QUANTITATIVE ASSESSMENT OF GLUTOSE-6-PHOSPHATE DEHYDROGENASE ENZYME AMONG INFERTILE MALES IN KHARTOUM, SUDAN

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ABSTRACT
Infertility is defined as inability to achieve conception in a period of one year in a couple, despite regular and adequate unprotected sexual intercourse. The condition affects 15% of couples. Oxidative stress has been attributed to affect the fertility status of males and thus, it has been studied extensively in recent years. Spermatozoa, like any other aerobic cell, is constantly facing the oxygen paradox. Oxidative stress has become the focus of interest as a potential cause of male infertility. Normally equilibrium exists between ROS production and antioxidant scavenging activities in the male reproductive tract. Under physiological condition, spermatozoa produces small amount of reactive oxygen species, which are needed for capacitation, acrosome reaction and fertilization. Objectives: This study aimed to investigate the G6PD level among infertile Sudanese patients.

Materials and Methods: This was an analytical descriptive case control study, included 45 infertile male cases, and 55 healthy recruited as control group. This study was conducted at reproductive health care center in Khartoum, Sudan. Results: All participants were males; the mean age of cases was 36 ± 8 years. Mean of G6PD level was significantly lower among cases than control group.

KEYWORDS: G6PD, Male Infertility, Sudan.

INTRODUCTION
Reproduction (or making a baby) is a simple and natural experience for most couples. However, for some couples it is very difficult to conceive. A man’s fertility generally relies on the quantity and quality of his sperm. If the number of sperm a man ejaculates is low or if the sperm are of a poor quality, it will be difficult, and sometimes impossible, for him to cause a pregnancy.

Male infertility is diagnosed when, after testing both partners, reproductive problems have been found in the male.[1]

The increased formation of ROS has been correlated with a reduction of sperm motility. The link between ROS and reduced motility may be due to a cascade of events that result in a decrease in axonemal protein phosphorylation and sperm immobilization, both of which are associated with a reduction in membrane fluidity that is necessary for spermatoocyte fusion. Another hypothesis is that H2O2 can diffuse across the membranes into the cells and inhibit the activity of some enzymes such as glucose-6-phosphate-dehydrogenase (G6PD). This enzyme controls the rate of glucose flux through the hexose monophosphate shunt, which in turn, controls the intra-cellular availability of nicotinamide adenine dinucleotide phosphate (NADPH). This in turn is used as a source of electrons by spermatozoa to fuel the generation of ROS by an enzyme system known as NADPH oxidase. Inhibition of G6PD leads to a decrease in the availability of NADPH and a concomitant accumulation of oxidized glutathione and reduced glutathione. This can reduce the antioxidant defenses of the spermatozoa and increase peroxidation of membrane phospholipids.[2]

Glutathione Peroxidases Reductase forms an excellent protection against lipid peroxidation of plasma membrane of spermatozoa. It scavenges lipid peroxides thereby arresting the progressive chain reaction of lipid peroxidation. It also scavenges H2O2, which is responsible for the initiation of lipid peroxidation. Glutathione reductase (GRD) stimulates the reduction of glutathione disulfide (GSSG) to reduced glutathione (GSH). This ensures a steady supply of the reductive substrate NADPH to GPX. Glucose-6-phosphate dehydrogenase (G6PD) is required for the conversion of NADP+ to its reduced form, NADPH.[3]
Glucose-6-Phosphate Dehydrogenase (G6PD) is a cytoplasmic enzyme affecting the production of the reduced form of the extra mitochondrial NADP + -Phosphate coenzyme G6PD activity, which is the only source of NADPH. Glucose-6-Phosphate Dehydrogenase acritical enzyme in the pentose phosphate pathway, exhibits diminished activity inadequate production of protective intracellular thiols during oxidative stress. The pentose phosphate pathway is the major generator of reducing power within the red blood cells, which protects erythrocytes against oxidative substances. There is considerable evidence that patients on Hemodialysis are in continuous state of oxidative stress.

The gene encoding G6PD is located in the telomeres region of the long arm of the X chromosome (band Xq28). More than 300 alleles with point mutation in the G6PD gene sequence have been identified.

Diagnosing male infertility problems usually involves General physical examination and medical history. This includes examining genitals and asking questions about any inherited conditions, chronic health problems, illnesses, injuries or surgeries that could affect fertility. The doctor might also ask about sexual habits and about sexul development during puberty.

Semen analysis provide a semen sample by masturbating and ejaculating into a special container at the doctor's office or by using a special condom to collect semen during intercourse. The lab will also check semen for signs of problems such as infections.

Might recommend additional tests to help identify the cause of your infertility. These can include Scrotal ultrasound. This test uses high-frequency sound waves to produce images inside the body. A scrotal ultrasound can help doctor see if there is a varicoceles or other problems in the testicles and supporting structures.

Hormone testing. Hormones produced by the pituitary gland, hypothalamus and testicles play a key role in sexual development and sperm production. Abnormalities in other hormonal or organ systems might also contribute to infertility. A blood test measures the level of testosterone and other hormones.

Post-ejaculation urinalysis. Sperm in urine can indicate the sperm are traveling backward into the bladder instead of out your penis during ejaculation (retrograde ejaculation).

This study aimed to assess the G6PD level among infertile males in Khartoum state, Sudan. And then measuring and comparing the mean of the G6PD level between cases and controls, and correlate its relationship with participant’s age.

### MATERIALS AND METHODS

This was an analytical descriptive case control study, included 45 infertile males and 55 apparently healthy subjects as controls. Infertile male remains for more than 5 years with out achieve conception of couple after make all necessary tests to exclude female infertility. This study conducted at the reproductive health care center in Khartoum, Sudan. Ethical approval was obtained from ethical committee of the faculty of medical laboratory sciences, Alneelain University, and informed consent was obtained from each participant before sample collection, then ethical conduct was maintained during data collection and throughout the research process. All statistical analyses were performed by SPSS software version. Continuous variables were expressed as mean and standard deviation. Comparison between variables was performed with t-test, p-value < 0.05 was considered significant.

#### Method

Five ml of blood samples were taken from each consenting voluntary participant in standard venipuncture into EDTA vacutainer tubes and labeled with unique identification number. Biochemical screening of samples for G6PD were performed at hematology laboratory unit of the Modern Medical Center, Khartoum, Sudan.

G6PD level was determined by Mindary Bs 480 which was already calibrated according to manufacture standardized procedure.

#### RESULTS

All participants were males, the mean age of cases was 36 ± 8 years. Mean G6PD level was significantly lower among cases (838±228.6) than control group (2214±1742.6) (table 1).

<table>
<thead>
<tr>
<th>G6PD</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>838±228.6</td>
<td>(454-1635)</td>
</tr>
<tr>
<td>Controls</td>
<td>2214±1742.6</td>
<td>(469-14361)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.000</td>
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</tr>
</tbody>
</table>

Table. (1) shows statistics and mean differences of G6PD in case and control groups.
DISCUSSION
Oxidative stress has become the focus of interest as a potential cause of male infertility. This study included 45 infertile males their G6PD levels were determined and compared with G6PD levels of 55 apparently healthy males as controls. Mean G6PD level was significantly lower among cases than control group, similar findings revealed by; Williams, A. C. (2001)\[12\] In contrast with our study, Roshankhah S. et al, (2016) revealed that; G6PD does not increase the susceptibility of sperm to oxidative stress induced by H2O2, and the reducing equivalents necessary for protection against H2O2 are most likely produced by other pathways. Therefore, G6PD cannot be considered as major risk factor for male infertility.\[13\]

According to the literature above, increased formation of ROS has been correlated with a reduction of sperm motility. This may be due to a cascade of events that result in a decrease in axonemal protein phosphorylation and sperm immobilization, which they play a role in the reduction of membrane fluidity that is necessary for spermatoocyte fusion, or by that H2O2, which can diffuse across the membranes into the cells and inhibit the activity of some enzymes such as glucose-6-phosphate-dehydrogenase (G6PD).\[13\]

CONCLUSION
This study concluded that mean G6PD level was significantly lower among cases than control group.

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2. Roshan Kumar Mahat, Sudeep Kumar, Manisha Arora, Dhananjay V. Bhale, Rachana Mehta, Jyoti Batra, Role of Oxidative Stress and Antioxidants in Male Infertility, IJHSR 324, March, 2015; 5(3).