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Impact of DNA Methylation and Gene Expression of *H19*, *SNRPN* and *LINE-1* Genes on Oligospermia

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Abstract: Male infertility is a complicated problem with a strong genetic and epigenetic foundation. Imprinted genes survive fertilization and retain their parent-specific germline patterns, while aberrant methylation imprints can be passed directly from the father's sperm to the developing embryo. Semen samples were collected from patients (number=100) (50 infertile oligospermia men and 50 fertile normospermia men) for epigenetic study (methylation pattern) after DNA isolation from seminal fluid samples using a kit for extraction DNA and converted to methylation pattern, then analyzed methylated DNA was used quantitative real-time polymerase chain reaction by high-resolution melting technique for *H19*, *SNRPN*, and *LINE-1* genes. Also, semen samples were used for the molecular study (gene expression) after RNA was extracted using a kit of extraction and converted to complementary DNA, then used quantitative reverse transcription real-time polymerase chain reaction for *H19*, *SNRPN*, and *LINE-1* genes to determine expression levels. The current study was present for DNA methylation was significant in the infertile group compared with fertile group, the defects on gene promoters and the affect on gene expression may serve as epigenomic biomarkers for the assessment of oligospermia infertility in men with multiple sperm defects.

Keywords: DNA methylation, gene expression, *H19*, *LINE-1*, male infertility, *SNRPN*.

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Introduction

Infertility is a genetically heterogeneous disease that has multifactorial etiology, and affects approximately 22% of couples of the reproductive age group and has a major effect on reproductive health (1, 2 ,3). The most common causes of infertility in men are anatomical defects, gametogenesis deficiency, endocrinopathies, immunological difficulties, perineal duct deficiency, and peripheral toxicity. Male infertility has been linked to spermatogenesis epigenetic events like chromosome condensation, genomic packaging in the spermatid nucleus, and the presence of retro-transposons (4, 5). Epigenetic reprogramming abnormalities and their links to fertility, ART-related hazards, testicular cancer, and the impact of environmental factors on epigenetic processes (6, 7). The epigenetics studies genome modifications that regulate gene expression without altering the content of the genetic sequence (8 ,9). Epigenetic changes encompass an array of molecular modifications of DNA or histones (post-translational histone

modifications) that are intimately associated with DNA by enzymes that regulate gene expression by modulating chromatin availability which affects how genetic variations and function inside a cell resulting in a constricted chromatin structure and transcriptional repression (10, 11). DNA methylation is a biochemical process that involves the addition of a methyl group to the 5′ position of the cytosine pyrimidine ring typically occurring in a cytosine pyrimidine guanin (CpG) dinucleotide by DNA methyltransferase (DNMT) activity. The changes are acquired in a gradual rather than by an abrupt process (12). Hypomethylation and hypermethylation can occur simultaneously at different regions in the genome. Oligospermia may exhibit abnormal sperm phenotypes (13). At the time of fertilization paternal genome delivered by the mature sperm has a haploid genome and is packaged densely with protamine's (mainly or partially with histones), whereas the maternal genome arrested at metaphase II is packaged with histones. In genomic imprinting, a parent-specific epigenetic modification, allele-specific expression is based on male vs female germline transmission (14). Most imprinted genes encode differentially methylated regions (DMRs), which are highly expressed as imprinting regulatory centers. Because epigenetic regulation is associated with effective cell fate determination, parent genomic imprints are established during gametogenesis, especially in the early stages of embryo development (15). Before male gametes enter meiosis, DNA methylation at imprinted genes is established and maintained throughout development (16). Paternal imprinting in sperm cells has been discovered in three genetic loci: the *H19* gene, maternal imprinting *SNRPN* gene, and *LINE-1* gene in this study, the methylation state and gene expression of the *H19, SNRPN*, and *LINE-1* genes were used to distinguish different groups of infertile males (oligospermia) and control males in seminal fluid (SF) samples and to investigate the links between DNA methylation and gene expression to assess epigenetic and molecular modification using qPCR and RT-PCR techniques.

Methodology

The study was designed case-control prospective study was done on 100 Iraqi men age range 20–50 years (50 for both oligospermia (infertile) men and healthy control men (fertile)) after a clinical examination of each patient diagnosed by andrology at Kamal Al-Samurai Specialized Hospital for Infertility and *in-veto* fertilization (IVF) in Baghdad for laboratory seminal fluid analysis (SFA) and for molecular study for DNA methylation and gene expression was subsequently compared the result between samples for both study groups. The exclusion criteria for this study referred to previous varicocele, genetic aberrations, endocrine system dysfunction. Oligospermia parameters (<15 million/ml) according to the World Health Organization standards of 2010 (17). The SF samples were collected after 3–4 days of sexual abstinence and used to study molecular analysis. In fertile men, all men had been married for at least two years and had never used contraception and were healthy.

Extraction and Purification of Genomic DNA

The genomic DNA purification technique kit (RIBO-Sorb-AM) (AmpliSens, Russia) was used to extract and isolate total DNA from all SF

samples, as directed by the manufacturer. The estimation of the concentration and purity for gDNA were used the Nano-Drop spectrophotometer for all samples (Nano-Drop Technologies, SA).

Bisulfite Conversional DNA (DNA Methylation Method)

The genomic DNAs were converted to methylated DNA patterns using the Qiagen EpiTect DNA Bisulfite Kit (Qiagen, Germany). Bisulfite conversion was used for quantitative real-time polymerase chain reaction (q-PCR) by EpiTect ® PCR control DNA kit as internal control human DNA methylated was used to provides a greatly improved ability to differentiate methylated (converted 5-methylcytosines) /+unmethylated compared (unconverted 5-methylcytosines). The ability of a certain primer to produce amplification was used to detect "methylated-specific,", or "unmethylated specific," with sequences matching thymine converted from unmethylated cytosine residues.
Then the methylation DNA was used for the High-Resolution Melting experiment (HRM) to detect methylations patterns by qPCR using Wizpure TM qPCR Master Mix (EVA). The qPCR program was performed of the following condition, initial denaturation step 95 ºC for 5min for 1 cycle, denaturation 95 ºC for 15 sec followed by annealing step 61 ºC for *H19-1*, *SNRPN*, and *LINE-1* and 62 for *H19-2* for 25 sec and extension 72 ºC for 15 sec for 5 cycles were used in the qPCR procedure. The denaturation step is 95ºC 15 sec and annealing 61-62 ºC for 25 sec, followed by Melt °C, 60-90 ºC for 5 sec for 1 cycle. The primers that were used in this study *H19-1, H19-2, LINE-1* and *SNRPN* genes were supplied by Alpha-DNA Company, (Canada).

Isolation and Purification of Genomic RNA

The genomic RNA was extracted and purified the total of RNA from sperm samples used kit (RIBO-prep) (AmpliSens, Russia), according to the manufacturer's instructions. Nanodrop was used to determine the amount and quality of the extracted RNA for each sample at 260 and 280 nm. Using a thermal cycler quantitative reverse transcription real-time polymerase chain reaction qRT-PCR (Eppendorf, Germany), the isolated mRNAs were reversed to cDNA using Reverta-L (RT-reagents Kit) (AmpliSens, Russia) according to the manufacturer's procedure. The qRT-PCR cycle consisted of one cycle of enzyme activation at 95°C for 1 min for one cycle, followed by 40 cycles for initial denaturation at 95°C for 15sec., annealing 60 for 20 sec., extension at 72°C for 30 sec., and final extension 72 for 7min. for one cycle. The primer was utilized for qRT-PCR using WizpureTM qRT-PCR Master Mix SYBR Green (Wizbio Solution, Korea) and evaluated using the $2^{\wedge-\Delta\Delta Ct}$ technique (18) for measuring threshold cycle (Ct value) (19, 20) after normalization Ct value with internal control (IC) mRNA *β-globin* serves as a housekeeping gene (HKG).

Statistical Analysis

All data obtained from this study were analyzed using International Business Machines Statistical Package for the Social Sciences (IBM SPSS©) Statistics Version for Windows software package version 26 (21) (SPSS Inc., USA). The results were utilized using unrelated t-tests, Z-test and Spearman's test, r: Correlation coefficient. The data

is presented as a mean standard deviation. The $P<0.05$, $P<0.001$ were considered to be statistically significant.

Result and Discission

The result of the demographic and general characteristics used in this study were showed in Table 1. The result showed there was no significant difference in the age and duration of married factors between infertile and fertile men. According to age factor mean± SD were 32.44 ±7.45 for infertile 31.79 ±6.79 for fertile. (P=0.650). In addition, the results of the duration of married factor mean ± SD were 4.47 ± 3.58 for infertile and 5.26 ± 5.18 for fertile groups (P=0.378). On the other hand, the results of types of infertility for infertile there were characterized into two types, primary infertility type (who have never had a child) was produced 76% (n=38) and secondary infertility type (inability to become pregnant or to carry a baby to term after previously giving birth to a baby) was 24% (n=12). Statistically, there was no significant difference between primary and secondary infertility types.

Table 1: General characteristics of the studied groups used independent t-test

Characteristics	Infertile (mean± SD) (N=50)	Fertile (mean± SD) (N=50)	*P*- value
Age (Y)*	32.44± 7.45	31.79 ± 6.79	0.650 ^{NS}
Duration of Married (Y)*	4.47 ± 3.58	5.26 ± 5.18	0.378 ^{NS}
Primary Infertility	38 (76%)	-----	----
Secondary Infertility	12 (24%)	-----	----

NS: Non-significant, (Y)*: year, SD: Standard division.

The results of the current study were similar to the result was found by Elbashir *et al*. (22) and Farah (23) who reported no significant differences between infertile and fertile men in age and duration of married factors for infertility status. Another study revealed that the infertility cases were observed in the early men's age were effect on sperm quality and infertility decrease within increased male age whereas older affected with male infertility and the genetic defects in sperm increase and altered, which leads to decreased infertility (24, 25). The usual physiological function of reproductive organs is slowly declining with advancing age due to permanent irregular variables that influence human spermatozoa's fertilization capacity. In addition, sperm from older men has an elevated risk of abnormalities and many children are born by older men who have an increased probability of abnormalities as mentioned by Conti and Eisenberg (26) and Durairajanayagam (27). Primary male infertility can result from a variety of conditions including congenital disorders, hypothalamic-pituitary disorders, systemic disorders, chronic diseases and nutritional deficiencies; thus, it will become more common than secondary infertility (28, 29). The results of the methylation level percentage (mean ± SD) of studied loci by q-PCR analysis that used in the current study listed in Table 2 revealed that the methylation levels mean ± SD for *H19-1* were 77.20 ± 15.6 for infertile and 88.25 ± 6.56 fertile (P= 0.001). On the other hand, the methylation levels mean ± SD for *H19-2* were 74.80 ± 15.20 for

infertile and 85.5 ± 12.5 for fertile (*P*=0.001). The result provides a lower significance difference for *H19-1* and *H19-2* methylation levels for infertile compared with fertile. The decrease methylation level for *H19* (hypomethylation) in infertile compared with fertile for *H19* gene (*H19-1* and *H19-2*). On the other hand, the methylation levels mean ± SD for *SNRPN* were 52.50 ± 12.1.1 for infertile and 47.8±10.8 for fertile (*P*=0.042). There was a highly significant difference in *SNRPN* methylation level for infertile compared with fertile. The increased of *SNRPN* methylation level (hypermethylation) was associated with decline SF parameters. Furthermore, the rustle of methylation levels means ± SD for *LINE-1* were 68.4 ± 15.6 for infertile and 74.40 ± 13.4 for fertile (P=0.043), which indicates there was a highly significant difference between infertile and fertile groups for *LINE-1* methylation (hypomethylation). Moreover, the results of the current study of methylation levels among *H19, SNRPN* and *LINE-1* genes was a hypomethylation level for *H19* and *LINE-1* genes and hypermethylation levels in *SNRPN* gene were seams with infertile and fertile. The hypo and hypermethylation were affected by sperm parameters, especially on oligospermia patients.

Table 2: Methylation levels (mean ± SD) for *H19, SNRPN* and *LINE-1* genes between the studied groups used Z-test

Gene	Methylation level (mean± SD) %		*P*-value
	Infertile (mean ± SD)	Fertile (mean ± SD)	
H19 -1	77.20 ± 15.6	88.25 ± 6.56	0.001*
H19 -2	74.80 ± 15.20	85.5 ± 12.5	0.001*
SNRPN	52.50± 12.1	47.8 ± 10.8	0.042**
LINE-1	68.4 ± 15.6	74.40 ± 13.4	0.043**

*Significant (P< 0.001), **Significant (P < 0.05). SD: Standard division.

Genomic imprinting determines a small but crucial subset of genes with parental-specific and monoallelic expression (30). The link between methylation abnormalities in imprinted genes and oligospermia and the majority of males with moderate or severe oligospermia have aberrant methylation patterns in both paternally and maternally methylated genes *H19* and *SNRPN* was reported by Kobayashi *et al*. (31). The *H19* hypomethylation levels were higher in infertile patients with low sperm counts than in fertile patients in the current study was similar to the result reported by Santi *et al*. (32). According to Marques *et al*. (33) and Dong *et al*. (34), the paternally imprinted gene was different at the SF parameters (sperm concentration and sperm motility), which were largely influenced by *H19* hypo/unmethylation and demonstrated male infertility and decrease in SF characteristics was linked to the increased of *SNRPN* methylation levels hypermethylation (high significant) for *SNRPN* level in infertile compared to fertile. This

suggests that the *H19* gene has an influence on the promoter region and might be used as a biomarker for sperm production defects (35). Santi *et al*. (36) found a significant connection between male factor infertility (low sperm quality) and hypermethylation (high significance) for *SNRPN* level in OZ in primordial germ cells (PGCs) was similar to the current study. Boissonnas *et al*. (37) reported that OZ sperm samples had abnormal methylation imprints, that aberrant methylation imprints had an effect on conception rate, and that average *LINE-1* methylation levels can serve as sensitive markers for genome-wide epigenetic alterations. Incentres study there was no link between *LINE-1* methylation and aberrant human spermatogenesis was found by Santi *et al*., (36). In addition, another study reported by Laqqan and Hammadeh (39) revealed that the proportion of aberrant methylation in infertile, Differences in DNA conversion to DNA methylation may be the main source of uncertainty in any methylation assay. In other studies, the methylation of maternally methylated genes *SNRPN* and paternally methylated genes *H19* that *H19* hypomethylated in oligospermia individuals was related with considerable with low sperm motility, which is particularly interesting in nonimprinted tumor suppressor genes compared with normal global DNA methylation according to the *LINE-1* gene in both the fertile and infertile groups were found by Hammoud *et al*. (40). Li *et al*. (41) found a significant association between male factor infertility and hypomethylation, particularly in the imprinting control region (ICR) of *H19*-ICR. Many illnesses have unique signatures based on the location of hyper- and hypo-methylated sites. According to Altuna, hypomethylation is linked to gene transcription (in the regulatory area of promoters) while hypermethylation is linked to gene repression (42). Imprint methylation abnormalities in sperm and severe oligospermia were linked to abnormal methylation and a specific lifestyle was linked to human environmental variables, according to Kobayashi *et al*. (31). In contrast to the current study, Nasri *et al*. (43) and Amena *et al*. (44) found no statistically significant difference between the case and control groups' median methylation percentage. Several studies found that utilizing a genome-wide approach, DNA methylation of sperm DNA of male infertility verified an association with modifications in the mature expression of genes (45). Methylation of sperm DNA has a variety of impacts on fertility. Following a loss in germ cell growth capacity, leading to reduced sperm counts and sterility on a genome-wide basis. While the reason for the various methylation patterns in sperm is unknown, advanced paternal age may be one of the variables that increase the likelihood of change, as well as variations in the methylome among infertile individuals (46). The result of the total RNA was extracted effectively from all SF samples for both studies groups (infertile and fertile) concentrations ranging from 78 to 199 ng/ɱl. In addition, total RNA purity is measured by the 260/A280 ratio, which ranges from 1.9 to 2. Moreover, the results of cDNA were reversed from RNA using the qRT-

PCR technique was documented the amplification on Ct-value for infertile and fertile produced different levels of Ct-value for *H19*, *SNRPN* and *LINE-1* gene expression levels compared with IC (*β-globin*) as shown in Figure 1,2,3, respectively. The photographs were obtained straight from the Rotor-Gene qRT-PCR device.

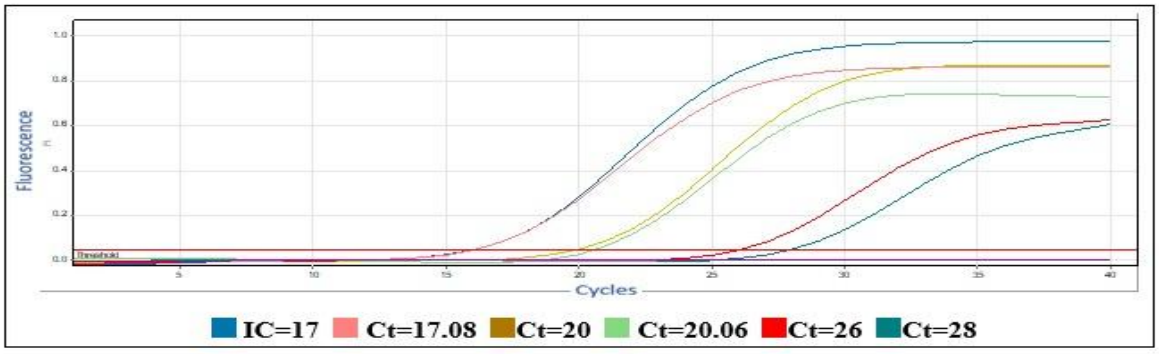

Figure (1): Amplification carves by RT-PCR. The Ct-value ranged from 17-28 for *β-globin* gene (IC) and different SF samples (in different colures) for infertile and fertile for *H19-1*gene.

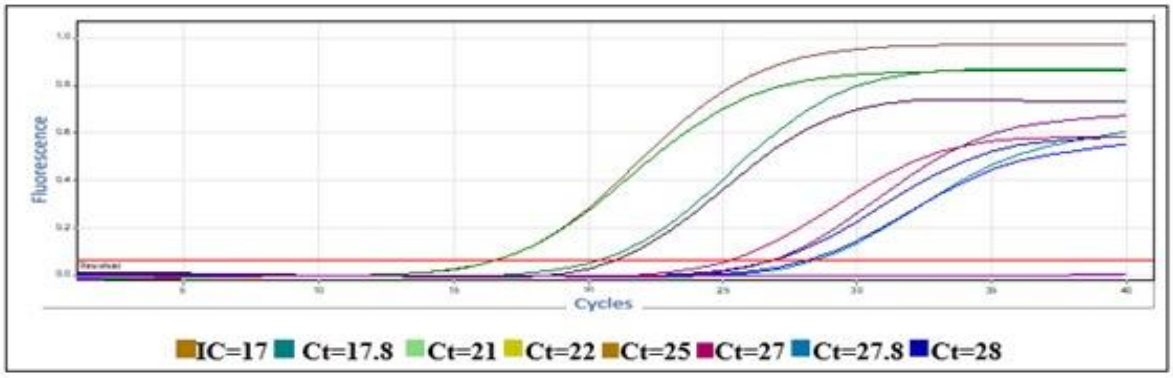

Figure (2): Amplification carves by RT-PCR. The Ct-value ranged from 17-28 for *β-globin* gene (IC) and different SF samples (in different colures) for infertile and fertile for *SNRPN* gene.

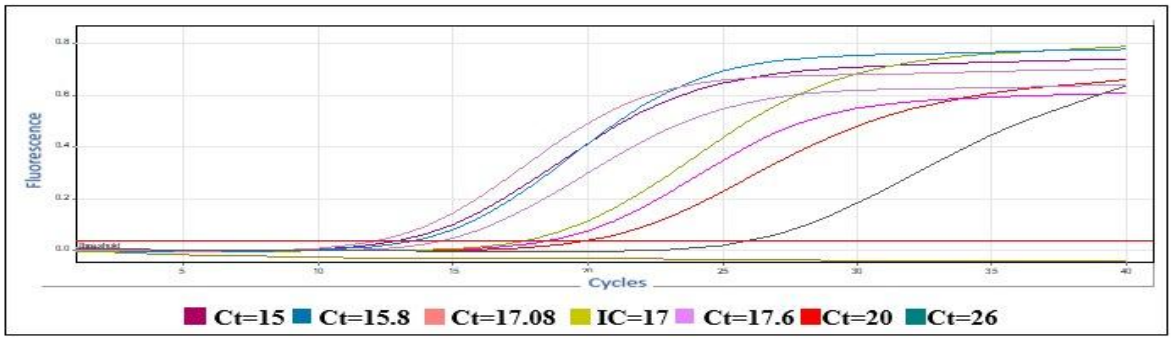

Figure (3): Amplification carves by RT-PCR. The Ct-value ranged from 17-28 for *β-globin* gene (IC) and different SF samples (in different colures) for infertile and fertile for *H19-1*gene.

The result of gene expression for *H19*, *SNRPN* and *LINE-1* genes were analyzed by qRT-PCR and used reference gene HKG *B-globin* (IC) used to quantification and compare Ct-value between infertile and fertile in different stages of gene expression levels (Ct, ΔCt, ΔΔCt and Fold Change) as shown in Table 3. The result of Ct-value calculation for gene expression for *H19* was 20.10 for infertile and 21.36 for fertile, (*P*-value=0.062), the Ct-value for *SNRPN* was 21.33 for infertile and 21.18 for fertile (*P* = 0.813) and the Ct-value for *β-globin* was 17.06 for infertile and 17.006 for fertile (*P*-value=1.00). The results were a significant difference between Ct-value for *H19*, *SNRPN* and *β-globin* genes between infertile and fertile. On the other hand, the results of Ct-value for *LINE-1* were 18.96 for infertile and 20.77 for fertile (*P*=0.007). The result showed low significant difference for Ct-value for *LINE-1* for infertile. On the other hand, the ΔCt-value was calculation as a result of the difference in Ct-value between the target and calibrator (*H19*, *SNRPN* and *LINE-1*) genes and the IC (*β-globin*) of every single case. The results of ΔCt for *H19* were 3.1 for infertile and 4.30 for fertile (*P*= 0.020). While, the results of ΔCt for *SNRPN* were 4.32 for infertile and 4.12 for fertile, (*P*=0.810). In addition, the result of ΔCt for *LINE-1* were 1.96 for infertile and 3.71 for fertile (*P*=0.002). The results showed there was no significant difference between infertile and fertile for ΔCt *H19* and ΔCt *SNRPN* genes. But, present a low significant difference for infertile compared with fertile for ΔCt *LINE-1* gene. Whereas, the result of ΔΔCt for *H19*, *SNRPN* and *LINE-1* genes for infertile were produced -1.206 for ΔΔCt *H19*, 0.2 for ΔΔCt *SNRPN* and -1.75 for ΔΔCt *LINE-1*. Moreover, the result of fold change ($2^{\wedge-\Delta\Delta Ct}$) for *H19*, *SNRPN* and *LINE-1* gene expression levels for infertile were produced for *H19* gene was 2.3 whereas indicating an up-regulation of *H19* gene expression level. On the other hand, the result of fold change for *SNRPN* gene was 0.98 with a down-regulation of gene expression level. Additionally, the result of the fold change of *LINE-1* gene was 3.36 whereas indicating an up-regulation of *LINE-1* gene expression level. Overall, their results demonstrated the up-regulation of gene expression level for *H19*, *LINE-1* and down-regulation for *SNRPN*. According to the current study, up-regulation for *H19*, *LINE-1*, and down-regulation for *SNRPN* that can inhibit spermiogenesis, led to reduction of semen quality were similar to results produced by Mahsa *et al*. (47). On the anther hand, infertile had lower methylation and produced upregulation for *H19* gene expression potential compared with fertile in the current study, methyl groups inhibit the infiltration of transcription factors and DNA polymerases, resulting in a down-regulation of *SNRPN* gene expression caused by the imprinted gene *SNRPN* hypermethylation, that is associated with decreased sperm motility and percentage of morphologically normal sperm and suppresses gene expression (48,49). In addition, the up-regulation of *LINE-1* gene expression level and the genetic profile of sperm mRNAs identified in ejaculated spermatozoa clarified what happened in gene expression during spermatogenesis

and was used as an indicator for efficient male infertility diagnosis (50). The *LINE-1* retrotransposons are silenced by multiple mechanisms including DNA methylation and de-silencing exhibits a binary expression pattern in up-regulation in spermatocytes but not in spermatogonia. The *H19*, *SNRPN* and *LINE-1* genes that play roles in the progression of spermatogenesis and aberrant expression of these genes may be responsible for arresting germ cell differentiation (51, 52). Upregulated expression of the *H19* gene and downregulated expression of *SNRPN* may be associated with sperm abnormalities due to reduced DNA methylation of *H19*- ICR and increased DNA methylation of *SNRPN*-ICR (53). Methylation eliminated the regulation, and an increase in *LINE-1* expression caused cells to become more genetically unstable, which had negative effects (54, 55).

Table 3: The gene expression calculation levels in different stages (Ct-value, ΔCt, ΔΔCt and $2^{\wedge-\Delta\Delta Ct}$) for *H19, SNRPN* and *LINE-1* for infertile group used independent t-test.

Sample	mean Ct	mean IC *β-globin*	mean ΔCt *H19*	mean ΔΔCt	$2^{\wedge-\Delta\Delta Ct}$	Fold change
Fertile	21.36	17.06	4.30	----	----	----
Infertile	20.10	17.006	3.1	-1.206	2.3	Up-regulation
***P*-value**	0.062 ^{NS}	1.00 ^{NS}	0.020*	----	----	----
			SNRPN			
Fertile	21.18	17.06	4.12	----	----	----
Infertile	21.33	17.006	4.32	0.02	0.98	Down-regulation
***P*-value**	0.813 ^{NS}	1.00 ^{NS}	0.810 ^{NS}	----	----	----
			LINE-1			
Fertile	20.77	17.06	3.71	----	----	----
Infertile	18.96	17.006	1.96	-1.75	3.36	Up-regulation
***P*-value**	0.007*	1.00 ^{NS}	0.002*	----	----	----

*: Significant (P< 0.001), NS: non-significant (P> 0.001)

Spearman's correlation coefficient between methylation patterns and gene expression fold change ($2^{\wedge-\Delta\Delta Ct}$) level for *H19*, *SNRPN* and *LINE-1* genes for infertile are shown in Table 4. The result of *H19* gene expression was positively correlated with *H19* methylation level (r = +0.31, *P* = 0.026). In contrast, present a negative correlated with *SNRPN* methylated level (r = -0.31, *P* = 0.029) and with *LINE-1* methylation level (r = -0.53, *P* = 0.001). On the other hand, the result of *SNRPN* gene expression has a negative correlation with *H19* methylation level (r = -0.31, *P* = 0.030) and *SNRPN* methylation level (r = -0.33, *P* = 0.021), With no found correlation with *LINE-1* methylation level (r = -0.28, *P* = 0.005). Moreover, the result of *LINE-1* gene expression was present a negative correlated with *LINE-1* methylation level (r = -0.34, *P* = 0.001) and with *SNRPN* (r = -0.46, *P* = 0.001) as well as *LINE-1* methylation level (r = -0.39, *P* = 0.005). In addition, the result of *LINE-1* gene expression was

present a negative correlated with *LINE-1* methylation level (r = -0.34, *P* = 0.001) and with *SNRPN* (r = -0.46, *P*=0.001) as well as *LINE-1* methylation level (r = -0.39, *P* = 0.005). The current study identified a link between the modification in the degree of sperm DNA methylation and the frequency of expression of the genes *H19, SNRPN* and *LINE-1*. This result conflicts with previous reports whether the methylation of DNA at the cell bodies or promoters is specifically correlated with gene expression is not exclusively accurate (56, 57). Other experiments have shown that not all changes in gene methylation cases are associated with levels of gene expression (39). Aberrant *LINE-1* methylation was reported in many aging and cancer studies by Suzuki *et al*. (58), who showed the influence of environmental factors or genome lifestyle patterns. Previous research shows that the modification in sperm DNA methylation influences gene expression and the shift in a transcriptional activity that occurs by delaying the activation of intragenic promoters or by altering the transcriptional unit activities of repetitive DNAs (59, 60).

Table 4: Correlation Coefficient between Gene Expression Level and DNA Methylation *H19, SNRPN* and *LINE-1* genes for infertile used Spearman's test.

Methylation / level		***H19***	***SNRPN***	***LINE-1***
H19	**r**	+0.31*	-0.31*	-0.53**
Fold Change	***P*-value**	0.026	0.029	0.001
SNRPN	**r**	-0.31*	-0.33*	-0.28
Fold Change	***P*-value**	0.030	0.021	0.048
LINE-1	**r**	-0.34*	-0.46**	-0.39**
Fold Change	***P*-value**	0.016	0.001	0.005

r: Correlation coefficient, *: Significant $P<0.05$, **: Significant. $P<0.001$

Conclusions

The result observed in this study for DNA methylation were significant in the infertile group compared with fertile group in SF samples and these results were associated with gene expression levels lower sperm parameters and were affected on that causes germ-cell arrest leading to infertility, and may serve as epigenomic biomarkers for the assessment of oligospermia infertility in men with multiple sperm defects.

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