in apparently healthy subjects *versus* Iraqi oligozoospermic patients.

Rs1048943 SNP	Frequency, n (%)		Chi square X^2	Odd Ratio (CI)
	Control	Patients		
Genotype frequency				
TT	24 (48%)	28 (56%)	2.127 NS	0.472 (0.187 – 0.894)
TA	19 (38%)	14 (28%)	3.065 NS	0.597 (0.237 – 1.063)
AA	7 (14%)	8 (16%)	0.791 NS	0.239 (0.098 – 0.764
Allele frequency				
T	0.67	0.70		
A	0.33	0.30		

CYP1A1*2C polymorphisms seem not to have a direct influence on male infertility, but a possible contribution to male infertility, alone or in combination with other genetic and environmental

factors, cannot be excluded (16).

Haplotype

The results of haplotype frequency of rs4646903 and rs1048943 SNPs of *CYP1A1* gene in Iraqi men with oligozoospermia and controls are shown in table 5.

The percentage of AT haplotype was significantly (p≤0.01) higher in apparently healthy subjects group than in oligozoospermia patients group (74% versus 46%, respectively). This result mean that AT haplotype represent a protective factor against the incidence of oligozoospermia in Iraqi men. In contrast, the percentage of GT haplotype was significantly (p≤0.01) higher in in oligozoospermia patients group than in apparently healthy subjects group (38% versus 12%, respectively). This result mean that GT haplotype represent a risk factor against the incidence of oligozoospermia in Iraqi men.

Table 5. The frequency of haplotypes of rs4646903 and rs1048943 SNPs of *CYP1A1* gene in Iraqi men with oligozoospermia versus controls.

Haplotype	Control	Patients	X^2	Odd Ratio
AT	37 (74%)	23 (46%)	12.58 **	1.618
GT	6 (12%)	19 (38%)	8.40 **	1.071
GA	4 (8%)	5 (10%)	0.634 NS	0.278
AA	3 (6%)	3 (6%)	0.00 NS	--

X^2: chi sequare ; ** mean significant at 0.01 level. ; NS mean no significant.

Haplotype combination

The results of haplotype combinations of rs4646903 and rs1048943 SNPs of *CYP1A1* gene in Iraqi men with oligozoospermia and apparently healthy controls are shown in table 6.

The frequencies of both AT/AT and AT/GA haplotype combinations were significantly (p≤0.01) higher in apparently healthy subjects group than in oligozoospermic patients group (32% versus 10% and 28% versus 10%, respectively). This result mean that both AT/ AT and AT/GA haplotype combinations represent a protective factor against the incidence of oligozoospermia in Iraqi men. In contrast, the frequency of AT/GT haplotype combination was significantly (p≤0.05) higher in oligozoospermic patients group than in apparently healthy subjects group (26% versus 12%, respectively). This result mean that AT/GT haplotype combination represent a risk factor against the incidence of oligozoospermia in Iraqi men.

Table 6. The frequency of haplotype combinations of rs4646903 and rs1048943 SNPs of *CYP1A1* gene in Iraqi men with oligozoospermia versus controls.

Haplotype combination	Control	Patients	X^2	Odd Ratio
AT/ AT	16 (32%)	5 (10%)	8.07 **	1.264
AT / GA	14 (28%)	5 (10%)	6.94 **	0.976
AT / GT	6 (12%)	13 (26%)	5.018 *	0.658
GT / GA	4 (8%)	9 (18%)	3.064 NS	0.339
GA / GA	4 (8%)	5 (10%)	0.634 NS	0.278
AA / GA	3 (6%)	3 (6%)	0.00 NS	--
GT / GT	2 (4%)	10 (20%)	5.492 *	0.703
AT / AA	1 (2%)	0 (0%)	0.634 NS	0.278

7. Conclusions

According to the results, this study concluded:

Semen volume was not differed between study groups and this result was in favor of the accurate determination for sperm count.

Reduced sperm count in oligozoospermic patients was accompanied with reduced motility percentage and increased percentage of abnormal and immotile sperm.

Homozygous mutant (GG genotype) of CYP1A1 rs4646903 represent a risk factor, whereas, the wild-type AA genotype represent a protective factor for oligozoospermia incidence.

Haplotype GT and haplotype combinations AT/GT and GT/GT defined by rs4646903 and rs1048943 of CYP1A1 represent a risk factor for oligozoospermia incidence in Iraqi men.

Haplotype AT and haplotype combinations AT/AT and AT/GA defined by rs4646903 and rs1048943 of CYP1A1 gene represent a protective factor against the incidence of oligozoospermia.

Reference

1- Babakhanzadeh, E., Nazari, M., Ghasemifar, S., & Khodadadian, A. (2020). Some of the Factors Involved in Male Infertility: A Prospective Review. International journal of general medicine, 13, 29–41.

2- Krausz, C., & Riera-Escamilla, A. (2018). Genetics of male infertility. Nature reviews. Urology, 15(6), 369–384.

3- Julio M. Castañeda, ... Martin M. Matzuk, in Encyclopedia of Reproduction (Second Edition), 2018.

4- Al-Quzwini, O. F., Al-Taee, H. A., & Al-Shaikh, S. F. (2016). Male fertility and its association with occupational and mobile phone towers hazards: an analytic study. Middle East Fertility Society Journal, 21(4), 236-240.

5- Patel, A. S., Leong, J. Y., & Ramasamy, R. (2018). Prediction of male infertility by the World Health Organization laboratory manual for assessment of semen analysis: a systematic review. Arab journal of urology, 16(1), 96-102.
6- WHO. (2010). WHO laboratory manual for the examination and processing of human semen.
7- Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). 'Molecular Cloning: A Laboratory Manual,''2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
8- SAS. 2012. Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.
9- Agarwal, A., & Said, T. M. (2011). Interpretation of basic semen analysis and advanced semen testing. In Male Infertility (pp. 15-22). Humana Press, Totowa, NJ.
10- Hassan ,GM. (2017). "Study of Genetic Apoptosis According to expression FAS and FASL genes In Severe Oligozoospermic Iraqi Patients".
11- Gopalkrishnan, K. A. M. A. L. A., Hinduja, I. N., & Kumar, T. C. (1992). Volume of semen as a parameter of its quality. The Indian journal of medical research, 96, 361-365.
12- Harraway, C., Berger, N. G., & Dubin, N. H. (2000). Semen pH in patients with normal versus abnormal sperm characteristics. American journal of obstetrics and gynecology, 182(5), 1045-1047.
13- World Health Organisation. (1992). WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. Cambridge university press.
14-Agúndez, J. A. (2004). Cytochrome P450 gene polymorphism and cancer. Current drug metabolism, 5(3), 211-224.
15-Shah, P. P., Saurabh, K., Pant, M. C., Mathur, N., & Parmar, D. (2009). Evidence for increased cytochrome P450 1A1 expression in blood lymphocytes of lung cancer patients. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 670(1-2), 74-78.
16- Fang, J., Wang, S., Wang, H., Zhang, S., Su, S., Song, Z., ... & Wang, Z. (2014). The Cytochrome P4501A1 gene polymorphisms and idiopathic male infertility risk: a meta-analysis. Gene, 535(2), 93-96.
17- Luo, H., Li, H., Yao, N., Hu, L., & He, T. (2014). Association between 3801T> C polymorphism of CYP1A1 and idiopathic male infertility risk: a systematic review and meta-analysis. PLoS One, 9(1), e86649.
18-Carreño Flórez, G., Escorcia Gamarra, R., Varela Prieto, L., Silvera Redondo, C., & Gutiérrez De Aguas, R. (2015). CYP1A1* 2A polymorphism and infertility in Colombian Caribbean male subjects. Revista Salud Uninorte, 31(1), 1-19-Cao, D., Ren, Z., Lu, D., Liu, L., Xu, P., Zhang, Q., & Wei, Q. (2019). Association between CYP1A1 rs4646903 T> C genetic variations and male infertility risk: A meta-analysis. Medicine, 98(31).
20-Salehi, Z., Gholizadeh, L., Vaziri, H., & Madani, A. H. (2012). Analysis of GSTM1, GSTT1, and CYP1A1 in idiopathic male infertility. Reproductive sciences, 19(1), 81-85.
21-Yarosh, S. L., Kokhtenko, E. V., Starodubova, N. I., Churnosov, M. I., & Polonikov, A. V. (2013). Smoking status modifies the relation between CYP1A1* 2C gene polymorphism and idiopathic male infertility: the importance of gene–environment interaction analysis for genetic studies of the disease. Reproductive Sciences, 20(11), 1302-1307.
22- Lu, N., Wu, B., Xia, Y., Wang, W., Gu, A., Liang, J., ... & Wang, X. (2008). Polymorphisms in CYP1A1 gene are associated with male infertility in a Chinese population. International journal of andrology, 31(5), 527-533.
23-Vani, G. T., Mukesh, N., Prasad, B. S., Devi, P. R., Prasad, M. H., Rani, P. U., & Reddy, P. P. (2009). Association of CYP1A1* 2A polymorphism with male infertility in Indian population. Clinica chimica acta, 410(1-2), 43-47.