Evaluation of Serum α-tocopherol and Anemia among Infertile Patients Attending Specialists Hospital, Sokoto

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

ABSTRACT

Infertility is the inability of a couple to achieve pregnancy over an average period of one year despite adequate, regular and unprotected sexual intercourse. Avitaminosis E has been implicated in the development of infertility and hemolytic anemia in animals. There is, however, little evidence that man is ever scanty in vitamin E. The aim of this study is to evaluate serum α-tocopherol levels and anemia among infertile patients attending Specialists Hospital, Sokoto. A total of fifty (50) infertile patients and fifty (50) apparently healthy fertile married as control were recruited for this study. The blood samples collected were analyzed for α-tocopherol using Hashim and Schuttringer (1996) method, and PCV using hematocrit reader. The data obtained were analyzed using the Student's T-independence test. The result shows that the level of serum α-tocopherol was significantly lower (p<0.05) in infertile patients (0.65±0.04) compared to controls (1.38±0.02). The result, however, shows no statistically significant difference of PCV and BMI (37.58±0.4 and 21.14±0.34 respectively) in infertile patients when compared to the controls (38.61±0.4 and 22.05±2.64 respectively).
1. INTRODUCTION

Infertility is the inability of a couple to achieve pregnancy over an average period of one year (in a woman under 35 years of age) or 16 months (in a woman above 35 years of age) despite adequate, regular (3-4 times per week), and unprotected sexual intercourse [1]. According to American Pregnancy Association, infertility is defined as trying to get pregnant for at least a year without success [2]. Contraception is normally achieved within 12 months in 80 to 85% of couples using no contraceptive measures [3].

In primary infertility, the woman has never conceived despite combination and exposure to sexual intercourse for at least two years, while in secondary infertility, the woman has previously conceived but subsequently unable to conceive despite combination and exposure to sexual intercourse for a period of two years [1].

Tocopherol was derived from two Greek words: tokos (=offspring) and phenol (=to bear). Tocopherol, therefore, literally means to bear children. This satisfactorily suggests the involvement of tocopherol in fertility [4]. Alpha-tocopherol, a potent antioxidant vitamin that protects cells damage from free radicals was designated as antisterility factor on account of the development of sterility in its deficiency [5].

The characteristic symptoms of experimentally-induced vitamin E deficiency vary from animals to animals. In mature female rats, sterility develops because of absorption of a fetus after conception. While in males, the germinal epithelium of the testes degenerates and spermatozoa become non-motile. Avitaminosis E has also been implicated in the development of hemolytic anemia in monkeys [5].

Global estimates suggested that nearly 72.4 million couples experience fertility problems [6]. World Health Organization [7], estimated that between 8% and 12% of couples experienced some form of infertility during their reproductive lives. Thus, affecting 50 to 80 million people worldwide, out of which 20 to 35 million couples in Africa are expected to experience this problem. This can be extrapolated to 3 to 4 million Nigeria couples suffering from infertility [8]. In Africa, the prevalence of infertility is higher particularly in Sub-Sahara Africa ranging from 20% to 60% [9]. An estimate of 19% infertile couples in Ile-Ife [10] and 15% from Usman Danfodiyo University Teaching Hospital, Sokoto has been reported [11]. There is, however, little evidence that man is ever deficient in the α-tocopherol. The aim of this study is to determine the serum levels of α-tocopherol and anemia among infertile patients attending Specialists Hospital, Sokoto.

2. MATERIALS AND METHODS

2.1 Chemicals and Equipment

All chemicals and equipment used are of analytical grade. The chemicals used include xylene, ethanol, α-dipyridyl and ferric chloride. The equipment used include bench-top universal centrifuge, spectrophotometer, Bench-top microhematocrit centrifuge, and hematocrit reader.

2.2 Study Population

A total of one hundred (100) subjects were recruited for this study. They consist of fifty (50) apparently healthy married fertile subjects as control and fifty (50) infertile patients attending Specialist Hospital, Sokoto.

2.3 Ethical Consideration and Clearance

The approval for this study was sought and granted from the ethics and research committee of the Specialists Hospital, Sokoto prior to the commencement of the study. The ethical clearance number of the study is SHS/HREC/2016/446.

2.4 Sampling Techniques

The arrangement was made with the clinicians where those that satisfy the study inclusion criteria were selected. The nature and reasons for the study were explained fully to the subjects in the appropriate language. Subjects consent was our priority and was obtained with their full consent.
history. Specimen collection was made and findings were documented in the proforma.

2.5 Anthropometric Measurements

The standard procedure [12] was employed for anthropometric measurements. Body mass index (BMI) was determined using the weight in kilogram (kg) divided by the square of the height in meters. The values of 20-25, <30 but >25, >30 and <20 were considered as normal, overweight, obese and underweight respectively.

2.6 Analytical Techniques

Serum α-tocopherol was estimated using the Hashim and Schuttringer method [13], while the pack cell volume (PCV) was read using a microhematocrit reader [14].

2.7 Statistical/Data Analysis

The analysis of the data obtained was treated accordingly using Grap and Instat3 © (2008) Statistical package. The results were expressed as Mean ± SEM. Paired comparisons were carried out using the Student's T-independence test. A P-value ≤0.05 were considered statistically significant.

3. RESULTS

The Socio-demographic characteristics of the study subjects are represented in Table 1. They consist of fifty (50) infertile patients and fifty (50) apparently healthy married fertile as controls, with mean age and BMI of (36.93±0.57 and 21.14±0.34) and (34.08±0.15 and 25.25±2.64) for patients and controls respectively. Table 2 shows serum α-tocopherol and BMI among patients and control. The result shows that the serum α-tocopherol of the patients were significantly lower (P<0.05) (0.65±0.04) than the controls (1.38±0.02). Table 3 presents pack cell volume (PCV) and body mass index (BMI) among patients and controls. The result showed no statistically significant difference (p>0.05) in PCV of infertile patients when compared to controls. Table 4 correlates α-tocopherol and PCV of the study subjects. Statistically, significant difference was observed (P<0.05) between the serum level of α-tocopherol of the patients when compared with controls.

### Table 1. Demographic and clinical characteristics (Mean±SEM) of the study subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>N</th>
<th>Age (yrs)</th>
<th>BMI (kg/M²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>50</td>
<td>34.08±0.15</td>
<td>25.25±2.64</td>
</tr>
<tr>
<td>Male</td>
<td>30</td>
<td>34.30±0.36</td>
<td>23.54±0.60</td>
</tr>
<tr>
<td>Female</td>
<td>20</td>
<td>33.94±0.10</td>
<td>26.31±4.26</td>
</tr>
<tr>
<td>Patients</td>
<td>22</td>
<td>35.75±1.21</td>
<td>20.47±0.64</td>
</tr>
<tr>
<td>Male</td>
<td>28</td>
<td>37.31±0.65</td>
<td>21.36±0.40</td>
</tr>
<tr>
<td>Female</td>
<td>20</td>
<td>33.94±0.10</td>
<td>26.31±4.26</td>
</tr>
</tbody>
</table>

N= Number of subjects, BMI= Body Mass Index, yrs= Years, Kg= Kilogram, M= Meter

### Table 2. Serum α-tocopherol and BMI (Mean±SEM) of the study subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>N</th>
<th>Age (yrs)</th>
<th>α-tocopherol (µmol/L)</th>
<th>BMI (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>50</td>
<td>34.08±0.15</td>
<td>1.38±0.02</td>
<td>22.03±2.64</td>
</tr>
<tr>
<td>Patients</td>
<td>50</td>
<td>36.03±0.57</td>
<td>0.65±0.04</td>
<td>21.14±0.34</td>
</tr>
<tr>
<td>P-Value</td>
<td>&gt;0.05</td>
<td>&lt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

N= Number of subjects, BMI=Body mass index, α=Alpha, Kg= kilogram, M= meter, µmol/L= micromole per liter, yrs=Years

### Table 3. Pack cell volume and BMI (Mean±SEM) of the study subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>N</th>
<th>Age (yrs)</th>
<th>PCV (%)</th>
<th>BMI (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>50</td>
<td>34.08±0.15</td>
<td>38.61±0.4</td>
<td>22.03±2.64</td>
</tr>
<tr>
<td>Patients</td>
<td>50</td>
<td>36.03±0.57</td>
<td>37.58±0.4</td>
<td>21.14±0.34</td>
</tr>
<tr>
<td>P-Value</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

N= Number of Subjects, BMI=Body Mass Index, %= Percentage, α=Alpha, µmol/L=Micromole Per Liter, PCV=Pack Cell Volume
4. DISCUSSION

Many studies have demonstrated the damaging effects of elevated free radicals (reaction oxygen species) to sperm function. Increasing vitamin E supplement in the hope to prevent the oxidative damage was a proposed solution. In the past decade, a number of studies were undertaken to ascertain whether such a proposal is truly helpful. Unfortunately, the few studies, the small sample size, and conflicting data have made it difficult for clinicians and researchers to agree on a recommendation. However, the existing studies do seem to be encouraging [15].

Oxidative stress, decreases antioxidant capacity and impaired sperm mitochondrial functions are the main factors contributing to infertility [16]. In this study, a significantly lower level of serum α-tocopherol was observed in infertility compared to control. This is similar to the study conducted by Serena et al. [17], who shows a significant decrease in serum α-tocopherol level in infertile patients when compared to control. This is, however, in contrary to the study of Sasikumar et al. [18], who reported an increased level of serum α-tocopherol in the test group in comparison with the controls.

Over the last decade, intensive research has been focused on various antioxidants and their optimal doses and combinations, for more effective and safe treatment of human fertility disturbances [15]. Although, reaction oxygen species (ROS) have been shown to be required for sperm capacitation, hyperactivation, and sperm-oocyte fusion [19], excessive levels of ROS can negatively impact sperm quality [20]. Improvement of sperm parameters after antioxidant therapy of infertility may result in higher pregnancy rate [21].

In a number of studies, it has been shown that oral supplement of Vitamin E significantly improved sperm motility [22]. There has been existing evidence that suggests a relationship between daily antioxidant intake and better semen quality among healthy men. Semen analysis was performed on 97 healthy male volunteers and results were correlated with the results of a dietary assessment questionnaire [2]. Higher levels of vitamin E intake were associated with higher levels of progressive sperm motility [2].

5. CONCLUSION

A reduced serum α-tocopherol in infertile patients was observed in this study. The α-tocopherol has been described to be an antisterility factor on the account of the development of sterility in its absence. We, therefore, commend the incorporation of serum α-tocopherol for both diagnosis and treatment of infertility in humans.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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