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Effect of Seminal Transforming Growth Factorß1 (TGFß1) and Glutathione on Apoptosis in Spermatozoa from Tunisian Infertile Men

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Abstract

We aimed to compare the effect of TGFB1 and glutathione on sperm necrosis in three groups of patients with increased necrospermia. The study included 120 men aged 37.6 ± 4.6 years consulting for sterility of the couple. Patients were subdivided into three groups according to the percentage of necrotic spermatozoa: necrospermia<30%; N=40), moderate necrozoospermia (50-80%, n=45) and severe necrozoospermia (>80%) For each patient, the sperm parameters were evaluated according to WHO standards, TGFB1 and glutathione Oxidized and reduced was carried out by the ELISA technique and by spectrophotometry respectively. We found a significant increase in the levels of TGFβ1 and DFI in moderate and severe necrospermia groups and positive correlations between these two factors and sperm parameters (numeration, mobility and morphology). In addition, a significant decrease in seminal GHSt was observed in the necrozoospermic groups compared to the control group. A strong correlation was observed between the degree of necrozoospermia and the fragmentation of sperm DNA (r=0.878, p=0.001). In addition, a significant positive correlation between TGFβ1 and DFI in the two groups of patients with moderate necrospermia (r=0.43, p<0.05) and severe necrospermia (r=0.52, p<0.05). This confirms that seminal TGFβ1 is a factor in the fragmentation of spermatozoa DNA. These results suggest that decreased glutathione and increased seminal TGFβ1 are important risk factors that can lead to a succession of morphological and functional alterations of spermatozoa resulting in necrozoospermia. Their perturbation in seminal plasma can lead to mediocre results of medically assisted procreation.

Keywords: Sperm DNA; Apoptosis; TGFß1; Glutathione; Necrospermia

Introduction

Mechanisms that regulate cell death are essential for normal development and maintenance of homeostasis. Cell death can be developmentally controlled, with apoptosis being the most common morphologic expression of such programmed cell death [1-4]. Lethal cellular programs that lead to apoptosis may be triggered by a variety of exogenous and environmental stimuli. Transforming growth factor-ß1 (TGFß1) is one of the best known physiological inhibitors of epithelial cell proliferation.

It is a member of a large family of structurally related factors that play a critical role during embryogenesis in mammals, frogs, and flies. TGFß1 is particularly multifunctional, being able to regulate cell proliferation, differentiation, and morphogenesis, and recently attention has turned to its possible role in cell death.

Localized cell death by apoptosis was described in Drosophila by mutation in a gene coding for a TGFß1 homolog. TGFß1 is one of the genes activated by the initiation of apoptosis during prostate regression [1]; it has been found to stimulate apoptotic cell death in cultured human gastric carcinoma cells and in endometrial stromal cells and, furthermore, it both inhibits proliferation and increases apoptosis in cultured uterine epithelial cells in rat hepatocytes and in hepatoma cells.

The mechanisms by which TGFß1 exerts its effect are only partially understood. Among these phenomena, it has been found that this factor induces the production of free radicals or reactive oxygen species (ROS) [2,3], which are produced by non-functional spermatozoa and leukocytes.

The TGFß1 causes an increase in the rate of oxidized glutathione (GSSG) by the reduced intracellular glutathione (GSHr). The latter is used by the spermatozoa to reduce many oxygenates, including ROS. The agents which cause oxidative stress in the sperm, such as tert - butyl hydroxyperoxide, can cause accumulation of GSSG because the capacity of the GSSG reductase depends on dinucleotide nicotinamide adenine phosphate (NADP) and becomes rate-limiting and the NADPH/NADP ratio decreases [4-7]. It has been proposed that the efflux of GSSG from the cell occurs to maintain a normal cellular redox state; such a depletion of glutathione levels is always observed. Thus, increased production of ROS and a decrease in the reduced glutathione concentrations indicate that TGFß1 induces an oxidative stress in the walls of spermatozoa [5-10,11-15].

The fragmentation of DNA is largely due to ROS. Furthermore, some membrane receptors, such as Fas receptor can, after binding to their ligand, trigger the biochemical cascade of apoptosis. Now, it has been shown that in infertile subjects, particularly oligozoospermics, the expression of this receptor on the surface spermatozoa is increased. The immature spermatozoa found in the ejaculate are programmed for death and consequently apoptotic.

It is also possible that during the spermiogenesis, the activity of an error endonuclease is responsible for setting route of the apoptotic process. This enzyme is physiologically involved in creating and repairing the break points on DNA and in the replacement of histones by protamines. Currently, available results on apoptosis sperm show

that the percentage of DNA fragmentation was higher in: Infertile men, the holders of anoligoasthenoteratozoospermia, the presence of high levels of free radicals in sperm.

This high rate is inversely correlated with the count, sperm motility and morphology and the fertilization rate in IVF and ICSI. The aim of our study was to evaluate the relationship of apoptosis with conventional semen parameters (count, motility, morphology and necrospermia), the rate of DNA fragmentation and seminal levels of TGFß1and of oxidized and reduced glutathione in the semen of infertile patients.

Patients and Methods

Semen collection and analysis

Semen samples were obtained from 120 men consulting for infertility evaluation at our laboratory of Cytogenetics and Reproductive Biology, Farhat Hached University Teaching Hospital, Sousse (Tunisia). Patients were subdivided into three groups according to the percentage of dead spermatozoa. The first group included 40 patients with normal level of dead spermatozoa (<30%).

The second group was composed of 45 patients with a percentage of sperm death between 50 and 80% (moderate necrozoo spermia). The third group consisted of 35 patients with necrozoo spermia more than 80% (severe necrozoo spermia) [6]. The criteria for defining normospermia and necrospermia were adopted according to WHO standards (WHO, 2010).

Inclusion criteria

Males of couples living together with regular unprotected coitus for a reasonable period of time but not less than one year without conception.

Exclusion criteria

A detailed medical history was performed for all studied cases. Subjects currently on any medication or antioxidant supplementation were not included [16-24]. In addition, subjects with testicular varicocele, genital infection, leukocytespermia, chronic illness and serious systemic diseases, smokers and alcoholic men were excluded from the study because of their well-known high seminal ROS levels and decreased antioxidant activity. For each patient, sperm viability and sperm DNA fragmentation were assessed within 1 hour after ejaculation because both parameters were shown to be increased rapidly after ejaculation.

Study consent

A written consent of each subject was taken after explaining the aims and objectives of the study and its benefits on individual and society [25-29]. Also, the study was approved by the Local Ethic Committee of the Farhat Hached University Hospital, Sousse (Tunisia).

Semen Analysis

Samples were produced by masturbation after 3 days of sexual abstinence. After liquefaction of semen, at room temperature, standard semen parameters were obtained according to WHO [2010] guidelines. Sperm viability was assessed 30 min after ejaculation [30-36].

Determination of Necrospermia

Sperm viability was assessed 30 min after ejaculation. The eosin-nigrosin viability test was performed by dissolving 20µl eosin with 20µl of fresh sperm and then 20µl nigrosin [37-39].

The percentage of viable sperm (sperm head unstained; living spermatozoa) and nonviable (sperm head stained; dead spermatozoa) was assessed by counting a minimum of 100 spermatozoa. (Figure 1).

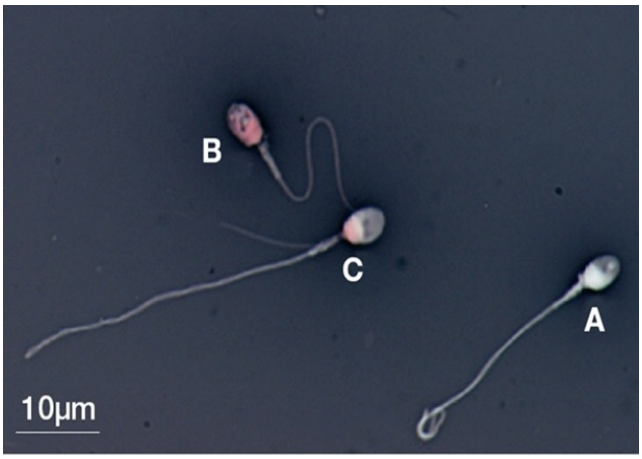

Figure 1: Appearance of sperm on smears. Unstained spermatozoa=live sperm count (A); Colored spermatozoa in pink=dead spermatozoa (B); Spermatozoa weakly colored pink=dead spermatozoa (C).

Measurement of DNA Fragmentation

The presence of apoptosis-related DNA strand breaks in spermatozoa was evaluated by the terminal desoxynucleotidyl transferase-mediated deoxyuridine triphosphate biotin nickend labeling (TUNEL) assay, using the ApopTag® Apoptosis Detection Kits (QBiogene, Paris, France) in controls and patients [40-44].

For cell per meabilization, slides were incubated in phosphate buffer saline (PBS) with a solution of 1% Triton X100 (Sigma) [45-47].

The procedure was carried out according to the manufacturer's instruction. Briefly, the specimens were washed twice in PBS 1X, equilibrated with the equilibration buffer at room temperature for 10 seconds and incubated in a dark moist chamber at 37°C, for 1 h, with the Terminal Desoxynucleotidyl Transferase (TdT) solution in order to allow DNA elongation [48-53].

After stopping the enzyme reaction, the slides were washed twice in PBS; and the DNA elongation was revealed by incubation of the cells with anti-digoxigenin antibody coupled to peroxidase, during 30 min in a dark moist chamber.

The peroxidase was revealed with DiAmino Benzidine (DAB). Slides were then counterstained with Harris' haematoxylin (RAL, Martillac, France) and finally mounted using Faramount mounting (Dako, Carpinteria, CA, USA). Slides were observed under a microscope (Zeiss, Oberkochen, Germany) equipped with a 100- magnification lens.[54-60] Spermatozoa with fragmented DNA had browncolored nuclei, whereas the other cells were blue-gray (counter coloration with

Harris's haematoxylin) [7,61-63]. On each slide, approximately 500 cells were counted, and the DNA fragmentation index (DFI) was calculated (Figure 2).

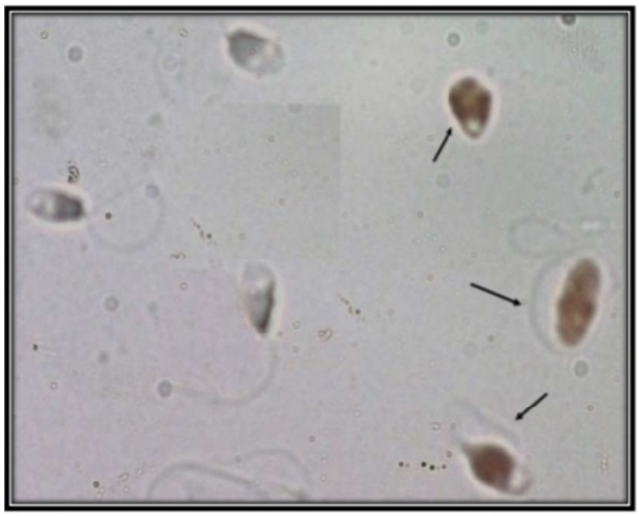

Figure 2: Tunnel Technique: Spematozoa non fragmented DNA (gray); spermatozoa fragmented DNA (brown).

Determination of GSH and GSSG contents

The total glutathione (GSHt), reduced glutathione (GSHr) and oxidized glutathione (GSSG) were measured spectrophotometrically in deproteinized supernatant fractions from the semen by the method of Akerboom and Sies (Akerboom & Sies, 1981) using 5,5'-dithiobis (2-nitrobenzoic acid) [9,64]. Absorbance values were compared with standard curves from known amounts of GSH standards.

Detection of TGFß1 in Semen

Human TGFß1 levels in semen were measured using a commercially available ELISA kit (Quantikine human IGF-I Immunoassay, R & D Systems). Before measurement, samples of sperm were pretreated and diluted to 1:100 with pretreatment buffers enclosed in the kit. The minimum detection limit of the assay was 0.094 ng/ml. The intraassay coefficients of variation (CVs) were 3.5% at 0.5 ng/ml, 4.3% at 1.2 ng/ml, and 4.3% at 2.4 ng/ml. The interassay CVs were 8.1% at 0.4 ng/ml, 8.3% at 1.1 ng/ml, and 7.5% at 2.3 ng/ml.

Statistical Analysis

Statistical analysis was performed using SPSS.18 (SPSS, Chicago, IL, USA). Data are represented as Mean ± Standard Deviation (SD). Group comparisons were made using students t-test. Pearson's correlation was performed to examine the relationship between the percentage of sperm with DNA fragmentation and standard semen parameters. All hypotheses testing were two sided with a probability value of 0.05 deemed as significant.

Results

Characteristics of study population

A total of 120 semen samples from infertile patients were analysed regarding the semen parameters, DNA fragmentation. The subjects' ages ranged from 24 to 76 years (Table 1). The mean age was 37.6 years.56.6% of the subjects were between 30 and 40 years old; 7.5% were <30 years and 35.8% were >40 years old. The results of our study were allocated to four age groups:

-Younger than 30 years (27.1 years in average; n=9)

-30–39 years (34.6 years in average; n=68)

-40–49 years (42.6 years in average; n=36)

-≥50 years (54.5 years in average; n=7)

For control group, subjects ranged in age from 25 to65 years with a mean of 37.34 years.

They were also distributed into four groups:

-Younger than 30 years (27.12 years in average; n=3)

-30–39 years (35.04 years in average; n=23)

-40-49 years (43.50 years in average; n=12)

-≥50 years (56.50 years in average; n=2)

Parameters	characteristics	Number (n)	Percentage (%)
Age (years)	≤ 30	36	30
	>30	84	70
Duration of infertility (years)	≤10	99	82,5
	>10	21	17,5
Type of infertility	Primary	90	75
	Secondary	30	25
Area	Rural	24	20
	Urban	96	80
Spermcriteria	Control[1]	40	33.3
	Moderate necrospermia[2]	45	37.5
	Severe necrospermia[3]	35	29.2

[1]: Patients with normal level of dead spermatozoa as recommended by WHO [2010] guidelines (<30%); [2]: patients with a percentage of sperm death between 50 and 80%; [3]: patients with necrozoospermia more than 80%.

Table 1: General characteristics of the study population.

Conventional semen analysis

The characteristics of sperm examination of the different groups of patients are reported in Table 2.

	Control	Moderate necrospermia	Severe necrospermia
	(n= 40)	(n= 45)	(n= 35)
Age (years)			
Mean ± SD	38.1 ± 3.4	38.4 ± 5.7	36.3 ± 4.8
(Min-Max)	(27 - 49)		(31 – 49)
Volume (ml)			
Mean±SD	2.1 ± 0.9	3.3 ± 1.4	3.2 ± 1.4
(Min - Max)	(2 – 5.5)	(1.5 – 6.5)	(2 – 7.5)
Sperm motility			
(a + b)			
Mean±SD	40.2 ± 3.8	26.1 ± 9.6	17.3 ± 8.7
(Min-Max)	(30 – 50)	(5 – 40)	(3 – 29)
Sperm Count (Million/ml)			
Mean±SD			
(Min-Max)	75.8 ± 20.8	45.6 ± 23.2	12.3 ± 6.5
	(36 – 105)	(20 – 85)	(5 – 18)
Abnormal morphology (%)			
Mean±SD			
(Min-Max)	70.5 ± 13.2	75.1 ± 14.9	81.5 ± 12.3
	(39 – 80)	(56 – 86)	(77 – 99)
Necrospermia (%)			
Mean±SD	16.5 ± 4.4	65 ± 8.8	84.7 ± 6.3
(Min-Max)	(5 – 29)	(51 – 70)	(80 – 100)
Note: Min=minimum ; Max=maximum ; ml=milliliter ; SD=standard deviation.			

Table 2: Means of age and conventional semen parameters of patients with moderate necrozoospermia, severe necrozoospermia, and controls.

No differences were observed in age, ejaculation volume, and sperm concentration and morphology between the different groups ($p>0.05$). According to WHO [1999] guidelines, these parameters were shown to be normal in all groups.

Only motility was shown to be lower in the second and third groups with a statistically significant difference compared to the control group ($p<0.05$).

As expected, the percentage of necrotic spermatozoa was higher in the second and the third group, compared to the first group ($p<0.01$). The mean value of necrospermia was 65.0 ± 8.8% in the second group

(range 51 to 70%) and 84.7 ± 6.3% in the third group (range 80 to 100%). However, this value did not exceed 16.5 ± 4.4% in the first group (range 5 to 29%).

The mean levels of seminal of GSHt (µmol/l), GSHr(µmol/l), GSSG (µmol/l) and TGFß1 (ng/ml) of all studied groups were shown in Table 4.

Biochemical analysis

Comparison of results between control group and patients with moderate or severe necrospermia

GSHt and GSSG showed significant elevation in seminal plasma of control group compared to pathological groups. However, the rate of GSSG in two pathological groups is slightly higher compared to control group.

Levels of GSH in the control group and pathological groups

Concentrations of different GSH forms in seminal plasma of the two groups of patients were in order GSHt>GSSG>GSHr. A significant increase in seminal GSSG ($p<0.001$) of control group has been noted [27]. Nevertheless, significant differences have also been seen in mean concentrations of GSHt and GSHr (Table 3).

Parameters	Control (n= 40)	Moderatenecrospermia (n=45)	Severenecrospermia (n=35)	p-value control/MN contrôle/SN
Necrospermia (%) Mean± SD (Min-Max)	16.5 ± 4.4 (5 – 29)	65 ± 8.8 (50– 77)	83.72 ± 6.3 (80 – 99)	0.02/0.04
DFI (%) Mean±SD (Min-Max)	8.28 ± 2.81 (5 – 25)	28.8 ± 5.21 (17 – 30)	32.11 ± 3.25 (31 – 48)	0.05/0.7

Note: DFI= DNA Fragmentation Index (%); min=minimum; max=maximum; SD= standard deviation; MN= moderate necrospermia; SN= severe necrospermia; Data are expressed as mean ± SD; $p<0.05$=Significance.

Table 3 : Necrospermia (%) and DNA Fragmentation Index : DFI (%).

Seminal transforming growth factor (TGFß1)

As regards to TGFß1, means of seminal concentrations were significantly different between the group of patients and control ($p<0.05$) [29] However, conversely to TGFß1 we found an increased concentration in patients with moderate and severe necrospermia compared to control group (145.3 and 130.7 *vs.* 92.4 ng/ml, respectively). Effectively, seminal TGFß1 in patients with necrospermia, showed also highly significant increase ($p \leq 0.01$) in comparison with control group (Table 4).

Parameters	Control (n= 40)	Moderate necrospermia (n= 45)	Severe necrospermia (n= 35)	p-value Control/MN control/SN
GHSt (µmole/l) Mean±SD (Min-Max)	53.6 ± 20.1 (25.6 – 99.5)	38.3 ± 13.1 (22.2 – 69.7)	45.4 ± 17.7 (14.2 – 68.4)	0.05 NS
GSSG (µmole/l) Mean±SD (Min-Max)	31.5 ± 19.2 (41 – 52)	29.6 ± 10.2 (7.1 – 59)	35.5 ± 16.1 (11.6 – 63.7)	0.04 0.02
GHSr (µmole/l) Mean±SD (Min-Max)	22.3 ± 12.3 (26 – 95)	15.3 ± 2.8 (3.1 – 59)	35.5 ± 16.1 (11.6 – 63.7)	NS
TGFß1 (ng/ml) Mean±SD (Min-Max)	92.4 ± 29.2 (43 –143.3)	145.3 ± 40.7 (110.1 – 205)	130.7 ± 61.2 (105.8–244.9)	0.01/0.05

Note: GHSt= Total glutathione; GSSG= oxidized glutathione; GHSr=reduced glutathione; TGFß1=transforming growth factorß1; MN=moderate necrospermia; SN=severe necrospermia. Data are expressed as mean ± SD; p<0.05=significance.

Table 4: Glutathione and TGFß1 levels in the study groups.

A highly significant correlation was observed between seminal TGFβ1 and necrospermia (r=0.62; p<0.05) (Figure 3).

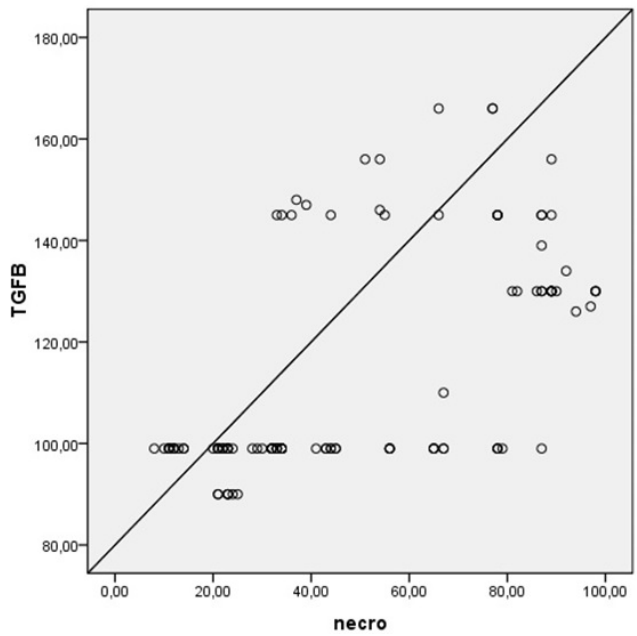

Figure 3: Correlation between necrozoospermia (necro) and the Sperm TGFβ1. A statistically significant positive correlation was found between the degree of necrozoospermia and sperm DNA fragmentation (r=0.62; p<0.05).

In fact, we noted an elevated rate of seminal TGFß1 in severe necrospermia (130.7 ± 61.2ng/ml) cases compared to control (92.4 ± 29.2ng/ml), the difference was significant. Additionally, elevated rates of seminal TGFß1 were strongly and negatively associated with sperm motility (r=-0.55, p<0.01).

Meanwhile, there has been no correlation between seminal TGFß1 and the percentage of abnormal morphology (r =0.70, p<0.01), but a negative relationship was found among TGFß1 and sperm count (r=-0.43, p<0.01).

On the other hand, we estimated correlations that can exist between TGFß1 and different forms GSH studied and we found high positive correlations with GSHt (r=0.490, p<0.001) and GSSG levels (r=0.369, p<0.004) [30].

Though, there have been no correlations noted between TGFß1 and GSHr.

For different forms of glutathione, only oxidized form showed a negative and significant correlation with abnormal morphology (r=-0.4, p<0.05).

Analysis of sperm DNA fragmentation

The sperm DNA fragmentation index (DFI) was 9.28 ± 2.98% in patients with a normal level of necrotic spermatozoa, 20.25 ± 3.21% in patients with moderate necrozoospermia, and 35.31 ± 5.25% in patients with severe necrozoospermia. Sperm DNA fragmentation was significantly higher in patients with necrozoospermia compared to normozoospermic men with the highest value in patients with necrozoospermia exceeding 80% (p<0.05) [36] Using Pearson's correlation test, a strong correlation was found between the degree of necrozoospermia and sperm DNA fragmentation (r=0.878; p=0.001) (Figure 4).

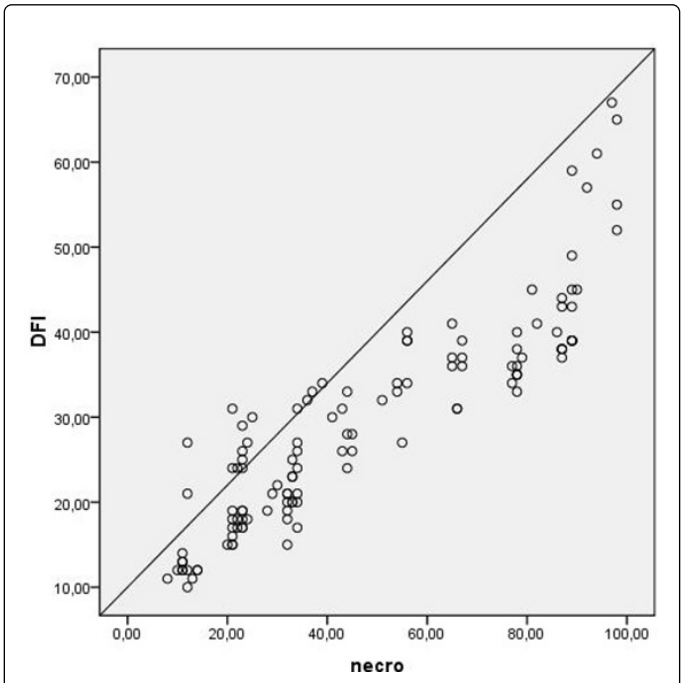

Figure 4: Correlation between necrozoospermia (necro) and the sperm DNA fragmentation index (DFI). A statistically significant positive correlation was found between the degree of necrozoospermia and sperm DNA fragmentation (r=0.878; p=0.001).

In addition, statistically significant negative correlations were found between the level of DNA fragmentation and sperm motility (r=-0.646; p=0.001), and abnormal sperm morphology (r=0.434; p=0.001). However no evident correlation was found between the percentage of sperm DNA fragmentation and sperm count, and paternal age (p>0.05) [39].

On the other hand, the DFI is increasing a relationship with the rate of seminal TGFß1. Indeed, there has been a significant positive correlation between the TGFß1 and DFI in both groups of patients with moderate necrospermia (r=0.43, p<0.05) and severe necrospermia (r=0.52, p<0.05) (Figure 5).

This confirms that the seminal TGFß1 is a factor favoring the fragmentation of sperm DNA [40].

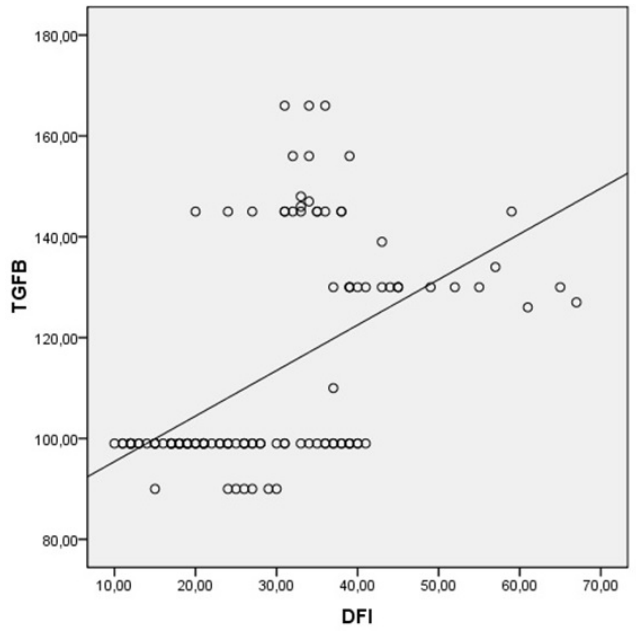

Figure 5: Correlation between the sperm DNA fragmentation index (DFI%) and Seminal Transforming Growth Factor β1(TGFβ1). A statistically significant positive correlation was found between the level of DFI and sperm TGFβ1 (r=0.52; <0.05).

Discussion

Apoptosis spermatozoa are mainly related to the problem of the stability of the membrane [9,26]. In this study, we examined the relationship between apoptosis, the rate of seminal TGFß1, the rate of the different forms of glutathione (GSHt, GSSG, GSHr) and fragmentation of the sperm DNA. As far as we know, this is the first analysis that examines all these parameters simultaneously [41-47].

The majority of previous studies have been done on heterogeneous groups with several affected sperm parameters.

In addition, no data were available in the literature concerning the relationship between the fragmentation of sperm DNA and the rate of seminalTGFß1 and/or the rates of the different forms of glutathione.

Using TUNEL and ELISA techniques, we observed that patients with moderate and severe necrospermia had high levels of DNA fragmentation of sperm and a high rate of seminal TGFß1 compared to the control group. In addition, the highest rate of TGFß1 and the highest value of the DNA fragmentation were observed in patients of greater than 80% necrozoospermia. A statistically significant correlation was observed between levels of TGFß1, fragmentation of DNA and necrozoospermia [10].

It seems that the necrozoospermia process or apoptosis was initiated from a specific threshold rate seminal TGFß1. This threshold value was the source of production of reactive oxygen species (ROS) [6,48,49] as a result of oxidative stress leading to cell death. The mechanisms of ROS-induced damage to spermatozoa included an oxidative attack on the sperm plasma membrane lipids, leading to the initiation of a lipid peroxidation cascade, as a consequence of which the spermatozoa lose their capacity for movement [11]. In addition, ROS may also affect the sperm axoneme, inhibit mitochondrial function, and affect the synthesis of DNA, RNA and proteins. The principal cytotoxic reactive oxygen intermediate involved in ROS-associated damage is probably hydrogen peroxide (H2O2) generated by the intracellular dismutation of superoxide anion (O2-) under the influence of superoxide dismutase (SOD). However, Cameroun and Bernard (2007) suggested that the hydroxyl radical (HOO°), formed by the protonation of O2-, could be a potent indicator of per oxidative damaging human spermatozoa [2,52]. Though, in addition to H2O2, lipid peroxides generated as consequence of the peroxidation process also appeared to be profoundly cytotoxic, as their degradation products do.

On the other hand, Glutathione is involved in a protective mechanism that involves inactivation of ROS, including peroxides formed in cellular oxygen metabolism. These toxic oxygen species maybe detoxified via reduction by glutathione peroxidase (GPX), which is converted to oxidized glutathione (GSSG) in the process. In turn, oxidized GSH is reduced by glutathione reducates (GRD), in the presence of NADPH. In addition, other electrophilic foreign compounds (xenobiotic) may be detoxified in a reaction catalyzed by a group of enzymes named glutathione S-transferases, by which they are conjugated with GSH. Ultimately, we can say that TGFß1-induced apoptosis in spermatozoa is preceded by an enhancement in reactive oxygen species production, an increase in the GSSG/(GSH +GSSG)ratio, and a decrease in the glutathione intracellular content. Reduced glutathione (GSH) issued intracellular to reduce numerous oxidizing compounds, including reactive oxygen species. Agents that induce oxidative stress in spermatozoa cause accumulation of GSSG because the capacity of NADP-dependent GSSG-reducates becomes rate-limiting and the NADPH/NADP ratio decreases. Indeed our results showed that in moderate and severe necrospermia, the rate oxidized glutathione (GSSG) was elevated compared to the control group, and the difference was statistically significant [23-25,55]. This confirms the fact that the oxidized form of glutathione is in favored the production of reactive species of oxygen as well as the TGFß1.

It has been proposed then that efflux of GSSG from the cell occurs in order to preserve the cellular normal redox state so that depletion in the glutathione levels is always observed. Thus, an increase in there active oxygen species production and a decrease in the glutathione concentrations indicate that TGFß1 induces an oxidative stress in spermatozoa. Support for this idea also comes from the observations that (i) H2O2 production by TGFß1 have already been found in bovine pulmonary artery endothelial cells. (ii) have described that TGFß1 suppresses the expression of antioxidative enzymes in adult rat hepatocytes, thereby showing that production of peroxides is increased in these cells, and (iii) have shown that TGFß1 down-regulates cytochromes P-450 *1A1* and *1A2*, two genes of which the expression is modulated by oxidative stress in adult hepatocytes. However, at present it is not clear if TGFß1-induced peroxide production may cause growth inhibition, apoptosis, or both things. The results presented clearly relate TGFß1 reactive oxygen species production to fetai hepatocyte cell death.

First, low concentrations of TGFß1 sufficient to completely block fetal hepatocyte growth do not induce reactive oxygen intermediate production in these cells. Second, studies performed to analyze c-fos expression, as a gene modulated by redox state, demonstrate that only high, apoptotic concentrations of TGFß1 produce an increase in its mRNA levels; this induction coincides with an increase in the nuclear

AP-1 binding activity. Finally, TGFß1-induced cell death in fetal hepatocytes may be either partially blocked by single radical scavengers or totally blocked by combinations of these. However, these agents do not preclude the TGFß1 growth inhibitory effect in these cells.

Conclusion

Results presented in this paper provide evidence for the involvement of an oxidative processing the apoptosis elicited by TGFß1 in spermatozoa. We can say that a high rate of seminal TGFß1 is an indicator of high sperm DNA fragmentation [12,13,25]. We can predict that the injection of these compromised spermatozoa may affect the outcome of assisted reproduction in multiple ways, including effects on the fertilization rates, embryo cleavage, and pregnancy rates. In addition, our data show that high level of seminal TGFß1 (>100 ng/ml) which is a critical semen parameter associated with infertility, is a predictive factor for an increased risk of sperm DNA damage [14-17].

The limit of our study is to know the exact rate of TGFB1 from which the spermatozoa become highly altered and therefore utilizable for fertilization [18-21,57]. Therefore we recommended the evaluation of seminal status of TGFß1 before any attempt of assisted reproduction.

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Declaration of Interests

The authors have declared that no conflict of interest exists

Authors' contributions

BH and HR conceived the idea and performed the research. HR conducted the literature review, analyzed the data and wrote the paper. AH, GH, KM, ES, HM and AM helped with the reviewing and editing of the manuscript. SA; approved the release to be published. All authors declared that they participated, read and approved the final manuscript.

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