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Research Article

Seminal Caspase 3, Cytochrome C and Total Antioxidant Capacity in Nigerian Male Subjects Undergoing Infertility Evaluation

Emokpae M.A¹, Chima H.N¹ and Ahmed M²

¹*Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin, Benin City.*

²*Department of Urology, Ahmadu Bello University, Zaria, Nigeria.*

ABSTRACT

Male factor infertility may be responsible for about half of cases of infertility. This study evaluated the activities of caspase 3, cytochrome c and total antioxidant capacity in normospermic males investigated for infertility. Seminal plasma cytochrome c, caspases 3 and total antioxidant capacity were determined by Enzyme linked Immunosorbent assay technique. Sixty (60) infertile males aged 39.6±0.28 and 30 males aged 38.9±0.52 with proven fertility were enrolled. The activities of cytochrome c ($p < 0.002$) and caspase 3 ($p < 0.01$) were significantly higher in infertile but normospermic than controls. There was however no significant difference in the level of total antioxidant capacity between the groups. The levels of cytochrome c ($p < 0.001$) and caspase 3 ($p < 0.02$) were significantly higher in the subjects with primary infertility than secondary infertility. Cytochrome c and caspase 3 correlated negatively with sperm morphology in normospermic infertile males. Seminal fluid caspase 3 and cytochrome c activities were higher in normospermic but infertile males than control subjects. The implication of these findings on fertility potential of the male subjects is discussed.

Keywords: *Seminal caspase 3, cytochrome c, infertility, normospermia and total antioxidant capacity.*

*Author for correspondence: E-mail: - mathias.emokpae@uniben.edu ; Tel. +2347055065994

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INTRODUCTION

Male infertility refers to the inability of a sexually active male to impregnate a non-contracepting fertile adult female within one year. No identifiable cause of the infertility may be found in more than 40% of infertile males (Jungwirth et al., 2015). Idiopathic infertile man is one who has difficulty impregnating his female partner even when his fertility status by routine semen analysis and physical examination are considered within normal reference ranges (Doaa and Eman, 2014). It was reported that about 15% of couples fail to achieve conception within a year and one in 8 couples do have difficulties achieving conception for the first child (primary infertility) while one in 6 couples may encounter problem attempting to achieve subsequent pregnancy after previous pregnancy (Jungwirth et al., 2015). Male factor infertility may be responsible for about 50% of cases and in 30-40% of subjects no identifiable cause may be observed. In this case, the male subject may present with no previous history of diseases that might affect fertility potential, no abnormal

physical examination and no hormonal, genetic or biochemical abnormalities may be observed. It was therefore suggested that the cause of idiopathic infertility could be multifactorial which may include endocrine disruption as a result of environmental pollution, reactive oxygen species, genetic or epigenetic disorders (Jungwirth et al., 2015).

In the etiology of male infertility, there is increasing evidence that damage caused by reactive oxygen species (ROS) to spermatozoa play key roles (Taylor, 2001; Lavranos et al., 2012). Most studies have implicated oxidative stress as a mediator of sperm dysfunction (Uadia and Emokpae, 2015; Uadia and Emokpae, 2016). Oxidative stress is a consequence of imbalance between the body's production of reactive oxygen species (ROS) and the antioxidant defense mechanism and this has been identified as one factor that affects fertility status (Makker et al., 2009).

There has been increasing evidence suggesting that oxidative DNA damage is implicated in male fertility and sperm function (Agarwal et al., 2005). Seminal parameters are influenced by oxidative stress of germ cells and ROS are

important in mediating apoptosis by inducing cytochrome c and caspases which can result in high frequency of single and double stranded DNA breaks (Said and Khosravi,2012).It could also be implicated in idiopathic infertility in males. Studies have shown that males with idiopathic infertility may present with higher seminal ROS levels than healthy fertile subjects (Pasqualotto et al., 2001; Zhang et al., 2014). In addition, apoptosis was reported to significantly correlate with ROS in infertile subjects (Wang et al., 2003). This study was therefore designed to evaluate the activity levels of caspase 3 and cytochrome c which are biomarkers of DNA damage as well as total antioxidant capacity in seminal fluid of infertile but normospermic male subjects undergoing infertility evaluation. Their identification as critical players of the death mechanism of cells has improved the understanding of molecular aspect of infertility.

MATERIALS AND METHODS

Study design: This was a cross-sectional case-control study involving males with history of idiopathic infertility for one year and above and males with proven fertility served as controls. The semen samples were collected from the infertile males attending Ahmadu Bello University Teaching Hospital fertility clinics and controls were males of proven fertility from the same locality.

Ethical Consideration: The study protocol was reviewed and approved by the Health Research Ethics committee (HREC) of Ahmadu Bello University Teaching Hospital in accordance with the Nigerian National Code for Health Research Ethics. All participants in this study gave informed consent before the samples were collected.

Inclusion and exclusion criteria: Male subjects aged 18-55years with a history of idiopathic infertility for one year and above and male subjects with proven fertility.

Infertile males with known endocrine disease, prolonged exposure to radiation and chemicals and any physical abnormality were excluded.

Sample Collection: A total of 60 semen samples were collected from idiopathic infertile males and 30 samples from males with proven fertility (controls).Subjects were instructed to collect samples by masturbation into sterile containers after a minimum of 3 days of sexual abstinence. The samples were

given laboratory code number and time of collection, thereafter the samples were transported to the laboratory within an hour of collection. Any loss of fraction of the semen was reported by the subjects.

Sample preparation: The freshly collected semen samples were examined for liquefaction at room temperature. Volume was measured and pH checked, sperm count, motility and morphology was done microscopically using standard protocol (WHO, 2010).Liquefied semen samples were centrifuged at 1000g for 5 minutes. Seminal plasma was aspirated into plain containers and kept frozen until analyses for cytochrome c, caspase3 and total antioxidant capacity were determined by Enzyme linked Immunosorbent assay technique using reagents supplied byElabscience and Wkea Medical supplies corporation, China.

Statistical analysis: The data were analyzed using SPSS version 20.0(Chicago, IL,USA). The differences between the mean±SEM were calculated using unpaired two tailed students' t – test and correlation studies between the sperm indices and cytochrome c, caspase 3 and TAC were done..

RESULTS

The results are as presented in tables 1 and 2. Sixty (60) infertile males on routine visit to infertile clinics, aged 39.6±0.28 formed the study group while 30 males aged 38.9±0.52 with proven fertility were enrolled as controls. Table 1 shows seminal plasma levels of cytochrome c, caspase 3 and total antioxidant capacity in normospermic but infertile subjects. There were no significant differences in the measured sperm indices between the infertile and control subjects. The activities of cytochrome c (p<0.002) and caspase 3 (p<0.01) were significantly higher in infertile but normospermia than controls. There was however no significant different in the level of total antioxidant capacity between the groups. Table 2 shows the levels of measured variables in seminal plasma of infertile but normospermic subjects, grouped into primary and secondary infertility based on whether the respondents had history of parity. There were no significant differences in the sperm indices but the levels of cytochrome c (p<0.001) and caspase 3 (p<0.02) were significantly higher in subjects with primary infertility than those with secondary infertility. Figures 1 and 2 show that cytochrome c and caspase 3 correlated negatively with sperm morphology in normospermic infertile males

Table 1:

Seminal plasma levels of cytochrome C, Caspase 3, total antioxidant capacity and measured sperm indices in infertile normospermic subjects and controls

Parameters	Normospermia (n = 60)	Controls (n = 30)	p-value
Concentration	95.56±9.45	97.44±13.89	P>0.05
Total motility	69.68±2.61	68.00±3.89	P>0.05
Morphology	68.01±1.93	67.06±2.84	P>0.05
Volume	3.60±0.27	3.40±0.28	P>0.05
Cytochrome c	94.31±7.02	70.98±7.57	P<0.002
Caspase 3	3.80±0.51	2.40±0.23	P<0.01
Total Antioxidant capacity	2.88±0.70	3.05±0.33	P>0.05

Table 2:

Seminal plasma levels of cytochrome C, Caspase 3, TAC and measured sperm indices in primary and secondary infertile normospermia

Parameter	Primary Infertility Normospermia <i>n</i> = 38	Secondary Infertility Normospermia <i>n</i> = 22	p-value
Concentration	88.97± 11.24	100.17 ± 17.32	p>0.05
Total motility	69.74 ± 3.14	67.27 ± 4.61	p> 0.05
Morphology	65.92 ± 2.29	66.46± 3.70	p >0.05
Volume	3.23± 0.29	3.69± 0.35	p >0.05
Cytochrome c	102.76 ± 3.07	80.76± 2.43	p <0.001
Caspase 3	4.59± 0.79	2.43± 0.29	p <0.02
TAC	2.19± 0.24	2.83± 0.38	p >0.05

TAC= total antioxidant capacity

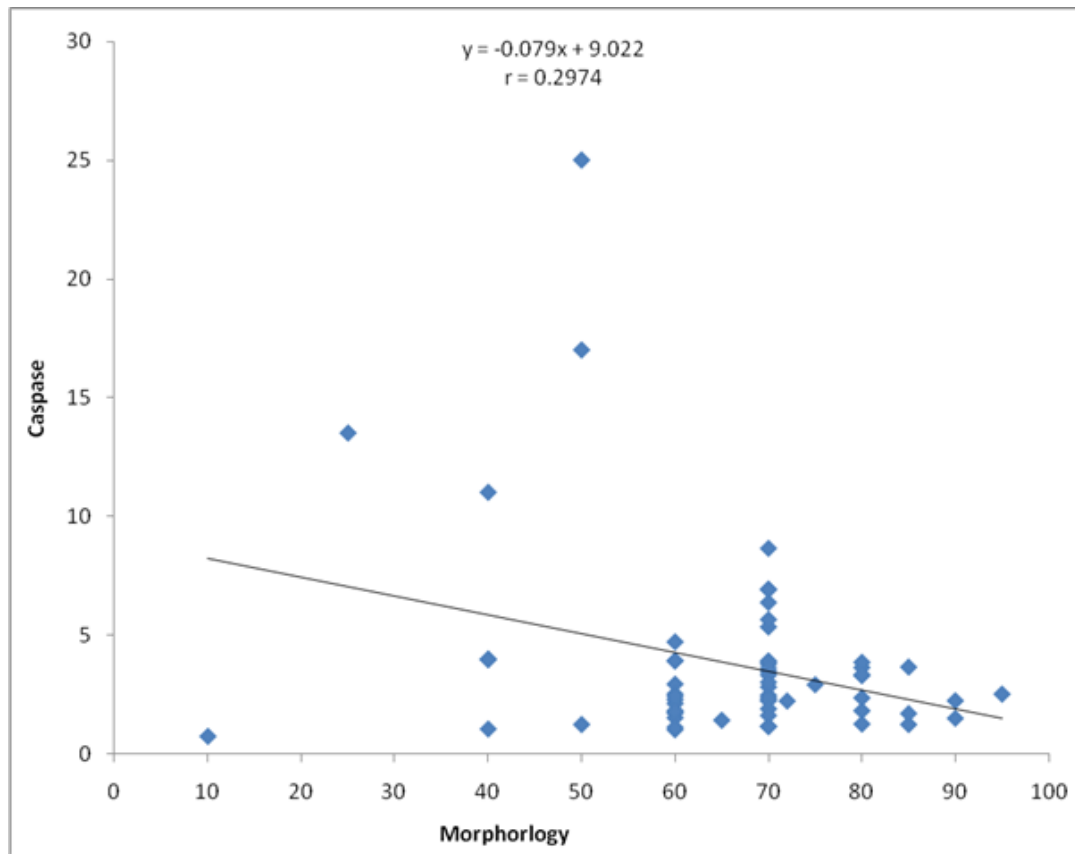


Figure1: Regression of caspase3 and morphology in infertile normospermic subjects

DISCUSSION

Seminal parameters may be affected by oxidative stress and caspase-dependent apoptosis which could lead to impaired spermatogenesis, decrease sperm motility, morphology and high frequency of single and double stranded DNA breaks (Said and Khosravi, 2012). It was observed from this study that the caspase 3 and cytochrome c activities in normospermic but infertile males were higher than in control males of proven parity. This observation is consistent with previous studies (Glander and Schuller, 1999; Duru et al., 2001; Weng et al., 2002; Paasch et al., 2004). Higher activity level of caspase 3 was reported in male subjects with infertility than controls.

It was observed that sperm with intact membranes contained low caspase activity (Paasch et al., 2004; Liu et al., 2015). Loss or reduced integrity of sperm membrane was reported to be common in sperm cells from infertile males. This reduced integrity may contribute to male factor infertility irrespective of normal sperm indices (Glander and Schaller, 1999; Duru et al., 2001; Said and Khosravi, 2012). This deterioration of membrane integrity was also related to the activation of caspase in somatic cells (Almeida et al., 2013). It was reported that within the cellular component of the testicular tissue, caspases are key players in the apoptotic process that result in DNA fragmentation of Sertolic cells (Cappallo-Oberman et al., 2013).

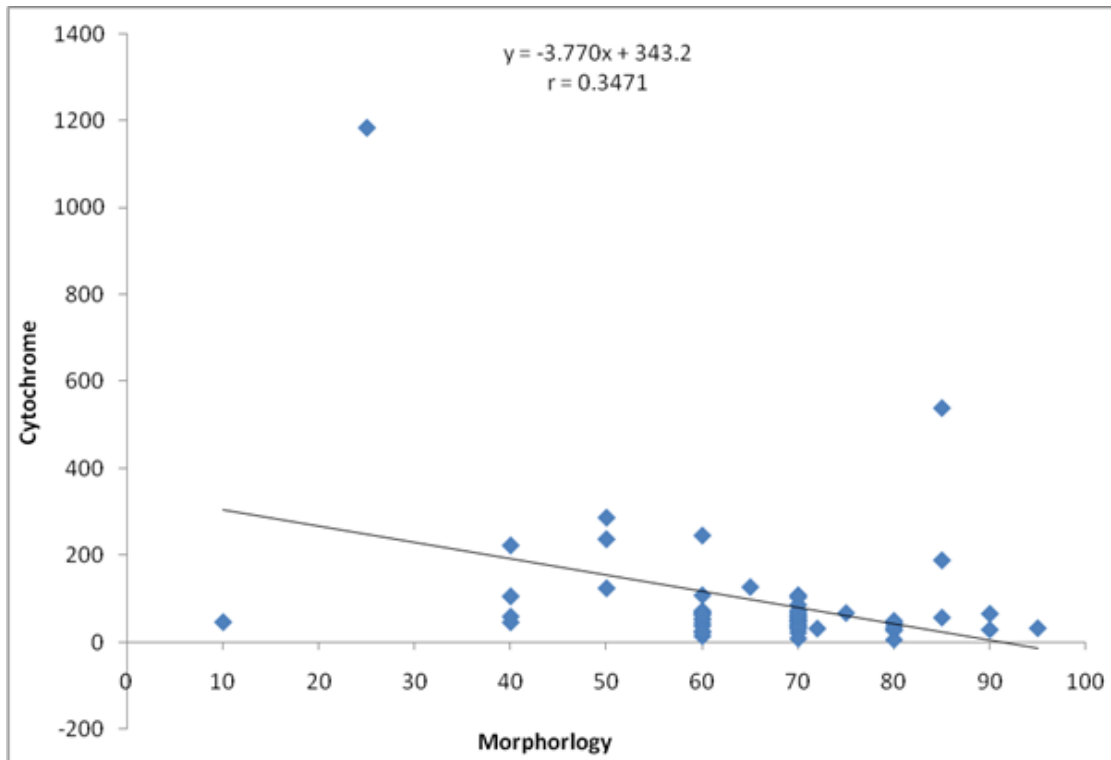


Figure 2:
Regression of cytochrome c and morphology in infertile normospermic subjects

From the functional perspective, caspases involved in apoptosis act either as initiators (caspase 8, caspase 9 and caspase 10) or as effector (caspase 3, caspase 6 and caspase 7) of apoptosis. Of the effector caspases, activated caspase 3 appears to induce activation of caspase-activated deoxyribonuclease which is also called DNA fragmentation factor-40 or caspase-activated nuclease (Said and Khosravi, 2012). This is involved in the in-vivo degradation of DNA. It was suggested that caspase is the most important of all effector caspase as the executioner of the final destruction of the cells by generating DNA strand breaks (Kim et al., 2001; Said and Khosravi, 2012). The effector caspases are usually activated proteolytically by an upstream caspase while initiator caspases are activated through regulated protein-protein interactions (Said and Khosravi, 2012). The consequences of caspase activation in mammalian testes, germ cells in normal physiological condition, are that the cells undergo several rounds of mitosis and differentiation that result in mature sperm cells. This clonal expansion is excessive and thus requires apoptotic process in order to equal the number of germ cells with the supportive capacity of Sertoli cells (Said and Khosravi, 2012). Regarding male reproduction, apoptosis controls the overproduction of spermatozoa and restricts the normal proliferating levels during unsuitable conditions for sperm development. In the presence of large number of spermatozoa with damaged DNA, the Sertoli cells express FasL which induces sperm cell apoptosis by Fas/FasL pathway. This process helps to ensure that only spermatozoa with normal integrity are allowed to develop to maturity (Pentikainen et al., 1999; Johnson et al., 2008; Emokpae and Uadia, 2017). Even animal studies have shown that apoptosis is an important modulator of spermatogenesis in normal and

pathological conditions (Xu et al., 2016). During apoptotic events in the mitochondria, there is a release of caspase activator- cytochrome c. Cytochrome c is a haem containing protein that resides in the space between the outer and inner membranes of the mitochondria where it transports electron from complex III to complex IV in the respiratory chain (Said and Khosravi, 2012). The higher activity levels of caspase 3 and cytochrome c in normospermic but infertile males may be an indication of increased apoptotic activities in these subjects.

Studies have reported that, the increased generation of reactive oxygen species may induce functional defects by inducing necrotic apoptosis which is accompanied by activation of caspase (Leist and Jaattela, 2001; Liu et al., 2015; Xu et al., 2016). We observed no significant difference in the levels of total antioxidant capacity between the infertile and control subjects. It is possible that the subjects were on antioxidant supplements prior to sample collection. In a previous study, apoptosis was significantly correlated with ROS within infertile subjects in the seminal fluid (Doaa and Eman, 2014). In subjects with idiopathic male infertility, a positive relationship was observed between sperm damage by ROS and higher levels of cytochrome c and caspase 3 (Wang et al., 2003).

A negative correlation was observed between caspase, cytochrome c and sperm morphology in normospermia but infertile subjects indicating that increase caspase and cytochrome c activities adversely affect sperm morphology. Caspase 3 and cytochrome c activities were significantly higher than those subjects with primary infertility than secondary infertility. This is an indication of more

fragmentation and apoptosis in subjects with primary than secondary infertile males.

In conclusion, seminal fluid caspase 3 and cytochrome c activities were higher in normospermic but infertile males than control subjects. This was shown to have positively correlated with sperm morphology.

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