

Study the effect of some biochemical enzymes at seminal plasma of infertile patients

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SUMMARY

AUTHORS' CONTRIBUTION: (A) Study Design - (B) Data Collection. (C) Statistical Analysis - (D) Data Interpretation - (E) Manuscript Preparation - (F) Literature Search - (G) Funds Collection

Current study included 49 semen samples collected from sub-fertile men, after the abstinence period (3 to 5 days); it was conducted in the laboratory of Al- Imam Al- Hussein at Karbala province for six month from 1/1/2023 to 1/6/2023

The purpose of this research was to evaluate the correlation between LDH activity in the seminal plasma of infertile men and some sperm parameters, and detected activity level of LDH in different sub-fertile patients. The results revealed significant increase ($P < 0.05$) in oligozoospermia compared to normospermia and Asthenospermic, also revealed a significant negative correlation ($P < 0.05$) between progressive motility percent and lactate dehydrogenase activity in asthenozoospermic men.

Concluded: No significant correlation between sperm concentration and lactate dehydrogenase activity, the significance of LDH activity to detecting the capacity of gland function.

INTRODUCTION

Seminal plasma is a liquid compound, which serves as a medium for moving extrusion spermatozoa from the testis to oocyte in the female reproductive canals [1]. Seminal plasma not only transports the spermatozoa but also equips the protection and nutrition to the spermatozoa in the female reproductive system [2].

Seminal plasma comprises of various biochemical components, such as cholesterol, glucose, proteins, antioxidant, mineral elements and enzymes [3]. These enzymes are vital components of seminal plasma and have important roles in the formation, activity and functions of spermatozoa. The levels of enzymes in the human seminal plasma are very important for the spermatozoal function [4].

One of these enzymes is lactate dehydrogenase enzyme is present in almost cells of the body and create only in the cytoplasm of the cell, the activities of this enzymes are existing in the several organs and tissues [5,6]. Lactate dehydrogenase is a hydrogen transmission enzyme that catalysis the oxidation of L-lactate to pyruvate with Nicotinamide-Adenine Dinucleotide (NAD)⁺ as hydrogen acceptor, the last step in the metabolic chain of anaerobic glycolysis [7]. Lactate dehydrogenase in the testis has important role because the interaction in the spermatogenesis and the activity of spermatozoa [8]; Also, it is present in the seminal plasma and have main role in providing energy for cell metabolism [9].

Seminal plasma enzymes are interacting with the presence or absence of spermatozoa and male infertility; many studies have been done for assessment of these enzymes activities in the seminal plasma and the relationship with infertility [10]. Male infertility caused by several reasons such as congenital malformations or external factors such as smoking, alcohol, medicines and anabolic steroids, other reasons idiopathic have ratio is about (40-50%) [11]. Infertility patients can be divided into several factors as sperm concentration, motility, and morphological abnormal sperm [12].

MATERIAL AND METHODS

Semen specimens' collection

The samples were collected from patients *via* masturbation after 3-5 days of sexual abstinences in the specialized sanitary container, then the specimens liquefy at 37C°, and the seminal fluid analysis was performed to detect the sperm parameters. According to the seminal fluid analysis the infertile patients classified into

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normospermia 8/49 samples, oligospermia 9/49 samples and asthenospermia 32/49 samples [12].

Sample preparation for examination: One droplet (10 µl) of sample was taken after liquefies and putting on the warm glass slides, covered by cover slip (22×22 Mm) and was examined under 40X power by the light microscope for assessment the sperms parameters which includes sperm concentration, 4ZL sperm percent motility, sperm viability percent, round cell concentration, morphological abnormal sperms and agglutination [12].

Sperm concentration: The concentration was determined from the average of the sperms number in ten random fields and multiplies by the factor 106. The count of sperms calculated by multiplying the concentration with the volume of ejaculate. The patient called oligozoospermia when sperm concentration less than 15 million sperm per ml [12].

Sperm motility: At least 200 sperms were calculated from each sample, then were recorded an average of motile sperms, which owns progressive forward movement, also was examined the percent of the sluggish motile, topical motile and immotile sperms [12].

It was classified according to world health organization and as below:

- A= progressive motility
- B= slow or sluggish progressive motility
- C= Immotile.

LDH ASSESSMENT

Lactate Dehydrogenase (LDH) activity: The concentration of lactate dehydrogenas enzyme was measured in serum of blood of 49 mens by observing the concentration of lactate was formed from reduced pyruvate by Lactate Dehydrogenase (LDH) at PH 7.4, temperature 30 °C using colorimetric method according

to the manufacture instruction (Randox Laboratories, England)

Pyruvate is reduced to lactate by Lactate dehydrogenase (LDH) at PH 7.4 and at 30 °C. The progress of the accompanying oxidation of NADH to NAD⁺ is monitored continuously by measuring the rate of absorbance decrease at 340 nm in a

Statistical analysis: It was done by using statistical package for social science (SPSS) (the version 22.0) according to Mallery P, et al. [13].

RESULTS

The estimation of LDH activity in seminal plasma of infertile patients showed significant increase (P<0.05) in oligospermia compared to normospermia and asthenospermia (Fig. 1.).

The results revealed positive correlation between progressive motility percent and lactate dehydrogenase activity in the group of oligospermia (Fig. 2.).

While, results revealed negative correlation between sperm concentration and LDH activity in oligospermic patients (Fig. 3.).

Also results showed negative correlation between progressive motility percent and lactate dehydrogenase activity in normospermic men (Fig. 4.).

And results showed positive correlation between sperm concentration and lactate dehydrogenase activity in normospermia (Fig. 5.).

The results revealed significant negative correlation between progressive motility percent and lactate dehydrogenase activity in asthenospermic men (Fig. 6.).

Also results showed negative correlation between sperm concentration and lactate dehydrogenase in asthenospermic patients (Fig. 7.).

Fig. 1. Lactate dehydrogenase activity levels different letters mean significant differences (mean ± S.D.).

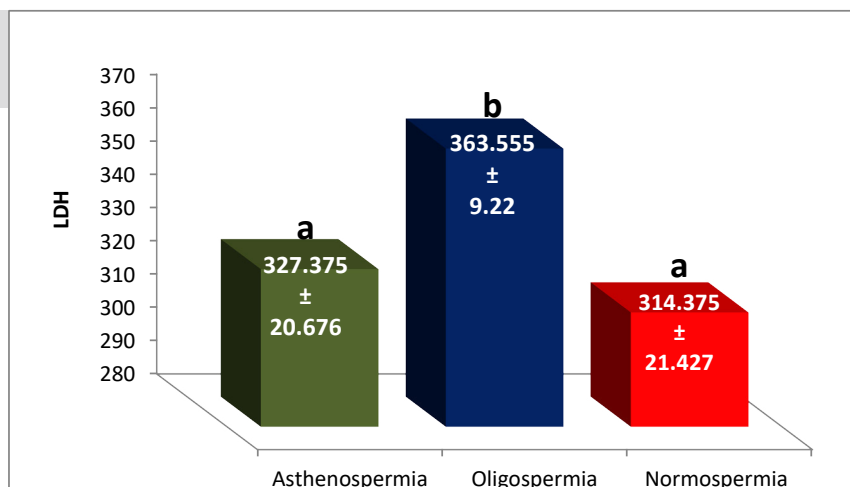


Fig. 2. The correlation between progressive motility percent and LDH activity in oligospermia.

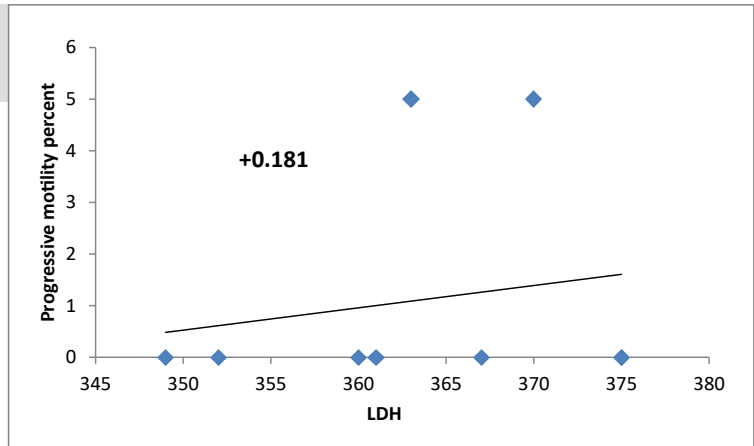


Fig. 3. The correlation between sperm concentration and LDH activity in oligospermia.

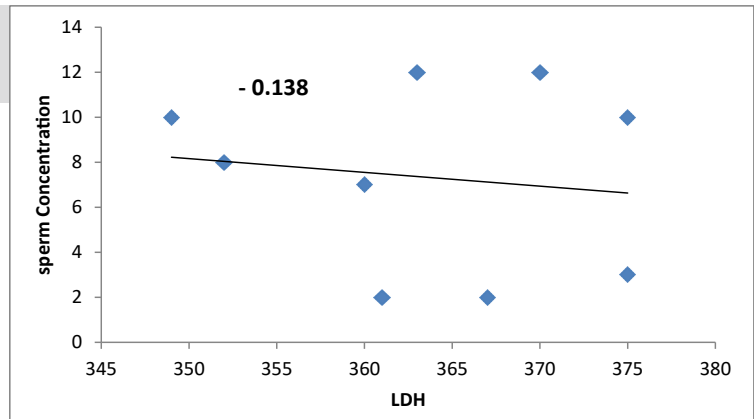


Fig. 4. The correlation between progressive motility percent and lactate dehydrogenase activity in normospermia.

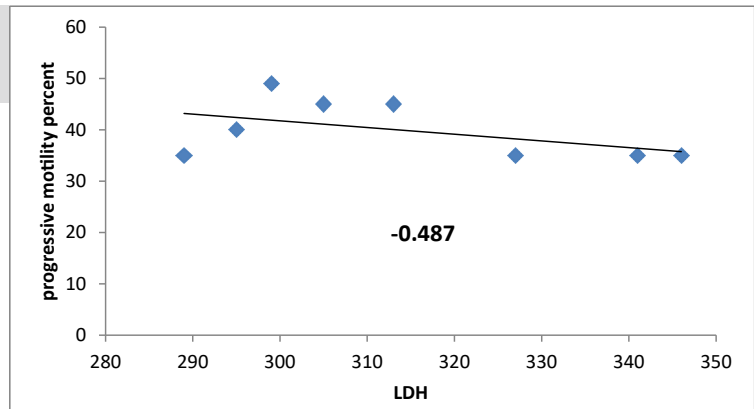


Fig. 5. The correlation between sperm concentration and lactate dehydrogenase activity in normospermia.

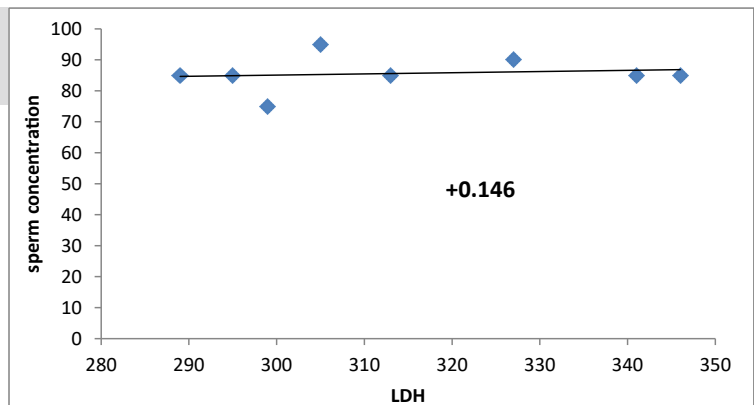


Fig. 6. The significant negative correlation between progressive motility percent and lactate dehydrogenase activity in asthenospermia (** Correlation is significant at the 0.01).

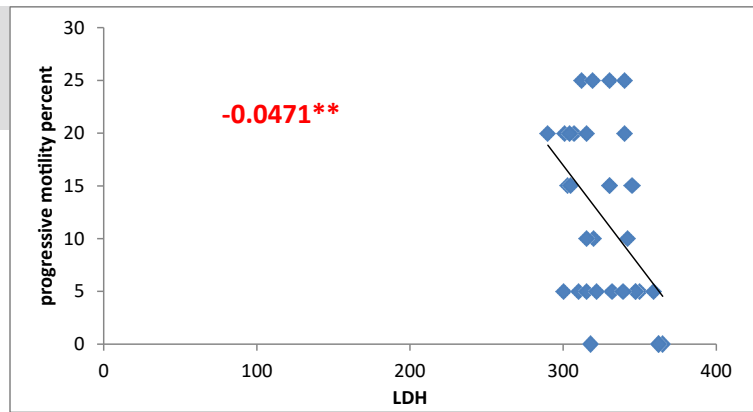
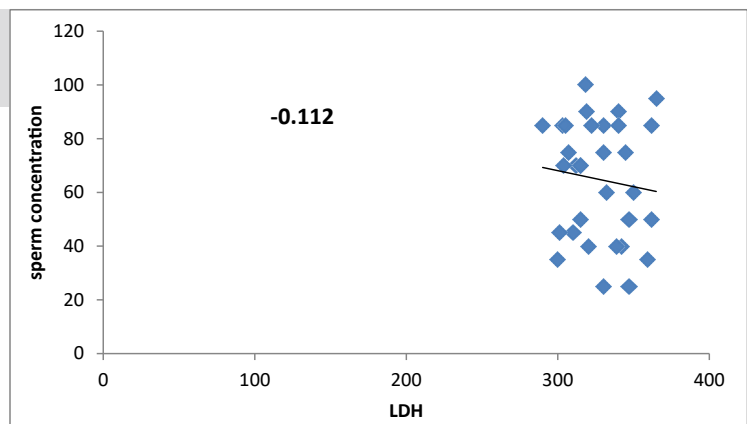


Fig. 7. The negative correlation between sperm concentration and lactate dehydrogenase in asthenospermic men.



DISCUSSION

The results of current study shown significant increase ($P < 0.05$) of LDH activity in seminal plasma of infertile patients in oligospermia compared to normospermia and asthenospermia (Fig. 1.), that may be refer to the normal activity of prostate secretion in normospermic and asthenospermic men, but in patients of oligospermia, the increment of LDH activity may be refer to the increasing in the percentage of live and normal sperm corresponded to an increasing in LDH activity in the seminal fluid. It has been stated that LDH plays an important metabolic role in sperm capacitation and fertilization [14]. Amadou CF, et al. [10] revealed non-significant differences ($P < 0.05$) between seminal plasma LDH activity of normozoospermia and azoospermia.

The results revealed positive correlation between progressive motility percent and lactate dehydrogenase activity in the group of oligospermia (Fig. 2.), but it was showed negative correlation between progressive motility percent and lactate dehydrogenase activity in normospermic men (Fig. 4.), also it was showed significant negative correlation between progressive motility percent and lactate dehydrogenase activity in asthenospermic men (Fig. 6.), that may refer to reduction in prostate secretion, or because the decrease of LDH activity by conversion from active shape to other with less activity, that effect on sperm count and motility. Asadpour R [14] and Goldberg E [15] observed the significant correlations were found between LDH levels and rat sperm viability, also mention every increase in the percentage of live and normal sperm corresponded to an increase in LDH activity in the seminal fluid. It has been stated that LDH plays an important metabolic role in sperm capacitation and fertilization [16].

Fig. 5. revealed positive correlation between sperm concentration and lactate dehydrogenase activity in normospermia, but Fig. 3. revealed negative correlation between sperm concentration and LDH activity in oligospermic patients, also results showed negative correlation between sperm concentration and lactate dehydrogenase in asthenospermic patients ((Fig. 7.), that may refer to function of this enzyme in the semen and effect on the sperm function. Hereng TH, et al. [17] conclude that exogenous pyruvate and glucose together speed up the glycolytic machinery to produce sufficient ATP to support progressive motility and capacitation, or may be refer to prostate secretion and the effect of this enzyme function on regular the semen component. Owen DH, et al. [18] mention several seminal plasma components present in substantial quantities and excluded from studies for many reasons of simplicity and practicality. These include lipids, choline, sialic acid, inositol, ascorbic acid, creatine, and pyruvate.

CONCLUSION

This study concluded the significance of LDH activity to detecting the capacity of gland function, and no significant correlation between sperm concentration and lactate dehydrogenase activity.

FUTURE STUDY

- Estimate these enzymes for infertility patients after the seminal fluid analysis.
- 2-Study the effects of seminal plasma enzymes in the female reproductive system on the capacitation, hyper activation and acrosome reaction.

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