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Research Paper

Fructose and Nitric oxide's Roles in the Development of Idiopathic Male Infertility

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ABSTRACT

The present research aimed to examine the involvement of fructose and nitric oxide in the development of idiopathic male infertility, while also exploring the potential correlation between their concentrations and sperm motility as well as pathological alterations. The research included the examination of sperm and blood samples from a total of 56 men diagnosed with asthenozoospermia and 30 men diagnosed with oligozoospermia. The control group consisted of a total of 20 essentially healthy individuals. The diagnosis is established by the morphological analysis of sperm. Nitric oxide and testosterone levels in the blood serum, as well as the fructose content in the semen of fertile and infertile men, were also measured. The content of fructose in sperm fluid has a significant association with the decline in both sperm count and motility, as shown by the observed values of 300.5±10.0 mg/dl in patients with asthenozoospermia and 388.6±10.0 mg/dl in patients with oligospermia. The study observed a significant rise in nitric oxide concentration by 25.0% ($p<0.001$) in individuals diagnosed with asthenozoospermia (135.1±12.5 nmol/l) compared to those diagnosed with oligozoospermia (108.1±3.9 nmol/l). This finding suggests that elevated levels of nitric oxide may contribute to a reduction in sperm motility. It is important to acknowledge that although there was no significant alteration in testosterone levels among those with oligozoospermia, there was a tendency for an increase in testosterone concentration among those with asthenozoospermia. The primary sign of a decline in the quantity and mobility of spermatozoa is an elevation in fructose content. An elevation in nitric oxide levels results in a rise in the quantity of morphologically modified pathogenic spermatozoa, subsequently resulting in a reduction in their functional capacity.

KEYWORDS: Fructose, Idiopathic Male Infertility, Nitric Oxide, Testosterone.

INTRODUCTION

Infertility is a significant socio-medical issue. Based on statistics provided by the World Health Organisation (WHO), the global prevalence of infertility is estimated to be 15% [1]. Based on demographic statistics, the global prevalence of infertility among couples is estimated to include around 190 million individuals. Additionally, some writers have reported that the incidence of infertility among couples, in their reproductive years, ranges from 8% to 12% [2,3]. Female infertility makes up 50% of the major causes of infertility, whereas male infertility makes up 30%–40%. Therefore, in around half of infertile couples, the male factor is a contributing element. Idiopathic infertility, also known as unexplained infertility, is the term used to describe infertility in males in 25–30% of instances [2, 4, 5, 6]. The primary factors considered in evaluating male infertility are the quality,

concentration, and motility of sperm. Disruption of spermatozoa's metabolic activities is one of the primary causes of

male infertility since it results in a drop in sperm quality and motility [7, 8]. In this context, research on the use of biochemical markers in the evaluation of male infertility might be very important both theoretically and practically. Fructose and nitric oxide (NO) have recently received a great deal of attention and have even been included in the list of clinical-diagnostic tests due to their function in the metabolism of spermatozoa. When the percentage of moving spermatozoa in the ejaculate is below 40% and the percentage of forward-moving spermatozoa is below 32%, it is known as asthenozoospermia, which indicates impaired sperm motility. Sperm motility is a significant contributor to male infertility in around 50.5% of instances. This component plays a crucial role in facilitating the movement of spermatozoa into the uterine tube and enabling their entry into the ovum, hence facilitating the process of fertilization [9, 10].

The primary metabolizable energy substrate of semen is assumed to be fructose, which is synthesized in seminal vesicles [11]. While fructose is crucial for spermatozoa's quick and rapid movement during the interface with the egg cell and the acrosomal response phase, glucose supports spermatozoa's long-term motility throughout their functional activity [12]. The increased energy requirement causes the concentration of fructose to drop in an environment where normal spermatozoa are active [13]. Since only motile spermatozoa utilize fructose, it is crucial to identify fructose when making a diagnosis of asthenozoospermia in a patient. Consequently, more spermatozoa with mitochondrial abnormalities are seen during idiopathic asthenozoospermia.

Furthermore, recent research has brought attention to the involvement of reactive oxygen species (ROS) and nitric oxide (NO) in the development of structural and morphological alterations in spermatozoa, particularly about the erection of the male genital organ [14]. Nitric oxide has a crucial role as a mediator in the functional activity of spermatozoa. Nitric oxide has a role in the regulation of cell permeability in testes, is particularly effective in testicular perfusion, activating gonadotropin and facilitating its migration to Leydig cells, maintains accumulation and blood circulation function in myofibroblasts, and controls the production of steroids [15].

Excessive synthesis of nitric oxide leads to gonadal dysfunction, as well as oxidative damage and apoptosis of spermatozoa. NO influences spermatozoa's ability to move controls how tyrosine is phosphorylated in different spermatozoa proteins and modifies the acrosomal reaction. In low concentrations, nitric oxide has a regulatory role in the motility, vitality, and metabolism of sperm. The rise in nitric oxide concentration, resulting from peroxynitrite generation, leads to a reduction in spermatozoa motility, a decrease in protein phosphorylation, and subsequent immobilization of the spermatozoa. Simultaneously, the decrease in intracellular glutathione content results in a diminished efficacy of sperm antioxidants. The disruption of the membrane structure is associated with the release of lytic enzymes, which subsequently cause cellular damage, leading to a decrease in sperm activity [15].

The objective of this research was to investigate the involvement of fructose and nitric oxide in the development of idiopathic male infertility.

MATERIALS AND METHODS

Fructose levels in the semen and nitric oxide levels in the blood serum of individuals with idiopathic male infertility were examined for this reason. The primary components of the research were sperm and blood samples from 106 patients, aged 25 to 35, who had not had intercourse in the previous three to five days. The primary study group consists of 86 infertile patients, whereas the healthy control group consists of 20 of them (29). The determination of sperm parameters was conducted by the microscopic inspection of sperm. Typically, the concentration of spermatozoa in one milliliter of semen should exceed 15 million. If the value of this indication is below 15 million, it is referred to as oligozoospermia, while its absence is referred to as azoospermia. The study participants were categorized into two groups based on the quantity and motility of their spermatozoa: asthenozoospermia (individuals with a normal sperm count but limited forward motion) - 56 individuals, and oligozoospermia (individuals with the number of spermatozoa in 1 ml of sperm <10 million) – 30 people.

The fructose and nitric oxide concentrations in semen were quantified using a colorimetric approach, using the B.I.R.D "semen fructose" and "Total Nitric Oxide and Nitrate/Nitrite" ELISA reagent kit provided by R&D Systems. Using an electrochemiluminescence immunoassay approach on a Cobas e411 analyzer, the testosterone concentration in blood serum was determined (Roche Diagnostics, USA).

The statistical analysis of the findings was done using the Excel-2017 software package. The study involved calculating the average numerical value (M) and average error (m) of the structural characteristic. To assess the extent of difference in the quantitative indicators of fructose and nitric oxide levels between the research groups and the control group, the Wilcoxon-Mann-Whitney nonparametric test was employed. Statistical significance was attributed to differences between groups when $p< 0.05$.

RESULTS AND DISCUSSION

The findings revealed that, in comparison to the control group, the average fructose content rose by 24.6% during asthenozoospermia and by 61.2% during oligozoospermia (p 0.001) (Table 1).

There exists a positive correlation between the decline in spermatozoa count and the rise in fructose concentration. The intake of fructose in the semen likewise declines as sperm activity decreases. Fructose in the semen thus becomes more concentrated. This suggests that the observed decline in fructose content may be attributed to a reduced uptake of fructose by spermatozoa. Therefore, fructose serves as a significant energy source and is used for metabolism and movement. The reduction in fructose content among individuals with asthenozoospermia may serve as a signal for the existence of morphologically modified and less motile spermatozoa. A decrease in fructose intake is seen because of an elevated occurrence of spermatozoa exhibiting morphological alterations and reduced motility. As a result, pathologically altered spermatozoa are less mobile and ingest less fructose [11].

Fructose serves as the primary source of energy for spermatozoa, both in aerobic and anaerobic conditions. The motility of spermatozoa is facilitated by the metabolic energy derived from fructose. The process of generating adenosine triphosphate (ATP) is attributed to oxidative phosphorylation occurring inside the mitochondria. Fructose

has a crucial role as a nutrition for spermatozoa, since it undergoes metabolic processes resulting in the production of lactate and pyruvate. Spermatozoa rely on the glycolytic cycle for ATP production and concurrently generate lactate, which serves as the primary supplier of acetyl-CoA inside the mitochondria. The metabolic process of fructose in spermatozoa takes place through the well-established Embden-Meyerhof glycolysis pathway. The synthesis of lactic acid involves the participation of intermediates such as phosphates of hexoses, triose phosphate, and pyruvic acid. Lactate serves as the primary carbohydrate substrate inside spermatozoa, whereas lactic acid undergoes degradation into carbon dioxide and water via the Krebs cycle, facilitated by the presence of oxygen. The movement of spermatozoa is facilitated by the energy they create. The process of fertilization occurs when motile spermatozoa undergo the acrosomal reaction and successfully penetrate an egg. When glucose is present, fructose is involved in the breakdown of the outer acrosomal membrane, which leads to the creation of the acrosomal reaction [12].

Nitric oxide concentration increased by 2.1 times in patients with asthenozoospermia, and by 64.3% in patients with oligozoospermia compared to the control group. The increase of nitric oxide in patients with asthenozoospermia and oligozoospermia compared to the control group leads to the appearance of pathological spermatozoa that have undergone various morphological changes. A 25.0% increase in nitric oxide in patients with asthenozoospermia compared to patients with oligozoospermia indicates that it plays an important role in the activity of spermatozoa.

When ROS levels are elevated, oxidative stress is known to develop. One of the most prominent ROS representatives is nitric oxide. The spermatozoa's resistance to the impacts of free radicals is weakened due to their tiny size and the fact that they contain a very limited quantity of cytoplasm. Each spermatozoid's membrane includes a considerable amount of unsaturated fatty acids in the form of docosahexaenoic acid (where six double bonds between the methylene groups are not conjugated), which ensures membrane fluidity and sperm motility. Because of the high concentration of unsaturated fatty acids, the membrane is more vulnerable to the impacts of free radicals. The sperm cell has a high energy need, and these cells are abundant in mitochondria. As a result, the mitochondrial membrane is the second target. Because of the free radical action, cell permeability rises, mitochondria enlarge, and spermatozoa motility ceases. Unsaturated fatty acids in the membrane of spermatozoa are changed into saturated fatty acids because of the action of a significant quantity of ROS. Consequently, the spermatozoa become immobile and are unable to assist in fertilizing the ovum. Simultaneously, free radicals can traverse the cellular membrane and induce DNA oxidation, decondensation, and subsequent harm inside the spermatozoa. The DNA helix is then broken by the subsequent apoptosis process, which causes the cell to die. Based on contemporary perspectives, it has been shown that oxidative stress has the potential to contribute to male infertility in around 40% of instances [14, 16, 17, 18, 19].

Numerous biochemical reactions in the tissues of the cavernosum include NO, which is released by nerve tergum or endothelial cells of blood vessels. Nitric

oxide (NO) diffuses from the muscle cells located in the blood arteries, where it binds with the guanylate acetylase enzyme. This interaction results in a significant augmentation of the enzyme's activity. Consequently, the acceleration of cGMP production leads to the activation of protein kinase, so facilitating the phosphorylation of diverse proteins. The intracellular concentration of Ca2+ falls due to the efflux of ions from the cell, leading to an accumulation of Ca2+ in the sarcoplasmic reticulum. Consequently, the relaxation of the sphincter cells in the veins of the genital organ leads to an augmentation in arterial blood flow [20].

Table 1: Sperm motility, Pathological Alterations, Blood Nitric Oxide Concentration, Testosterone Levels, and Fructose Levels in Semen in Individuals with Idiopathic Male Infertility (M±m), min-max.

Patient groups	Spermatozoa Quantity, mln	Forward motion, (%)	Pathological spermatozoa (%)	Fructose, mg/dl	Nitrous oxide (NO), mmol/l	Testosterone nmol/ml
Control group, n=20	64,6±3,1 (20–84)	34,6±0,9 (30–45)	21,2±1,0 (16–32)	241,1±3,9 (217–277)	65,8±7,7 (34-128,3)	12,4±0,7 (6,69-17,2)
Asthenozoospermia, n=56	45,9±2,4 (15–104)	17,9±0,9 (2–28)	28,2±1,3 (14–50)	300,5±10,0 (201–482)	135,1±12,5 (2,8-324,2)	13,1±0,7 (5,69-30,5)
Oligozoospermia, n=30	7,2±0,7 (0,4–13)	7,6±1,0 (0–18)	37,3±2,9 (21–61)	388,6±10,0 (309–526)	108,1±3,9 (86-134)	14,0±1,0 (7,38-32,5)
p	<0,001	<0,001	<0,001	<0,001	0,008	0,491

In patients with asthenozoospermia compared to the control group, testosterone levels did not differ significantly, but its concentration did, rising by 12.9%. The outcomes weren't statistically significant. Testosterone is produced in the Leydig cells situated in the interstitial tissue of the male gonads, and it has a paracrine influence on the seminal ducts through diffusion. Testosterone has a crucial role in both the start and maintenance of the spermatogenesis process [21]. It is well known that a reduction in fructose levels within sperm is associated with a decline in spermatozoa motility under situations of androgen insufficiency. Based on the findings of our research, it has been observed that individuals with oligozoospermia exhibit normal levels of testosterone concentration. Furthermore, patients with asthenozoospermia tend to display a slight increase in testosterone concentration. This correlation suggests that the elevated fructose concentration in sperm is associated with a decline in sperm motility. In the context of elevated nitric oxide levels, the motor activity of spermatozoa is diminished and there is a decrease in fructose utilization, despite spermatogenesis proceeding normally.

CONCLUSION

The primary sign of a decline in the quantity and mobility of spermatozoa is a rise in the concentration of fructose. An

elevation in nitric oxide levels increases morphologically deformed pathogenic spermatozoa, subsequently resulting in a reduction in their functional efficacy. The quantification of fructose and nitric oxide levels has significant relevance in the identification and assessment of idiopathic male infertility.

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