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COULD THERE BE AN ASSOCIATION OF SERUM ALBUMIN ON SEMINAL PARAMETERS?

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AUTHORS' CONTRIBUTIONS
This work was carried out in collaboration between all authors. Author DALM designed the study, wrote the protocol, collected, analyzed and interpreted the data. Author HW contributed in supervision and clinical supporting. Authors BW and JL contributed in supervision and technical support. All authors read and approved the final manuscript.

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ABSTRACT

A cross sectional study (n = 51) was carried out on written consent in a fertility clinic of Sri Lanka during the period of August 2014 to April 2016 to find out whether there is a relationship of serum albumin concentration with seminal parameters such as seminal volume, count, morphology and motility. The study was aimed to find out the biological causes for male infertility due to the fact that the male infertility has become increased in the modern society irrespective of the economic situation. The selected subjects were evaluated for seminal analysis and serum albumin analysis with WHO method of semen analysis and BCG dye binding method of albumin analysis respectively. The relationship between the independent (serum albumin) and each dependent (seminal parameters) was analyzed with Spearman correlation test statistically. Under the outcome, except others the seminal volume was positively correlated to the serum concentration of albumin ($P = 0.005$, $r_s = 0.572$). Anyway, this could be due to the composition of seminal volume which compose most of the protein and amino acids such as enzymes as well as coagulators. Even though some clinicians consider the seminal count and motility as the most important parameters which decide the quality of semen, seminal volume also plays a certain role in the function. Thus, it's important to maintain the serum level of albumin in the normal reference range to have better quality semen, hence fertility.

Keywords: Relationship; serum albumin; semen parameter; male infertility.

1. INTRODUCTION

Male factor infertility, which contributes 30-40% for the infertility [1] has become a psychosocial issue in the modern society. The fertility of men manly depends on the quality of seminal parameters such as seminal volume, count, motility and morphology. The deviation of these parameters from their normal reference range could cause poor quality semen, which ultimately give rise to male infertility. Various conditions and situations can cause for poor quality semen. Varicocele, infections (gonorrhea), hormonal

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imbalance (low level of testosterone), lifestyle factors (obesity, smoking), environmental factors (radiation, heat) are some of the examples for them [2,3]. Anyway it's considered that mostly the male infertility is idiopathic. The association of biological factor with male infertility is poorly attended. Thus, the study was set up to fill the gap to a certain extent. Thus, in the present study it was setup to find out the association between serum protein and seminal parameters.

The protein including albumin is required for the semen as a structural compound of spermatozoa. Further, albumin may act as an antioxidant component, which can prevent the sperm from oxidative attack. Thus, the morphology as well as motility of spermatozoa could be maintained. Furthermore, the semen is inclusive of albumin and amino acid that is secreted from prostate and seminal vesicles. Anyway, as the accessory glands received their nutrients from blood, the volume of semen may be indirectly dependent on the content of serum protein (albumin). Even though the studies carried out on the topic are not reported, a fairly similar study which was carried by Elzanaty et al. [4], had found that the seminal albumin concertation (not the serum albumin) was positively associated with the semen count and morphology. Anyway in the particular study a negative relationship was seen between semen albumin and seminal volume. However, the body fluid component of the Elzanaty et al. study is different from the present study.

2. METHODS AND METHODOLOGY

All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

The ethical approval was obtained from the ethic review committee of the institute where the study was carried out.

2.1 Design: Cross Sectional Study [5]

The cross sectional design was selected due to the reasons such as;

Ability to access many outcomes and risk factors

Inexpensive feature

Less time consumption

Possibility of generating hypothesis to build up relevant studies.

2.2 Method

The male partner of infertile couples who visited the fertility clinic of an institute of Sri Lanka during the period of August 2014 – April 2016 was involved in the study. The individuals who wished to take part in the study were evaluated on exclusive and inclusive criteria on the consent.

Inclusive criteria:

(1) All the male, who were over 18 years old.

Exclusive criteria [6,7,8]:

(1) Individuals, who had been suffering from systemic diseases such as diabetes, hypertension, cancer, arthritis during the period (the conditions and the drugs used for could affect the reproductory hormones and sperm production in the testis).
(2) Individuals, who had been on drugs relevant to above disease conditions.
(3) Individuals, who had addicted to recreational drugs such as marijuana, abin and ganja (chemicals in the drug could cause negative effect on the sperms synthesis).
(4) Individuals, who had been on anti-gastric drugs such as cimetidine or any steroidal drugs (increase the hormone, prolactin which negatively affect on sperm production).
(5) Individuals, who were with pathological issues in reproductive system (varicocele, testicular problems, varicocele may raise the temperature in the area which could be unfavorable for the production of sperm).
(6) Individuals, who were unable to communicate (dumb, deaf and mentally handicapped).
(7) Individuals, who were on fertility treatment at the time (the semen quality could be changed on the ongoing treatment)
(8) Individuals, who were unwilling to participate in the study.

The subjects who were satisfactory according to the criteria, were selected for the study and interviewed orally to gather the data such as age, residence, lifestyle behaviors (smoking, food pattern, alcoholism, exposures to rays and heat) which could affect the synthesis of sperm anyway.

Then, the subject were asked to provide a semen sample (3 days abstinent from ejaculation).

Finally, a blood sample was collected in to a plane tube from the subject under well aseptic condition.

2.2.1 Measuring of serum albumin concentration [9]

Bromo cresol green dye binding method (BCG dye binding method) was sued as the method of detecting the serum albumin concertation due to the facts of rapidity, cost effectiveness and high accuracy over the other methods (salt fractionate and cellulose acetate methods) [10].

The basic concept of the dye binding method is to form a soluble, colorful dye-albumin complex which is quantified spectrophotometrically.

In the experiment BCG was selected as the dye due to high specificity and sensitivity toward albumin [11]. Further, in the BCG method solubility of the complex is also increased by introducing brij 35 which is a surfactant.

2.2.2 Preparation of reagent for Bromo cresol green (BCG) dye binding method [9]

2.2.2.1 Sodium hydroxide/NaOH (1 M)

Sodium hydroxide, 2 g was measured and placed in a 50 ml of volumetric flask. The amount was dissolved with a small volume of distilled water and the final volume was adjusted to 50 ml by adding distilled water while mixing the solution.

2.2.2.2 Brij solution (30 g/dl)

Brij, 1.5 g was dissolved in a small volume of hot distilled water in a volumetric flask (5 ml) and the final volume was adjusted up to 5 ml by adding distilled water while the mixing continues.

Finally, the prepared Sodium hydroxide, 25 ml was placed in a 1 liter of volumetric flask and 5.6 g of Succinic acid and 56 mg of BCG dye were added to the flask respectively. Compounds were mixed well together and total volume was adjusted upto1 liter by adding distilled water while the mixing continues. pH of the prepared solution was adjusted to 4.15 with 1 M NaOH. Finally 100 mg of Sodium azide and 3.5 ml of freshly prepared Brij solution also were in cooperated to the same solution to make BCG solution.

2.2.3 Measuring of albumin level of serum

2.2.3.1 Standard (4 g/l)

Standard serum, 0.2 ml was measured and dissolved in 1.8 ml of distilled water. Then, a 0.1 ml of the dissolution was placed to a separate tube and mixed up with 2.5 ml of BCG solution. The mixture was kept in the room temperature for 10 mints and absorption was measured at 630 λ.

2.2.3.2 Test

0.1 ml of direct test serum was treated on the same way as above without any dilution and the absorption was measured eventually after 10 mints.

Each sample was duplicated and the average was obtained

The serum concertation of albumin was obtained under following equation

Absorption of test sample / Absorption of standard sample X Concentration of standard

A QC (quality control) sample (a serum of known concentration of albumin) was run with the test procedure several times and a Levey-Jennings chart was drawn to check the accuracy of the test procedure.

2.2.4 Analysis of semen volume [12]

After the liquefaction was taken place, the volume of semen was measured with 10 ml of measuring cylinder.

2.2.5 Analysis of sperm count

The liquefied semen mixture was gently shaken to mix the specimen and using a Sahli pipette semen was drawn up to 0.5 micro liter mark. Then the semen diluting fluid was placed up to 11 micro liter mark and placed the pipette on a rotator to mix the interior contents well.

Thereafter, the Improved Neubauer counting chamber was loaded with the mixture and allowed the sperm to settle in. Eventually, the number of sperms in four corner squares was counted.

$$\text{Number of sperm/ml} = \frac{n \times 10 \times 20 \times 1000}{4}$$

n = number of sperm counted in all four corner squares

2.2.6 Analysis of sperm motility

A drop of liquefied semen (10 µl) was placed on a clean slide and covered with a coverslip and rimed the edge with petroleum jelly to prevent evaporation. It was observed the proportion of motile to non-motile sperms under high power field (× 40) in several

microscopic field to obtain the average percentage of motile sperm.

2.2.7 Analysis of sperm morphology

A drop of liquefied semen (10 µl) was placed on a clean slide and made a thin smear and the smear was air dried. The dried smear was washed thoroughly with semen diluting fluid to remove the mucous. Then the smear was covered around 8 mints with the diluted Leishman stain which was prepared by mixing 10 ml of stain and 20 ml of distilled water. Thereafter the stain was washed off well with buffered distilled water. Finally, the slide with stained smear was made to dry. The slide was observed for morphology under high power field and the ration of normal to abnormal spermatozoa was observed in different microscopic fields to have the final average percentage of normal spermatozoa.

On the seminal analysis, individuals with normal seminal parameters were categorized as nomospermic group and ones with either one more defective seminal parameters were categorized as non nomospermic group

3. DATA PROCESSING AND STATISTICAL METHODS

All the results were subjected to normality test and non-normal distribution was indicated. Thus, non-parametric test was used in the analysis of results. The each average serum albumin values of the two groups such as non normospermic and normospermic was compared under Wilcoxon signed rank test. Further, the albumin values of whole group was compared with their relative and respective seminal values separately with Spearman correlation test and Linear regression analysis. All the statistical analysis were done with the IBM SPSS 20 versions.

4. RESULTS AND DISCUSSION

4.1 Quality Control for the BCG Dye Binding Test Method

Mean	= 4.47 g/dl
Median	= 4.50 g/dl
Standard deviation	= 0.12 g/dl
Coefficient of variance	= 2.69%
The value of quality control standard	= 4.50 g/dl

According to the graph, the quality control results have clustered within the mean ± 2 SD (4.22 – 4.70 g/dl) with little variation in the upward and downward direction. Further mean is nearly equal to the median and the coefficient variance has been less than 5%, all which indicate the accuracy of the test procedure.

In the whole study group the average serum albumin value was 4.07 ± 0.32 g/dl (2.33 – 4.76 g/dl) and almost all except one individual (2.33 g/dl) were within the normal reference range of 3.5 – 5.5 g/dl.

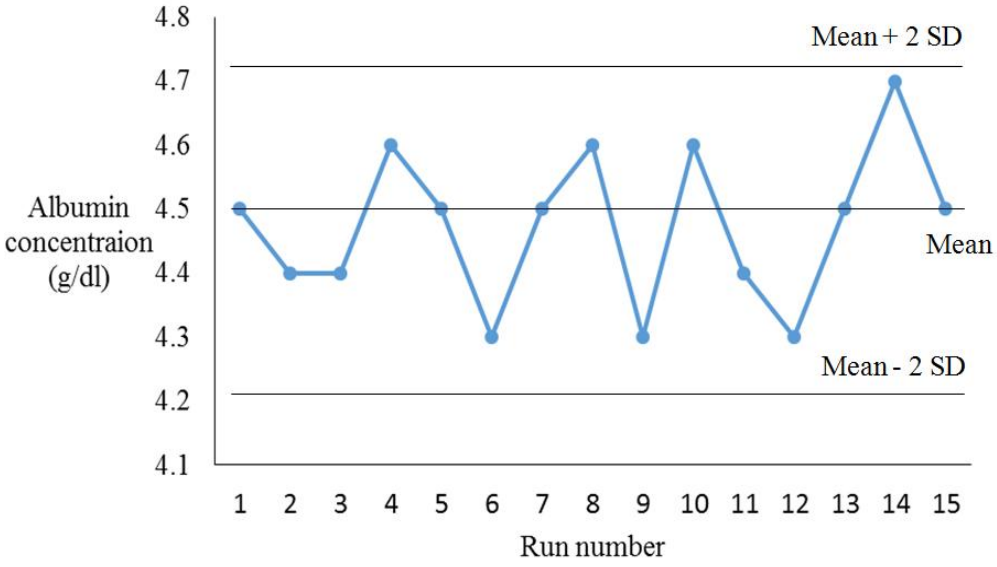

Fig. 1. Levey-Jennings chart for quality control

Table 1. The average serum albumin levels of the two groups of individuals with normal and abnormal seminal parameters

Type of body fluid	Average serum albumin concentration (g/dl)		P value (Wilcoxon signed rank test)
	Group with normospermic parameters containing individuals (n = 21)	Group with abnormal seminal parameters containing individuals (n = 30)	
Serum	4.24 ± 0.23	3.98 ± 0.35	P < 0.05

Table 2. Model summary of the analysis

Model	R	R square	Adjusted R square	Std. error of the estimate
1	.390[a]	.152	.134	1.33390

The average serum albumin levels of the two groups with normal seminal parameters and non-normal seminal parameters were compared according to the Wilcoxon signed rank test and found to have a significant increase ($P < 0.05$, $P = 0.01$) of the particular value in the group with normal seminal parameters.

The average age and BMI of both groups was 35 years and 25 Kg/m^2 respectively. Exposures to mobile rays, heat rays and Food habits of both groups were fairly similar and individuals with extremely high and low seminal parameter values were removed to have a smooth outcome.

Anyway, since the average albumin concentrations of both group were within the normal reference range, the increase cannot be considered as a true value.

4.2 Spearman Correlation Test

Under the test which measures the strength of association between two ranked variables, it was found that the serum albumin level was positively related to the semen volume ($P < 0.05$, $P = 0.005$, $r_s = 0.572$). No significant relationship was seen between the serum albumin and other parameters such as sperm count, motility and morphology.

4.3 Linear Regression Analysis

The multiple linear regression analysis was run considering each seminal parameter as dependent variables and serum albumin level as the independent variable and found to have a significant moderate linear association with semen volume ($r = 0.39$, $r^2 = 0.15$, $p < 0.05$). Thus, among the factors which affect the level of seminal volume the serum albumin is occupied by 15%.

According to the whole result the level of serum albumin, which indicates the level of nutrition of human beings had a positive association with the volume of semen. However, it's obvious that the serum albumin has a significant positive relationship with the seminal volume. This could be due to the fact of occurrence of numbers of protein in the semen fluid such as enzymes and structural proteins, which assists in expanding the volume. Thus, semen volume indirectly depends on the serum albumin.

As it is rare to find the similar studies on the topic, it is difficult to compare the outcome of the present study with the same of other studies. However, certain few other studies were carried out to find the relationship between the seminal level of albumin and semen parameters. A study (n=916) which was carried out with the mentioned objective, had enclosed that there was a positive relationship between the seminal albumin and sperm count as well as morphology. Anyway, an opposite relationship was seen with the seminal volume [4]. Nevertheless, the study had investigated semen instead of serum, which was little different from the objective of the present study.

Sometimes to have such a relationship in the study of Elzanaty et al. [4], the higher level of seminal protein/albumin might have affected negatively the technical accuracy of measuring of volume of semen by making the semen into a semi solid mass.

5. CONCLUSION

The serum level of albumin has a positive relationship with the seminal volume. This could be due to the composition of semen, which consist of a bulk amount of proteins and amino acids. Nevertheless, as the volume plays a less important role in male infertility compared to the sperm count and motility, the outcome of the result is important to improve the fertile ability of men who are with proffer count and motility including less volume. Anyway, it's better to carry out more and more studies considering the present study as a platform.

Further, the power of this study is limited by the relatively small overall sample size. This could be due to the strict adherence to exclusive criteria. The outcome is dependent on the exclusive criteria, hence the data gathered from the subjects.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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