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FAS and FASL genes polymorphisms and their relationship with the incidence of severe oligozoospermia in a sample of Iraqi patients.

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Abstract: The interaction between Fas and FasL plays an important role in triggering the apoptotic pathway. Both Fas and FasL exist as membrane bound and soluble forms. It is suggested that Fas may be a marker of overall apoptosis triggering, at the same time regulating apoptosis by competing with the cell surface receptor. The aim of the present study was to detect the FAS and FASL gene polymorphisms and to demonstrate their relationship with incidence of severe oligozoospermia in a sample of Iraqi patients. Following semen analysis, blood samples were collected from severe oligozoospemic patients (n=50) and normozoospermic subjects (control, n=50). DNA was extracted using DNA extraction Kit (Geneaid Biotech). The genotypes of FAS (-670G/A) and FASLG (-844T/C) were determined by using Taqman (RT-PCR) Kit (WizPure™ qPCR Master (PROBE) , south Koria). The results of the present study, as related with Fas -670G to A SNP, indicated that the frequencies of both GA and AA genotypes were significantly ($p<0.01$) higher in severe oligozoospermia group than in normozoospermia group (30% versus 20% and 60% versus 0%, respectively). Also, as related with FasL -844C to T SNP, the frequencies of both CT and TT genotypes were significantly ($p<0.01$) higher in severe oligozoospermia group than in normozoospermia group (38% versus 16% and 50% versus 0%, respectively). In conclusion, there was an A allele-related risk factor for Fas -670G to A SNP and T allele-related risk factor for FasL -844C to T SNP with the incidence of severe oligozoospermia in Iraqi patients.

Key Words: Severe Oligozoospemia, FAS, FASL, polymorphism.

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Introduction:

Fertility is one of the most tragic marital problems. It was estimated that nearly 8-12% of couples are infertile (1). Despite recent advances in the treatment of infertility, the problem could not be satisfactorily tackled so far for varied reasons (2,3,4,5). Poor semen quality is correlated with low fertilization rate, impaired preimplantationdevelopment, increased abortion and elevated incidence of disease in the offspring (6,7). In humans, a strong association has been found between abnormal semen parameters and the abortive apoptosis in ejaculated sperm (8,9). Germ cell apoptosis is physiologically involved in various stages of mammalian testicular development (10). It plays an important role in regulating germ cell number and eliminating defective germ cells and thus in maintaining normal spermatogenesis(11).

Apoptosis appears to have an essential role in the control of germ cell number in testis (12). During spermatogenesis, germ cell death via apoptosis has been estimated to result in the loss of up to 75% of the potential number of mature sperm cells (10). This apoptotic wave appears necessary for normal spermatogenesis to develop, probably because it maintains a proper cell number ratio between maturing germ cell stages and Sertoli cells (13). The Fas (CD95) system is considered to be one of the key regulator systems of testicular germ-cell apoptosis (12). The expression of Fas has been reported in the apoptotic spermatocytes and spermatids (14,15,16,17,18) and also in Sertoli cells (19). The presence of Fas on ejaculated sperm was first described by Sakkas *et al*. (8) and it was explained by the abortive apoptosis theory. This theory suggests that in some cases of infertility, the normal apoptotic mechanisms have malfunctioned, have been overridden or have not been completed and Fas positive sperm have failed to be eliminated (8). It was also shown that men with abnormal semen had a higher percentage of Fas positive sperm than men with normal semen (8).

The interaction between Fas (CD95/Apo-1) a type I transmembrane glycoprotein receptor and cellular death inducing ligand a type II transmembrane glycoprotein (FasL) plays an important role in triggering the apoptotic pathway (13, 14). Both Fas and FasL exist as membrane bound and soluble forms. It is suggested that sFas may be a marker of overall apoptosis triggering, at the same time regulating apoptosis by competing with the cell surface receptor. The apoptotic cell death is a highly regulated process that in many cases requires activation of caspases, a superfamily of cysteine aspartyl-specific proteases. Caspase-3, an executioner caspase is thought to play a central role in apoptosis in a wide variety of cells (19). The presence of activated form of caspase-3 marks the point of no return within the complex apoptotic signaling. This paper was designed to describe a case–control study that aimed to examine the contribution of aforementioned FAS and FASL polymorphisms to the risk of severe oligozoospermia and found that the FAS and FASL polymorphisms are associated with the risk of severe oligozoospermia with Iraqi patients.

Material and Methods

After semen analysis which done in Baghdad Specialist Fertility Centre during a period from 15 September to 10 February 2016, then study groups were classified according to the semen analysis. Blood samples were collected from severe oligozoo-spemic patients (n=50) and apparently healthy subjects (Control, n=50) in Baghdad Specialist Fertility Centre. Mixing blood samples by vortex for 10 min. and transfer up to 200 µL of blood sample to a 1.5 ml microcentrifuge tube. Thereafter, adding 20 µL of protease K and mix by pipetting. Incubate at 60°C for 5 min. then adding 200 µL of GSB Buffer and mix by shaking vigorously, incubate at 60 C for 20 min. then adding 200 µL of absolute ethanol to the samples lysate and mix immediately by shaking vigorously for 10 sec., transfer all of the mixture to the GS column then centrifuge at 14.000 xg for 1 min., after that discarding the 2ml collection tube containing the flow through then and transferring the GS column to a new collection tube. Adding 400 µL of W1 Buffer to the GS column and centrifuge at 14.000 xg for 30 sec. then

discarding the flow through and place the GS column back in the 2ml collection tube. Then adding 600 µL of Wash Buffer to the GS column and centrifuge at 24.000 xg for 30 sec. then discarding the flow through and place the GS column back in the 2ml collection tube then centrifuge again for 3 min. at 14.000 xg to dry the column matrix. Transfer the dried GS column to a clean 1.5 ml microcentrifuge tube and adding 50 µL of Elution buffer into the center of the column matrix, and let stand for 5 min to allow elution buffer to be completely absorbed, then centrifuge at 14.000 xg for 1 min to elute purified DNA.

Genotypes of FAS-670G/A, FASLG-844T/C polymorphisms were determined by Taqman (RT-PCR) Using Kit (WizPure™ qPCR Master (PROBE), south Korea, FAS gene forward primer:

5- GGGCTATGCGATTTGGCTTAA -3 and reverse primer:
5- GTAGTTCAACCTGGGAAGTTG GG -3, FASL gene forward primer:
5-TGCAGTTAACTACGATAGCAC CAC-3 and reverse primer:
5-AGGAGGAAAACATCTGTTGCC-3.

Probe sequences for target FAS gene. Dye Fam-BHQ:
5- TGTCCATTCCAGAAACGT -3 and Dye Vic-BHQ:
5-ACTGTC CATTCCAGGAAC -3.

Probe sequences for target FASL gene. Dye Fam-BHQ:
5-TGCAGTTAACTACGATAGCCA CCAC-3 and Dye Vic-BHQ:
5-AGGAGGAAAACATCTGTTGCC-3.

Application program RT-PCR: Hold 50 C for 15min.Denaturation 95 C for 15min. for one cycle, annealing 95 C for 5 sec., 60 C for 20 min., 72 C for 15 min, for five cycles. Melting curve analysis 95 C for 5 min., 60 C for 20 min., 72 C for 15 min. for 40 cycles.

The Statistical Analysis System-SAS program was used to effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage, and estimate of Odd ratio in this study(20).

Results and Discussion:

According to the results achieved by RT-PCR using TaqMan with two dye (Fam + Vic). Genotypes of FAS gene are presented in figure (1). Samples with wild genotypes were appear reaction toward Fam dye and not reaction to Vic dye with single curve. The heterozygous genotypes were appearing reaction toward Fam + Vic dyes and two curves. The homozygous mutant genotypes were appearing reaction toward Vic dye and not reaction to Fam dye with single curve beads.

The distribution of genotypes and allele frequency at – 670 site of FAS gene presented in table 1. As related with GG genotype (wild-type), the frequency of this genotype was significantly (p<0.01) lower in severe oligozoospermia group than apparently healthy control group (10 % *versus* 80%, respectively, X^2=13.548; OR=2.415). In contrast, GA genotype frequency was significantly (p<0.01) higher in severe oligozoospermia group than apparently healthy group (30 % *versus* 20%, respectively, X^2=8.259; OR=0.973).

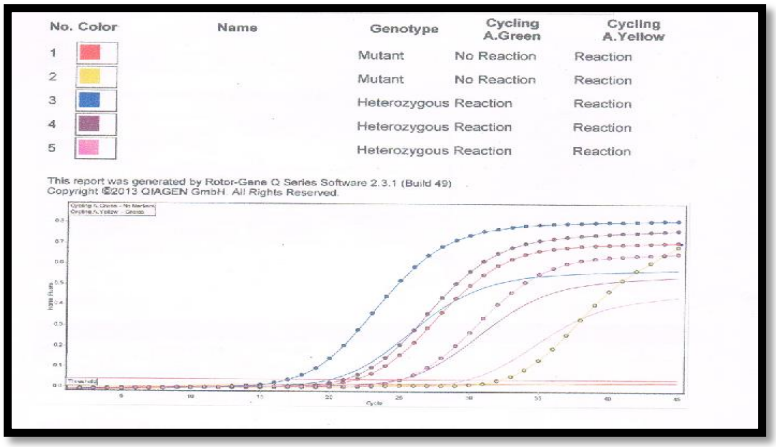

Figure (1): Determination of FAS and FASL gene polymorphism by RT-PCR using TaqMan genotyping kit (Fam + Vic) dyes.

Table (1): The genotype and allele frequencies of – 670 G to A SNP in FAS gene of Iraqi severe oligozoospermic patients.

Genotypes	Control[1] (n=50)		Patients[2] (n=50)		Chi square X^2	Odd ratio
	n	%	n	%		
Wild-type GG	40	80	5	10	13.548 **	2.415
Heterozygous GA	10	20	15	30	8.259 **	0.973
Homozygous AA	0	0	30	60	12.713 **	2.309
Allele frequency						
G	0.90		0.25			
A	0.10		0.75			

[1]Control: Normozoospermic, subjects with normal semen criteria. [2]Patients: severe oligozoospermic patients. ** means the difference is highly significant at 0.01 level.

Also, AA genotype frequency was significantly (p<0.01) higher in severe oligozoospermia group than apparently healthy group (60 *versus* 0%, respectively, X^2=12.713; OR=2.309). The frequencies of G and A alleles were 0.90 and 0.10 in apparently healthy control group and 0.25 and 0.75 in severe oligo-zoospermia group, respectively.

In this study as noted in the results of table 1, there is an A allele-related risk factor with the incidence of severe oligo-zoospermia (OR=0.973 and 2.309 in GA and AA genotypes, respectively).

The results of the present study are in agreement with Guixiang *et al.* (21) who found that FAS -670 G to A (rs1800682) was associated with sperm apoptosis and semen quality and the individuals carrying FAS -670 GG genotype had a low apoptosis rate associating with poor sperm motility and decreased sperm concentration compared with the AA genotype. In contrast, Wang *et al*. (22) found no association of FAS -670 G to A SNP with susceptibility to severe oligozoospermia in Han Chinese men. Using the immune activity assay, McVicar *et al.* (23) found a high percentage of FAS-positive sperms in infertile men supporting the role of the

FAS pathway in regulating sperm apoptosis.

FAS -670G variant allele, which is locating in the sequence of STAT1 transcription factor binding site, was demonstrated to be associated with low apoptosis capacity (24). Also, FAS -670G allele disrupted the binding of STAT1 transcription factor resulting in a decreased FAS gene expression. The presence of FAS on ejaculated sperm was first described by Sakkas *et al.* (8). They demonstrated that semen samples from subfertile men who have low semen parameters were more likely to show high levels of FAS expression.

Sun *et al.* (25) reported that the FAS gene promoter polymorphisms were significantly associated with increased risk of esophageal SCC in Chinese population. No association between FAS -670 G to A and breast cancer risk was reported by several studies (26,27,28). The frequency of FAS gene genotypes at promoter position – 670 was determined in 249 patients with systemic lupus erythematosus (SLE) and 212 apparently healthy controls and the results found no significant differences between patients and controls (29). Ying Huang *et al.* (30) found an association between the increased risk of acute myeloid leukemia (AML) and FAS promoter polymorphisms and that the distribution of FAS -670 GG, GA and AA among the AML patients were not significantly different from those of the healthy controls.

The results of FASL – 844 C to T SNP were achieved by RT-PCR using TaqMan with two dyes (Fam + Vic). Samples with wild genotypes (CC) were appear reaction toward Fam dye and not reaction to Vic dye with single curve. The heterozygous genotypes (CT) were appearing reaction toward Fam + Vic dyes and two curves. The homozygous mutant genotypes (TT) were appearing reaction toward Vic dye and not reaction to Fam dye with single curve beads (as shown in figure 1).

The distribution of genotypes and allele frequency at – 844 site of FASL gene presented in table 2. As related with CC genotype (wild-type), the frequency of this genotype was significantly (p<0.01) lower in severe oligozoospermia group than subjects with normozoospermic semen (12 % *versus* 84%, respectively, X^2=13.468; OR= 2.561). In contrast, CT genotype frequency was significantly (p<0.01) higher in severe oligozoospermia group than apparently healthy group (38 % *versus* 16%, respectively, X^2=8.933; OR=2.047). Also, TT genotype frequency was significantly (p<0.01) higher in severe oligozoospermia group than apparently healthy group (50 *versus* 0%, respectively, X^2=10.75; OR=2.368).

The frequencies of C and T alleles were 0.92 and 0.08 in apparently healthy control group and 0.31 and 0.69 in severe oligozoospermia group, respectively.

As shown from the results of table (2), there is a T allele-related risk factor with the incidence of severe oligozoospermia (OR= 2.047 and 2.368 in CT and TT genotypes, respectively).

Table (2): The genotype and allele frequencies of – 844 C to T SNP in FASL gene of Iraqi severe oligozoospermic patients.

Genotypes	Control[1] (n=50)		Patients[2] (n=50)		Chi square X^2	Odd ratio
	n	%	n	%		
Wild-type CC	42	84	6	12	13.468 **	2.561
Heterozygous CT	8	16	19	38	8.933 **	2.047
Homozygous TT	0	0	25	50	10.75 **	2.368
Allele frequency						
C	0.92		0.31			
T	0.08		0.69			

[1]Control: Normozoospermic, subjects with normal semen criteria. [2]Patients: severe oligozoospermic patients. ** means the difference is highly significant at 0.01 level.

The results of this study confirmed the results of Wang *et al*. who indicated that *FASLG* –844C/T SNP was significantly associated with risk of idiopathic azoospermia or severe oligozoo-spermia, suggesting it might be a genetic predisposing factor of idiopathic azoospermia or severe oligozoospermia in Han Chinese men. In the study of Wang *et al.* (22) the frequency of –844T allele was 22.8%, which was also close to southern Han Chinese (23.0%), but lower than that of African Americans (82.0%) and American Caucasians (36.0%) (31, 32) and Iraqis in the present study (69%). Also, Wang *et al.* found that the men with *FASLG* –844TT genotype had an increased risk of idiopathic azoospermia or severe oligozoospermia compared with those with CC+CT genotypes. The present work found that –844C allele had twice the basal activity of the –844T allele, and basal expression of FASL on peripheral blood fibrocytes was also significantly higher in –844C than in –844T homozygous donors. Therefore, the data suggest that *FASL* –844TT genotype might express less FASL which results in reduced germ cell apoptosis and increased germ cell production so that limited amount of Sertoli cells cannot provide enough structural and nutritional support to the excessive germ cells, thus leading to spermatogenical retard (22). In contrast, the results of the present study disagree with other study which found no association between FASL -844C>T SNP and altered sperm apoptosis and poor semen quality in Chinese population (21). Also disagree with Deepika *et al.* who found that the frequency of alleles and genotypes of FASL -844C>T were not significantly differ between infertile azoospermic patients and control subjects (33).

The *FASL* –844C>T polymorphism is located in a binding motif of CAAT/enhanced-binding protein beta (C/EBP-beta) element (28). There has been evidence indicating the –844T allele may decrease basal FASL expression, suggesting that the *FASL* –844C>T polymorphism may influence the FASL expression and FASL-mediated apoptotic signaling.

Apoptosis is a mechanism that selects sperm cells and controls the overproduction of gametes to numbers that can be supported by the Sertoli cells. It is a process naturally occurring in spermatogenesis (34). Therefore, apoptosis may be commonly observed in spermatocytes and spermatids (35). Apoptosis may be activated through

extrinsic mechanisms involving the interaction of Fas and its ligand, FasL (36, 37), a system which controls homeostasis in various tissues (38). Fas is a type I transmembrane protein that belongs to the tumor necrosis factor receptor superfamily. FasL is produced as a type II membrane protein and is a member of the tumor necrosis factor family of proteins (39, 40). Upon binding to FasL, Fas induces apoptotic activation of intracellular caspases, effector proteins, which in turn activate the cascade of events leading to cell death (8, 38).

In the testis, Fas-induced apoptosis plays an important role during spermatogenesis. Testicular germ cells which are defective or in excessive numbers externalize Fas and, when in contact with FasL from Sertoli cells, undergo apoptosis and are resorbed. Thus, apoptosis plays an important role in (i) selecting germ cells and (ii) maintaining a proper amount of germ cells available for maturation by the Sertoli cells. In some cases, apoptosis may initiate (externalization of Fas) but, due to lack of trigger signals from the Sertoli cells, abort the process and mature into abnormal sperm, in a process known as abortive apoptosis (8). Sakkas *et al.* (8) described the presence of Fas on ejaculated sperm, suggesting that abortive apoptosis may indeed be a major stakeholder in male infertility.

In addition, FASL -844C>T SNP correlated with other diseases, Crew et al. (27) reported no association between breast cancer and FASL -844C>T SNP. FASL -844T allele has a possible protective effect on cancer risk (41).

In conclusion, both FAS -670G>A and FASL -844C>T were correlated with the risk of severe oligozoospermia in a sample of Iraqi patients.

References:

1. Barbara, B. (2003). Men contribute to and suffer from infertility. Family Health International. *23*:17-20.
2. Al-Faisal A.H.M. ,Ali Fadel Alnajar and Abdul Ameer M.Ghareeb .2010. AZF, SRY microdeletions and hormonal disturbances among azoospermic Iraqi men. *ISAN Journal of Pharmaceutical Science*,6(2): 92-97.
3. Al-Faisal A.H.M. ,Ali Fadel Alnajar and Wafaa Hazim Saleh .2009. Cytogenetical-Molecular and Hormonal Study on Male Infertility in Iraq.*J.Med.Clin.Associ. of Thialand*,37(3):59-63.
4. Hussein,A.Z., Al-Faisal,A.H.M and Al-Jiboori B.M.2009. Y chromosome azoospermia factors (AZF) microdeletions in azoospermic men. *Iraqi J.Med.Sci.*, Vol 7(1): 102-108.
5. Jadhav, S.; Afroz, S.; Bhore, U.; Karadkhekar, S. and Dudhmal, V. (2012). Correlation of physical and biochemical parameters of human semen in addicts and functional impotents. IOSR Journal of dental and medical sciences (JDMS), 2(4):25-28.
6. Brinkworth, M.H. (2000). Paternal transmission of genetic damage: findings in animals and human. Int. J. Androl., 23(3):123-135.
7. Jensen, M.A.; Charlesworth, B. and Kreitman, M. (2002). Patterns of genetic variation at a chromosome 4 locus of *Drosophila melanogaster* and *D. simulans. Genetics*, 160(2): 493-507.
8. Sakkas, D.; Mariethoz. E. and St John, J.C. (1999). Abnormal sperm parameters in humans are indicative of an abortive apoptotic mechanism linked to the Fas-mediated pathway. *Experimental Cell Research* 251, 350–355.
9. Shen, H.M.; Dai, J.; Chia, S.E.; Lim, A. and Ong, C.N. (2002). Detection of apoptotic alterations in sperm in subfertile patients and their correlations with sperm quality. Hum. Reprod., 17:1266–1273.

10. Dunkel, L.; Raivio, T., Laine, J. and Holmberg, C. (1997). Circulating luteinizing hormone receptor inhibitor(s) in boys with chronic renal failure. Kidney Int. Mar, 51(3): 777-784.
11. Koji, T. (2001). Male germ cell death in mouse testes: possible involvement of Fas and Fas ligand. *Medical Electron Microscopy*, 34, 213–222.
12. Lee, J.; Richburg, J.H.; Younkin, S.C. and Boekelheide, K. (1997). The Fas system is a key regulator of germ cell apoptosis in the testis. *Endocrinology*, 138: 2081–2088.
13. Rodriguez, I.; Ody, C.; Araki, K. *et al.* (1997). An early and massive wave of germinal cell apoptosis is required for the development of functional spermatogenesis. *EMBO Journal*, 16: 2262–2270.
14. Pentikainen, V.; Erkkila, K. and Dunkel, L. (1999). Fas regulates germ cell apoptosis in the human testis in vitro. Am. J. Physiol.,276:310–316.
15. Francavilla, S.; D'Abrizio, P.; Rucci, N. *et al.* (2000). Fas and Fas ligand expression in fetal and adult human testis with normal or deranged spermatogenesis. *Journal of Clinical Endocrinology and Metabolism*, 85: 2692–2700.
16. Francavilla, S.; D'Abrizio, P.; Cordeschi, G. *et al.* (2002). Fas expression correlates with human germ cell degeneration in meiotic and postmeiotic arrest of spermatogenesis. *Molecular Human Reproduction*, 8: 213–220.
17. Kim, H.S.; Ryu, J.Y. and Lee, B.M. (2007). Identification of differentially expressed genes in the testis of Sprague–Dawley rats treated with di(n-butyl) phthalate. Toxicology, 234: 103–112.
18. O'Neill, D.A.; McVicar, C.M.; McClure, N.; Maxwell, P.; Cooke, I.; Pogue, K.M. and Lewis, S.E. (2007). Reduced sperm yield from testicular biopsies of vasectomized men is due to increased apoptosis. Fertil. Steril., 87:834–841.
19. Sugihara, A.; Saiki, S.; Tsuji, M.; Tsujimura, T.; Nakata, Y.; Kubota, A.; Kotake, T. and Terada, N. (1997). Expression of Fas and Fas ligand in the testes and testicular germ cell tumors: an immunohistochemical study. Anticancer Res., 17:3861–3865.
20. SAS. (2012). Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.
21. Ji, G.; Gu, A.; Hu, F.; Wang, S.; Liang, J.; Xia, Y.; Lu, C.; Song, L.; Fu, G. and Wang, X. (2009). Polymorphisms in cell death pathway genes are associated with altered sperm apoptosis and poor semen quality. Hum. Reprod., 24 (10): 2439-2446.
22. Wang, W.; Lu, N.; Xia, Y.; Gu, A.; Wu, B.; Liang, J.; Zhang, W.; Wang, Z.; Su, J.; Wang, X.; (2009). *FAS* and *FASLG* polymorphisms and susceptibility to idiopathic azoospermia or severe oligozoospermia. *Reproductive BioMedicine*, 18(1): 141-147.
23. McVicar, C.M.; McClure, N.; Williamson, K.; Dalzell, L.H. and Lewis, S.E. (2004). Incidence of Fas positivity and deoxyribonucleic acid double-stranded breaks in human ejaculated sperm. Fertil. Steril., 81:767–774.
24. Wang, L.E., Cheng, L., Spitz, M.R., Wei, Q. (2003). Fas A670G polymorphism, apoptotic capacity in lymphocyte cultures, and risk of lung cancer. *Lung Cancer.*, 42, 1–8.
25. Sun, T.; Miao, X.; Zhang, X. *et al.* (2004). Polymorphisms of death pathway genes FAS and FASL in esophageal squamous-cell carcinoma. *Journal of the National Cancer Institute* 96, 1030–1036.
26. Kripple, P.; Langsenlehner, U.; Renner, W.; Koppel, H. and Samonigg, H. (2004). Polymorphisms of death pathway genes FAS and FASL in esophageal squamous-cell carcinoma. *J. Natl. Cancer Inst.*, 96: 1478–1479.
27. Crew, K.D.; Gammon, M.D.; Terry, M.B.; Zhang, F.F.; Agrawal, M. *et al.* (2007). Genetic polymorphisms in the apoptosis-associated genes FAS and FASL and breast cancer risk. Carcinogenesis 28: 2548–2551.
28. Bebenek, M.; Duś, D. and Koźlak, J. (2008). Fas expression in primary breast cancer is related to neoplastic infiltration of perilymphatic fat. Advances in Medical Sciences, 53(1): 49-53.
29. Arasteh, J.M.; Sarvestani, E.K.; Aflaki, E. and Amirghofran, Z. (2010). Fas gene polymorphisms in systemic lupus erythematosus and serum levels of some apoptosis-related molecules. Immunol. Invest., 39(1):27-38.
30. Huang, Y.; Deng, D.; Li, H.; Xiao, Q.; Huang, L.; Zhang, B.; Ye, F.; Ye, B.; Mo, Z.; Yang, X. and Liu, Z. (2016). Fas-670A>G polymorphism is not

associated with an increased risk of acute myeloid leukemia development. Biomed Rep. 4(2): 153–160.

31. Wu, J.; Metz, C.; Xu, X. *et al.* (2003). A novel polymorphic CAAT/enhancer binding protein beta element in the FasL gene promoter alters Fas ligand expression: a candidate background gene in African American systemic lupus erythematosus patients. *Journal of Immunology* 170, 132–138.
32. Li, C.; Wu, W.; Liu, J. *et al.* (2006). Functional polymorphisms in the promoter regions of the FAS and FAS ligand genes and risk of bladder cancer in south China – a case–control analysis. *Pharmacogenetics and Genomics* 16, 245–251.
33. Deepika, J.; Neeraj, K. and Kiran. (2015). Association of polymorphism in cell death pathway gene FASLG with human male infertility. Asian Pacific Journal of Reproduction 4(2) :112-115.
34. Fujisawa, M.; Hiramine, C.; Tanaka, H.; Okada, H.; Arakawa, S. and Kamidono, S. (1999). Decrease in apoptosis of germ cells in the testes of infertile men with varicocele. *World J. Urol.*,17:296–300.
35. Hikim, AP.; Wang, C.; Lue, Y. *et al.* (1998). Spontaneous germ cell apoptosis in humans: evidence for ethnic differences in the susceptibility of germ cells to programmed cell death. *Journal of Clinical Endocrinology and Metabolism.*, 83, 152–156.
36. Arnoult, D.; Parone, P.; Martinou, J.C.; Antonsson, B.; Estaquier, J.; Ameisen, J.C. (2002). Mitochondrial release of apoptosis-inducing factor occurs downstream of cytochrome c release in response to several proapoptotic stimuli. *J. Cell Biol.*,159:923–929.
37. Chipuk, J.E. and Green, D.R. (2005). Do inducers of apoptosis trigger caspase independent cell death? Nat. Rev. Mol. Cell Biol.,6:268–275.
38. D'Alessio, A.; Riccioli, A.; Lauretti, P.; Padula, F.; Muciaccia, B.; De Cesaris, P.; *et al*. (2001). Testicular FasL is expressed by sperm cells. Proc. Natl. Acad. Sci. USA., 98(6):3316–3321.
39. Suda, T.; Takahashi, T.; Golstein, P. and Nagata, S. (1993). Molecular cloning and expression of the Fas ligand, a novel member of the tumor necrosis factor family. *Cell* 75, 1169–1178.
40. Nagata, S. and Golstein, P. (1995) The Fas death factor. *Science* 267, 1449–1456.
41. Zhang, Z.; Qiu, L.; Wang, M.; Tong, N. and Li, J. (2009). The FAS ligand promoter polymorphism, rs763110 (2844C.T), contributes to cancer susceptibility: evidence from 19 case-control studies. *Eur. J. Hum. Genet.*, 17: 1294–1303.