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Original Research

The relationship between clinical parameters and hepatocyte growth factor/c-Met levels in the sperm of infertile males

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Abstract

Objective: Hepatocyte growth factor is a pleiotropic cytokine with potent mitogenic activity in many organs and tissues. The receptor for hepatocyte growth factor is c-Met, a transmembrane glycoprotein with tyrosine kinase activity. We investigated the relationships between hepatocyte growth factor and c-Met expression levels on sperm motility and male infertility. **Methods**: The study was designed as a prospective cohort, single-center clinical trial and was conducted between March 2015 and June 2016. The control group consisted of 31 healthy male volunteers with children, while the study group consisted of 61 men who were diagnosed with male infertility after presenting to the *In Vitro* Fertilization Unit of our Institute. All participants in the study were aged 18–60 years. Sperm samples were taken from each participant and divided in two. The fresh sample was examined immediately for expression of c-Met, while the other was stored frozen and evaluated later for hepatocyte growth factor. Primary outcome measures were the levels of hepatocyte growth factor and c-Met expression, while secondary outcome measures were the correlation of these levels with sperm count, morphology and motility. **Results**: The c-Met expression level in the control group (26.53 $\pm$ 3.50, 19.43–32.73) was significantly lower than in the infertile group (27.95 $\pm$ 2.86, 21.58–33.07) ($p = 0.039$). HGF expression was also significantly lower in the control group (3.25 $\pm$ 1.76, 0.83–8.25) than in the infertile group (4.87 $\pm$ 4.11, 1.01–21.58) ($p = 0.043$). In the overall group, c-Met expression showed a positive correlation with sperm motility percentage and a negative correlation with sperm count and sperm morphology percentage. **Conclusions**: The c-Met receptor and its ligand, hepatocyte growth factor, appear to be fundamental regulators of spermatogenesis, sperm motility and fertilization capacity.

Keywords: Male infertility; Sperm motility; c-Met; Hepatocyte growth factor

1. Introduction

Hepatocyte growth factor (HGF) is a pleiotropic cytokine previously identified as a 'scatter factor' and potent mitogen for hepatocytes that provides a connection between different target cells [1–4]. HGF is expressed in various immunoreactive organs in the male reproductive system, including the seminiferous tubules, epididymis, ductus deferens, epidermis, prostate and seminal vesicle epithelium [5,6]. It can act as a mitogen (stimulator of cell growth), a motogen (stimulator of cell motility) and a morphogen (inducer of a structure similar to multicellular tissue). It also has features that allow it to play a role in the functional regulation of many systems involved in movement, such as the spermatogenic epithelium [7–9]. HGF has a unique receptor called met, with the encoding oncogene referred to as c-Met. The c-Met receptor has tyrosine kinase activity and a heterodimer structure composed of an α-chain of 50 kD and a β-chain of 155 kD. Both subunits are formed by glycosylation and proteolytic cleavage of the main 170 kD precursor. The c-Met oncogene was first discovered following transformation of human osteosarcoma cells with N-methyl-N'-nitro-nitroguanidine, a chemical carcinogen [10]. The activity of HGF on testicular tissue is different between the prenatal and postnatal developmental stages. Modulation of steroidogenesis and apoptosis directs mitosis, morphogenesis and differentiation [7]. HGF is one of the growth factors that supports development of the male reproductive system and plays a role in controlling the spermatogenetic process. A significant amount of HGF is also present in human seminal plasma. The detection of HGF in vasectomized men has led to the understanding that HGF is not testicular in origin. The main sources of HGF in humans are the epididymis, prostate and seminal vesicle. Using immunohistochemistry, c-Met is detected in spermatogonia, spermatocytes, spermatids and spermatozoa cell membranes, but not in Sertoli and Leydig cells. c-Met is also present in peritubular myoid cells and interstitial compartments, in mitotic and meiotic germ cells, and in spermatozoa where it is thought to play a role in differentiation and migration during human spermatogenesis [11,12].

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The aim of this study was therefore to evaluate the levels of c-Met oncogene and its ligand HGF in relation to the sperm count and to sperm morphology and motility parameters in infertile males and healthy volunteers.

2. Materials and methods

The study design was a prospective cohort study and was conducted between March 2015 and June 2016 at a single center following approval from the institutional ethics committee.

A control group and a study (infertile) group were created. The control group consisted of healthy males aged 18-60 years who had children, had no known chronic disease, had not undergone any urological surgery, had normal sperm count, motility and morphology as revealed by sperm analysis, and had volunteered to participate in the study. The infertile group consisted of males aged 18–60 years who were admitted to the Institute's IVF Andrology Laboratory, had at least one value for sperm count, motility and morphology that was below the reference range from the WHO 2010 Semen Analysis Parameters on sperm analysis (Table 1) [13], and had volunteered to participate in the study.

Table 1. Lowest reference values for semen analysis (5th percentile, 95% confidence intervals).

Parameter	Lowest reference value
Semen volume (mL)	1.5 (1.4–1.7)
Total sperm count (millions)	39 (33–46)
Sperm concentration (million/mL)	15 (12–16)
Total motility (%)	40 (38–42)
Progressive motility (%)	32 (31–34)
Sperm morphology (normal forms, %)	4 (3.0–4.0)

All participants signed an Informed Volunteer Consent Form after being provided with detailed verbal and written information on the study.

A sperm sample was taken from all study participants under appropriate conditions at the Institute's IVF Andrology Laboratory after 2–5 days of sexual abstinence. Individuals with a sperm sample under volume of 3 mL were excluded from the study.

Samples were subjected to a spermiogram following a liquefaction process lasting 30 minutes at 37 °C. The spermiogram procedure was performed by an embryologist at the IVF Andrology Laboratory after placing a 10 μL sample of sperm into a Makler Chamber. The sperm volume, sperm concentration, motility and morphology percentage were recorded.

Following the spermiogram procedure, the first sample of one milliliter was placed into a Falcon 2003 tube without any treatment and sent to the Institute's Genetics Laboratory for the evaluation of c-Met expression on the same day. The second one milliliter sample was stored frozen at –10 °C in a Falcon 2003 tube for later HGF measurement.

Total RNA was extracted from 1 ml of fresh ejaculate using a tissue total RNA Isolation Kit (HibriGen, Turkey). It was then transcribed *in vitro* to cDNA using MuLV reverse transcriptase (High Capacity cDNA Reverse Transcription Kit, Applied Biosystems, USA) and random hexamers as primers, according to the manufacturer's instructions. The presence of transcripts for the target gene *c-Met* and the reference gene *HPRT1* was shown by Real Time PCR under the experimental conditions recommended by the manufacturer and using the Human c-Met Expression Kit (HibriGen, Turkey) and LightCycler 2.0 device (Roche Diagnostics, Germany). The amplification mixture for each Q-PCR consisted of PCR mix and 50 ng of template cDNA in a final volume of 20 μL. The amplification protocol consisted of denaturation at 95 °C for 5 min, followed by 36 cycles of amplification at 95 °C for 10 s, annealing at 55 °C for 10 s, and extension at 72 °C for 15 s. *HPRT1* was used as the house keeping gene in an independent Q-PCR reaction. Results were obtained in the form of threshold cycle (CT) values. c-Met and *HPRT1* copy numbers were calculated using the $2^{-\Delta\Delta Ct}$ method. All laboratory personnel were blinded to the pertinent characteristics of the samples, the study subjects, and to the hypotheses being investigated.

HGF levels were measured with a commercial kit (Human HGF Instant ELISA, Affymetrix eBioscience) that had been stored at –20 °C. The previously stored frozen samples were thawed at 24 °C and sent to the Institute's Biochemistry Laboratory for measurement of HGF. Each plate from the kit contained 96 small compartments, including14 standard and 2 empty compartments. Antibodies coated with anti-human HGF had been absorbed into these compartments. Distilled water (100 μL) was added to each compartment, followed by 50 μL of the sample. The compartment was then closed with adhesive film and shaken for 3 hours at room temperature on a microplate shaker. Human HGF was present in each sample in the standard small compartments and was bound to the absorbed HGF antibodies during the first incubation. Anti-human HGF antibody conjugated with biotin was then bound to the human HGF bound to the initial antibody. Streptavidin-HRP was subsequently bound to the anti-human HGF antibody conjugated with biotin, and the adhesive film removed and the compartments emptied. Any unbound streptavidin-HRP and anti-human HGF antibody conjugated with biotin was thoroughly washed away by rinsing 6 times with the 'wash buffer' provided in the commercial kit and then completely removed by tapping the plate during the last wash. Substrate solution (100 μL) was then added to all compartments and the plates incubated at room temperature for 30 minutes while preventing direct light exposure. The HRP was activated by the substrate solution added to the compartments (**Supplementary Fig. 1**). Blue color changes were

Table 2. Sperm count, sperm motility percentage, sperm morphology percentage, c-Met expression level, and HGF level in the total, infertile and control groups.

		Total (n = 92)	Infertile (n = 61)	Control (n = 31)	p
Basal Sperm Count (x million)	Mean ± Sd	39.75 ± 31.12	25.99 ± 23.00	66.84 ± 27.12	[a]0.001**
	Min–Max (Median)	2–116 (33.5)	2–110 (21)	18–116 (64)	
Sperm Motility (%)	Mean ± Sd	46.91 ± 15.56	45.43 ± 17.39	49.84 ± 10.77	[b]0.138
	Min–Max (Median)	15–86 (45)	15–85 (45)	40–86 (45)	
Sperm Morphology (%)	Mean ± Sd	2.70 ± 1.87	1.80 ± 1.65	4.45 ± 0.62	[a]0.001**
	Min–Max (Median)	0–6 (3)	0–5 (2)	4–6 (4)	
c-Met	Mean ± Sd	27.47 ± 3.15	27.95 ± 2.86	26.53 ± 3.50	[a]0.039*
	Min–Max (Median)	19.43–33.07 (28.35)	21.58–33.07 (28.76)	19.43–32.73 (27.89)	
HGF (ng/mL)	Mean ± Sd	3.79 ± 2.86	3.25 ± 1.76	4.87 ± 4.11	[a]0.043*
	Min–Max (Median)	0.83–21.58 (3.10)	0.83–8.25 (2.98)	1.01–21.58 (3.63)	

[a]Mann-Whitney U Test. [b]Student t-Test. *$p < 0.05$. **$p < 0.01$.

observed at different levels of intensity depending on the amount of soluble human HGF initially added to the compartments. The reaction was terminated by adding 100 μL of stop solution to each compartment and the plate was then incubated for 1 hour at 2–8 °C in the dark. The absorbance of the reaction color in each compartment was measured at 450 nm using a spectrophotometer (**Supplementary Fig. 2**). The standard curve was determined using 7 human HGF standard compartments and the human HGF sample concentrations. Samples were diluted at a ratio of 1:2 and this was considered when calculating the results using the standard curve.

Statistical analysis: The NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) program was used to perform the statistical analysis. Descriptive statistical methods (mean, standard deviation, median, frequency, percentage, minimum, maximum) were used to evaluate study data. Pairwise group comparisons of quantitative data were conducted with the Student's t-test when the variables showed a normal distribution, and the Mann-Whitney U test otherwise. The Wilcoxon Signed Rank test was used for intragroup comparison of variables that did not show a normal distribution. Spearman correlation analysis was used to evaluate the relationships between variables. Significance was accepted at the $p < 0.01$ and $p < 0.05$ levels.

4. Results

The study was conducted on a total of 92 male patients (61 infertile and 31 fertile) who presented to the Florence Nightingale Hospitals Group's IVF department between March 2015 and June 2016.

The sperm count, sperm motility percentage and sperm morphology percentage reported in the WHO 2010 Semen Analysis Parameters (Table 1) were considered when assigning participants to either the control or infertile groups. Participants with values below the reference range for any of the three parameters (sperm count, motility percentage and morphology percentage) were assigned to the infertile group.

The sperm count and sperm morphology percentage were significantly higher in the control group (Mann-Whitney U test). The sperm motility percentage was also higher in the control group but did not reach statistical significance, possibly because only one of the three semen analysis parameters had to be lower than the reference range in order to assign an individual to the infertile group.

Basal sperm count was 66.84 ± 27.12 (18–116) in the control group and 25.99 ± 23.00 (2–110) in the study group and a statistically significant difference was present (p = 0.001).

Sperm morphology percentage was 4.45 ± 0.62 (4–6) in the control group and 1.80 ± 1.65 (0–5) in the study group with a statistically significant difference (p = 0.001).

The c-Met expression level was 26.53 ± 3.50 (19.43–32.73) in the control group and 27.95 ± 2.86 (21.58–33.07) in the study group with a statistically significant difference (p = 0.039).

HGF levels were 4.87 ± 4.11 (1.01–21.58) in the study group and 3.25 ± 1.76 (0.83–8.25) in the control group, again with a statistically significant difference (p = 0.043) (Table 2).

No statistically significant correlations were found between c-Met expression and HGF levels in the total cohort, the infertile group or the control group (each $p > 0.05$) (Table 3). Similarly, no significant correlations were observed between basal sperm count and HGF level in the total cohort, the infertile group or the control group (each $p > 0.05$) (Table 4).

However, a significant negative correlation was found between the basal sperm count and c-Met expression level in the total cohort (Table 4). The correlation between these factors did not reach statistical significance in either the infertile or control groups. Fig. 1 shows the correlation between sperm count and c-Met expression level in the total cohort.

Table 3. Correlation between c-Met expression and HGF levels.

	c-Met - HGF	
	r	*p*
Total (n = 92)	0.130	0.219
Infertile (n = 61)	0.146	0.263
Control (n = 31)	0.287	0.117

r = Spearman's Correlation Coefficient.

Table 4. Correlation between sperm count and HGF and c-Met expression levels.

	Sperm count (millions)					
	Total (n = 92)		Infertile (n = 61)		Control (n = 31)	
	r	*p*	r	*p*	r	*p*
HGF	0.191	0.068	0.154	0.236	0.054	0.774
c-Met	–0.309	0.003**	–0.180	0.164	–0.220	0.235

r = Spearman's Correlation Coefficient.
*$p < 0.05$. **$p < 0.01$.

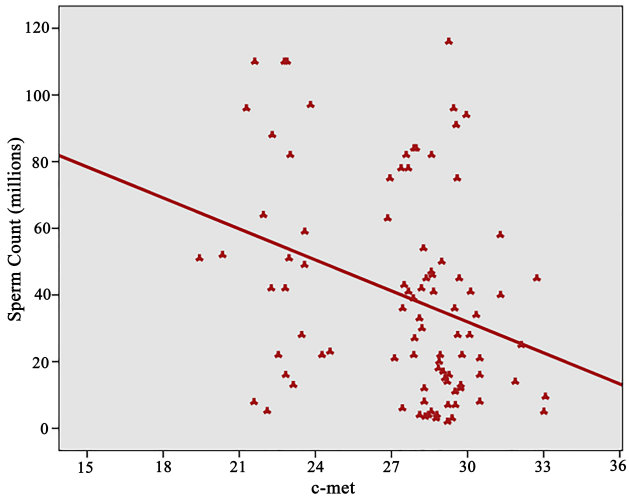

Fig. 1. Correlation between basal sperm count and c-Met expression level in the total cohort (r = –0.309; *p* = 0.003).

No significant correlations were found between the sperm motility percentage and HGF level in the total cohort, the infertile group or the control group (each $p > 0.05$) (Table 5).

However, significant positive correlations were observed between the sperm motility percentage and the c-Met expression level in the total cohort (Fig. 2) and in the infertile group (Fig. 3) (Table 5).

No statistically significant correlations were found between sperm morphology and HGF level in the overall cohort or in the infertile group (each $p > 0.05$) (Table 6). However, a significant negative correlation was observed in the control group (r = –0.378, $p = 0.036$).

Table 5. Correlation between sperm motility percentage and the HGF and c-Met expression levels.

	Sperm motility percentage					
	Total (n = 92)		Infertile (n = 61)		Control (n = 31)	
	r	*p*	r	*p*	r	*p*
HGF	0.129	0.221	0.145	0.265	0.014	0.942
C-Met	0.421	0.001**	0.488	0.001**	0.259	0.159

r = Spearman's Correlation Coefficient.
*$p < 0.05$. **$p < 0.01$.

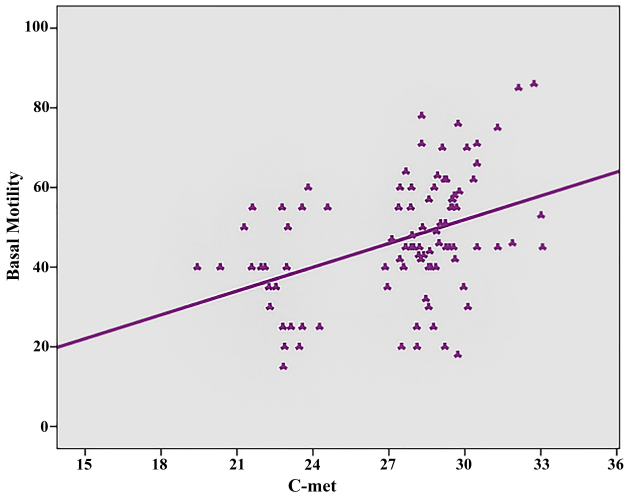

Fig. 2. Positive correlation between sperm motility percentage and c-Met expression level in the total cohort (r = 0.421, *p* = 0.001).

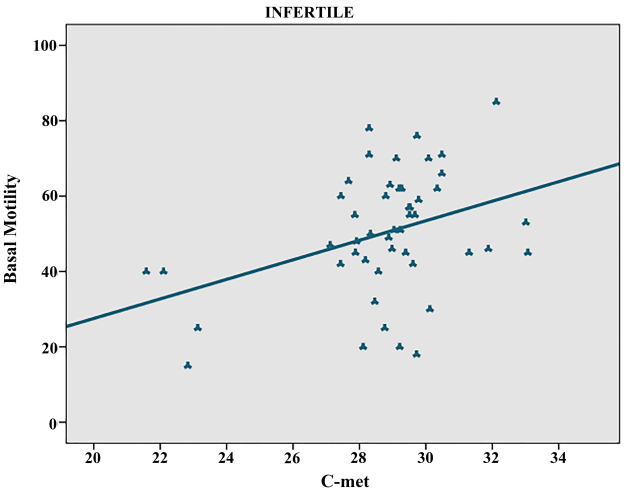

Fig. 3. Positive correlation between sperm motility percentage and c-Met expression level in the infertile group (r = 0.488, *p* = 0.001).

Significant negative correlations were also found between the sperm morphology percentage and c-Met expression level in the total cohort (Fig. 4) and in the infertile

group (Fig. 5), but not in the control group ($p > 0.05$) (Table 6).

Table 6. Correlation between sperm morphology percentage and HGF and c-Met expression levels.

	Sperm morphology percentage					
	Total (n = 92)		Infertile (n = 61)		Control (n = 31)	
	r	*p*	r	*p*	r	*p*
HGF	0.089	0.397	0.052	0.691	–0.378	0.036*
c-Met	–0.441	0.001**	–0.422	0.001**	–0.176	0.342

r = Spearman's Correlation Coefficient.

*$p < 0.05$. **$p < 0.01$.

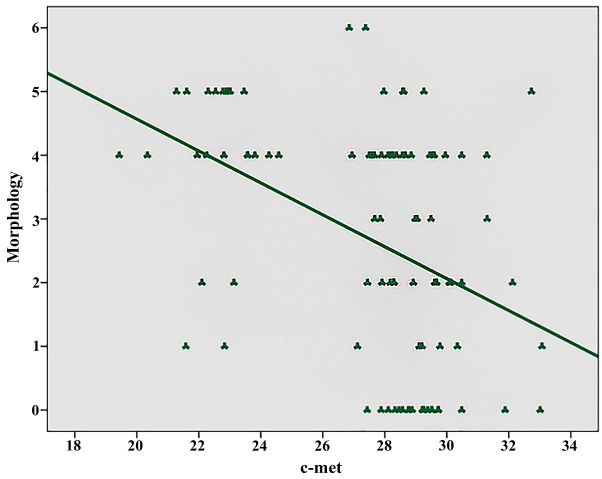

Fig. 4. Negative correlation between sperm morphology percentage and c-Met expression level in the total cohort (r = –0.441, *p* = 0.001).

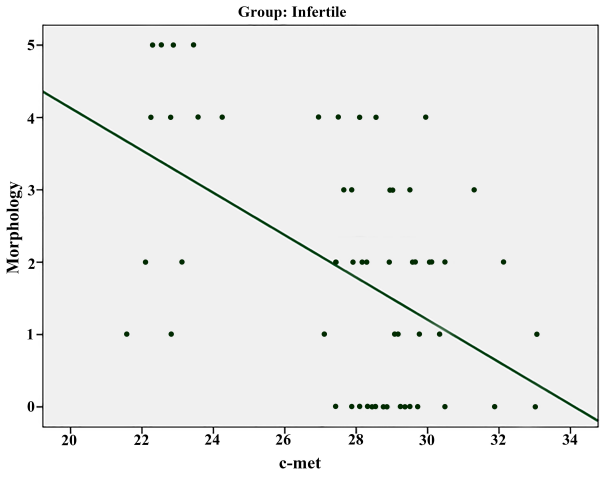

Fig. 5. Negative correlation between sperm morphology percentage and c-Met expression level in the Infertile group (r = –0.422, *p* = 0.001).

5. Discussion

Male infertility is responsible for 25–30% of all cases of infertility. It can be caused by many factors, but changes in sperm count, motility and morphology are the most common problems.

HGF regulates many different motile systems, including the spermatogenic epithelium. It is a pleiotropic cytokine known to be a potent mitogen for hepatocytes, and is also thought to be a 'scatter factor' that connects target cells [1–4]. HGF binds with high affinity to c-Met, a transmembrane receptor with tyrosine kinase activity that is only expressed in mesenchymal cells.

HGF acts as both a paracrine and an autocrine modulator to support testicular functions. In this study we analyzed the sperm of infertile and healthy males for the parameters of basal sperm count, sperm morphology percentage and sperm motility percentage. We then analysed the relationships between these parameters and the levels of c-Met expression and HGF.

Depuydt *et al.* [11] reported the presence of significant amounts of HGF in human seminal plasma. The detection of HGF in vasectomized men indicates that it is not testicular in origin, with the main source in humans found to be the epididymis, prostate and seminal vesicle [11,12].

Catizone *et al.* [14] was the first to investigate the role of HGF in male fertility by evaluating the *in vitro* motility of spermatozoa isolated from the caput of the epididymis. They found that motility in culture medium was significantly lower in the absence of HGF, and that sperm motility could be preserved for long periods in the presence of HGF. The HGF/c-Met key-lock system was therefore shown to have a positive role in preserving sperm motility during transition of the sperm from the epididymis to an *in vitro* environment.

However, another study did not find a positive correlation between the HGF level and sperm motility. Kitamura *et al.* [15] added recombinant HGF to washed spermatozoa from infertile males and measured sperm motility, as well as the HGF concentration in seminal plasma. The results were compared with the hormone profile and with various semen analysis parameters. Recombinant HGF had no effect on the linear motility or motility frequency of the sperm that would cause hyperactivation in the spermatozoa. Moreover, no correlation was observed between the HGF concentration in the seminal plasma and any of the clinical parameters investigated.

Wiltshire *et al.* [16] evaluated HGF and semen analysis parameters in three different patient groups: those with normozoospermia, subfertility or azoospermia. They found no significant correlations between serum HGF concentration and sperm concentration, sperm motility percentage, total sperm count or total motile sperm count. There was also no significant difference in the HGF level between the three patient groups.

Catizone *et al*. [14] reported that c-Met in rat spermatogonia, pachytene spermatocytes and round spermatids could increase germ cell proliferation in conjunction with HGF. C-Met also influences male germ cell homeostasis and consequently male fertility by decreasing the number of apoptotic cells in prepubertal experimental rats [14].

In the present study, c-Met expression in the infertile group was significantly higher than in the control group (Table 2). This suggests a role for c-Met in the regulation of spermatogenesis and sperm quality, either by directly affecting sperm germ cells or through tubular and interstitial somatic cells in the testis. HGF is a growth factor that induces sperm motility [17]. The effect of HGF on spermatozoa increases during passage through the epididymis and this is directly correlated with high c-Met receptor expression [18].

Catizone *et al*. and others [14,19,20] have reported that c-Met is expressed in both human and rat germ cells and that in rats it is always present during the spermatogenetic process from spermatogonia to spermatozoa.

Using *ex vivo* organ culture of testicles from 8–30 day old rats, it has also been shown that HGF controls germ cell mitotic activity by increasing spermatogonial cell proliferation [21]. Mitotic germ cells need this HGF signal, but germ cells must also be inhibited to allow the orderly entry of germ cells at the beginning of the meiotic process.

The above findings support our notion that the factors responsible for low HGF levels in the infertile group could be increased c-Met expression. C-Met upregulation is a response to the low HGF level. Elevated c-Met expression affect the HGF/c-Met key-lock system and could attempt to increase intracellular signaling.

The HGF level in the control group was significantly higher than in the infertile group (Table 2). The presence of sufficient ligand in the control group allows an appropriate amount of HGF/c-Met complex to form and therefore satisfactory intracellular signaling can occur. This finding may also explain the higher sperm motility percentage observed in the control group compared to the infertile group, despite the lack of statistical significance. Increased HGF levels appear to downregulate the c-Met receptor, to physiological levels of expression. Spearman's correlation coefficient analysis did not reveal any significant correlations between c-Met expression and HGF levels in the total cohort, the infertile group or the control group (Table 3).

A positive correlation was found between the c-Met expression level and sperm motility percentage in the overall cohort and in the infertile group, thus supporting a positive effect of c-Met expression on sperm motility. However, no such correlation was observed in the control group where all parameters for the WHO 2010 Semen Analysis reference ranges were met. In the presence of healthy spermatozoa, sperm maturation and differentiation are consisted with the seminal plasma. If the sperm motility sufficient, there is no need to increase the c-Met expression.

Goda *et al*. [22] reported that adenovirus-mediated HGF gene transfer into the testis of experimental cryptorchid rat models stimulated the overexpression of HGF and significantly decreased the number of apoptotic germ cells by regulating spermatogenesis and testicular weight.

HGF is known to synergize with endocrine signals to support the homeostasis of male germ cells, decrease germ cell apoptosis, and act as a survival factor for male germ cells [23–27].

Naz *et al*. [28] reported that the highest specific localization of HGF in the rat epididymal tract where the sperm becomes motile was in the distal corpus and cauda. HGF was found to slightly induce cell motility in immotile sperm, to have a protective effect on epididymal sperm motility *in vitro*, but not to improve the motile sperm percentage [23,24]. However, the absence of this motogenic effect in human sperm, although previously reported in rodents [26], means the role of HGF in human sperm physiology is still controversial.

We found significant negative correlations between the c-Met expression level and sperm morphology percentage in the total cohort and in the infertile group (Table 6). We believe that better sperm morphology indicates higher quality sperm within the fertile range, thus eliminating the need to increase intracellular signaling and resulting in decreased c-Met expression.

A negative correlation was found between c-Met expression and sperm concentration in the overall cohort (Table 4). Increased c-Met expression to stimulate sperm and increase its activation is therefore not required in the presence of a sufficient sperm concentration. This suggests there is a need to consider the function of the epididymis during evaluation. Since HGF expression and the regulation of c-Met expression occur in this region, it is possible that a "main control area" is active here to correct the sperm quality.

We have demonstrated in this study that HGF levels in healthy males are high and support sperm motility during passage of the ejaculate through the epididymis. The necessary stimulation is therefore provided by HGF without the requirement for c-Met upregulation.

The high c-Met levels observed in the infertile group suggest this receptor may be overexpressed in response to low HGF in order to stimulate sperm. This could be an attempt to compensate for functional defects by upregulating c-Met receptor expression when HGF-related functions in the cell are inadequate, particularly those concerning motility.

The observed increase in c-Met expression as sperm motility increases demonstrates the association between these two factors and is consistent with the results of earlier studies. Since we do not have a normogram for the ligand-receptor relationship between HGF and c-Met in healthy males and because this is variable even during spermatogenesis, it may be more useful to consider these two factors

together in future work.

The negative correlations observed here between sperm count and c-Met expression (Table 4) and between sperm morphology percentage and c-Met expression (Table 6) suggest that upregulation of c-Met occurs in order to increase intracellular signaling. When the cause of infertility is other parameter than sperm motility, the relationship between the HGF ligand and the c-Met receptor is regulate with the activity of various processes trying to activate or increase it within the cell.

6. Conclusions

When the cause of infertility is a parameter other than sperm motility percentage (e.g., decreased sperm count or sperm morphology percentage), we believe the c-Met receptor controls the key-lock mechanism throughout the life of the sperm and starting in the epididymis in order to compensate for the defect. It is already well known that c-Met is upregulated in many other tissues, and especially in neoplastic tissue. As factors other than the sperm motility percentage deteriorate, the c-Met receptor is overexpressed in order to increase intracellular signaling. This improves the functional properties of sperm and leads to fertilization as an end result. It will be interesting to further clarify the relationship of various Semen Analysis Parameters such as sperm count and sperm morphology with c-Met and HGF levels. In future work, we plan to evaluate c-Met expression and HGF levels in cases where asthenospermia is suspected as the cause of male infertility and to monitor the effect of recombinant HGF.

Further studies in this area should advance our understanding of the pathogenesis and treatment of male infertility.

Author contributions

BOK, VSH and UG designed the research study. CO performed the research. FA provided help and advice on the ELISA experiments. CMB analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the Ethics Committee of T.C. Istanbul Bilim University (approval number: 44140529-2015, 15/04).

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Conflict of interest

The authors declare no conflict of interest.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at http://doi.org/10.31083/j.jomh1808177.

References

[1] Nakamura T, Nawa K, Ichihara A. Partial purification and characterization of hepatocyte growth factor from serum of hepatectomized rats. Biochemical and Biophysical Research Communications. 1984; 122: 1450–1459.

[2] Nakamura T, Nishizawa T, Hagiya M, Seki T, Shimonishi M, Sugimura A, *et al*. Molecular cloning and expression of human hepatocyte growth factor. Nature. 1989; 342: 440–443.

[3] Stoker M, Gherardi E, Perryman M, Gray J. Scatter factor is a fibroblast-derived modulator of epithelial cell mobility. Nature. 1987; 327: 239–242.

[4] Weidner KM, Hartmann G, Sachs M, Birchmeier W. Properties and Functions of Scatter Factor/Hepatocyte Growth Factor and its Receptor c-Met. American Journal of Respiratory Cell and Molecular Biology. 1993; 8: 229–237.

[5] Toshiro K, Hagiya M, Nishizawa T, Seki, Shimonishi M, Shimizu S, *et al*. Primary structure of rat HGF deduced from the complementary DNA and its expression in rat tissues. Proceedings of the National Academy of Sciences of the United States of America. 1990; 87: 3200–3204.

[6] Zanegar R, Muga S, Raija R, Michalopoulos G. Tissue distribution of hepatopoientin A: a heparin binding polypeptide growth factor for hepatocytes. Proceedings of the National Academy of Sciences. 1990; 87: 1252–1256.

[7] Nakamura T, Mizuno S. The discovery of Hepatocyte Growth Factor (HGF) and its significance for cell biology, life sciences and clinical medicine. Proceedings of the Japan Academy Series B: Physical and Biological Sciences. 2010; 86: 588–610.

[8] Kim ES, Salgia R. MET Pathway as a Therapeutic Target. Journal of Thoracic Oncology. 2009; 4: 444–447.

[9] Trusolino L, Bertotti A, Comoglio PM. MET signalling: principles and functions in development, organ regeneration and cancer. Nature Reviews. Molecular Cell Biology. 2010; 11: 834–848.

[10] Matsumoto K, Nakamura T. Hepatocyte growth factor: molecular structure and implications for a central role in liver regeneration. Journal of Gastroenterology and Hepatology. 1991; 6: 509–519.

[11] Depuydt CE, De Potter CR, Zalata A, Baekelandt E, Bosmans E, Comhaire FH. Levels of hepatocyte growth factor/scatter factor (HGF/SF) in seminal plasma of patients with andrological diseases. Journal of Andrology. 1998; 19: 175–182.

[12] Catizone A, Ricci G, Arista V, Innocenzi A, Galdieri M. Hepatocyte growth factor and c-MET are expressed in rat prepuberal testis. Endocrinology. 1999; 140: 3106–3113.

[13] World Health Organization. WHO Laboratory Manual for the Examination and processing of Human Semen. 5th ed. World Health Organization. 2010. Available at: https://apps.who.int/iris/handle/10665/44261 (Accessed: 15 January 2022).

[14] Catizone A, Ricci G, Galdieri M. Functional role of hepatocyte growth factor receptor during sperm maturation. Journal of Andrology. 2002; 23: 911–918.

[15] Kitamura M, Matsumiya K, Nishimura K, Yamanaka M, Matsumoto K, Okuyama A. Effect of hepatocyte growth factor on sperm motility. American Journal of Reproductive Immunology.

2000; 44: 193–196.
[16] Wiltshire EJ, Flaherty SP, Couper RT. Hepatocyte growth factor in human semen and its association with semen parameters. Human Reproduction. 2000; 15: 1525–1528.
[17] Ricci G, Catizone A, Galdieri M. Expression and functional role of hepatocyte growth factor and its receptor (c-met) during fetal mouse testis development. The Journal of Endocrinology. 2006; 191: 559–570.
[18] Tsarfaty I, Rong S, Resau JH, Rulong S, da Silva PP, Vande Woude GF. The Met proto-oncogene mesenchymal to epithelial cell conversion. Science. 1994; 263: 98–101.
[19] Depuydt CE, Zalata A, de Potter CR, van Emmelo J, Comhaire FH. The receptor encoded by the human C-MET oncogene is expressed in testicular tissue and on human spermatozoa. Molecular Human Reproduction. 1996; 2: 2–8.
[20] Catizone A, Ricci G, Galdieri M. HGF and postnatal testis development. Molecular and Cellular Endocrinology. 2005; 241: 32–40.
[21] Yamauchi M, Itoh H, Naganuma S, Koono M, Hasui Y, Osada Y, *et al*. Expression of hepatocyte growth factor activator inhibitor type 2 (HAI-2) in human testis: identification of a distinct transcription start site for the HAI-2 gene in testis. Biological Chemistry. 2002; 383: 1953–1957.
[22] Goda K, Fujisawa M, Shirakawa T, Dobashi M, Shiota G, Zhang ZJ, *et al*. Adenoviral-mediated HGF expression inhibits germ cell apoptosis in rats with cryptorchidism. The Journal of Gene Medicine. 2004; 6: 869–876.
[23] Parvinen M, Kangasniemi M, Kaipia A, Mali P, Soder O, Pollanen P, *et al*. Local regulation of seminiferous epithelium: the role of cytokines. Bulletin De L'Association Des Anatomistes. 1991; 75: 163–165.
[24] Pöllänen P, Söder O, Parvinen M. Interleukin-1 alpha stimulation of spermatogonial proliferation in vivo. Reproduction, Fertility, and Development. 1989; 1: 85–87.
[25] Boitani C, Stefanini M, Fragale A, Morena AR. Activin stimulates Sertoli cell proliferation in a defined period of rat testis development. Endocrinology. 1995; 136: 5438–5444.
[26] Kubota H, Avarbock MR, Brinster RL. Culture conditions and single growth factors affect fate determination of mouse spermatogonial stem cells. Biology of Reproduction. 2004; 71: 722–731.
[27] Puglisi R, Montanari M, Chiarella P, Stefanini M, Boitani C. Regulatory role of BMP2 and BMP7 in spermatogonia and Sertoli cell proliferation in the immature mouse. European Journal of Endocrinology. 2004; 151: 511–520.
[28] Naz RK, Joseph A, Lee Y, Ahmad K, Bhargava MM. Expression of scatter factor/hepatocyte growth factor is regionally correlated with the initiation of sperm motility in murine male genital tract: is scatter factor/hepatocyte growth factor involved in initiation of sperm motility? Molecular Reproduction and Development. 1994; 38: 431–439.

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