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The Alkaline Phosphatase Levels in the Seminal Plasma and Sperms of Sub-Fertile Patients and Normospermic Men

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Abstract

This study examined 110 semen specimens collected from sub-fertile and Normospermic men after a period of abstinence from 3-5 days. The samples were collected in Fertility Center Laboratories/ Al- Saader Hospital/ Najaf province in the period from November 2009 to May 2010.

This study aimed to comparison between the concentrations of the enzyme alkaline phosphatase (ALP) in seminal plasma of different in sub-fertility groups include: Asthenospermia (AS), Oligoasthenospermia (OAS), Azoospermia and Normospermia (NS) to study the effect of ALP levels in the quality of sperm in sub-fertile patients.

The results revealed that a significant increase ($p < 0.01$) in the concentration of ALP in the seminal plasma compared to the levels of this enzyme in sperm per each group of this study: NS, AS and OAS. The comparison study of the seminal plasma ALP enzyme level indifferent groups of this study, revealed a significant decrease in the levels of ALP enzyme in the seminal plasma of Oligoasthenospermic ($p < 0.05$) and Azoospermic ($p < 0.01$) patients compared to seminal plasma of Normospermic men, while this decrease was insignificant in the seminal plasma of Asthenospermic patients. The results showed non significant decrease ($p > 0.05$) in ALP enzyme activity of the asthenospermic patient's sperm, while a significant decrease ($p < 0.05$) of this enzyme in Oligoasthenospermic sperms compared to the levels of this enzyme in Normospermic sperm.

It was concluded that the level of ALP in the seminal plasma and sperms correlated with the concentration of the sperms.

1. Introduction

The alkaline phosphatase secret in seminal fluid by Epididymis of rabbit and dogs (1;2), while it was secreted by prostate and testis in human (3). All Isoenzymes of this enzyme is encoded by three genes in the body, which includes the placental alkaline phosphatase, intestinal and Osteohepatic, this differences of the isoenzymes refer to the addition of gluco groups to the peptide chains to form glucoproteins and thus these isoenzymes differ in their different characteristics such as behavior which is expressed during the Electrophoresis (4). According to the differences of isoenzymes of alkaline phosphatase can diagnosis of seminal fluid quality (5). The alkaline phosphatase

is intended to be more effective in the Leyding cell , transitional and Fibroblast in seminiferous tubules (6), but on the other hand not see any effectiveness of this enzyme in Sertoli cell.

The alkaline phosphatase from the enzymes that cause loss of the phosphorus Group, which is effective in several tissues, including bone, liver, kidney, bowel, lung, and placenta in addition to the reproductive system. The amount of most alkaline phosphatase in the bulls excreted from the seminal vesicles in addition to the testis and epididymis, which constitutes a small percentage of concentration in semen (7). Another study indicated the existence of significant difference in the concentration of the alkaline phosphatase in dogs that underwent a process of Vasectomization for those that did not take place in this process (8). Also observed significant decrease in the levels of one isoenzyme of alkaline phosphatase secreted from germ cells, which called Placental-like alkaline phosphatase, in men who have undergone the process of Vasectomy So that it is possible to use the measuring of this enzyme concentration in the semen of these patients, to see the success of vasectomy (3;5). The strains of rabbits that have high fertility, have higher levels of seminal phosphatase enzymes compared to those that have low fertility (9;10).

The seminal alkaline phosphatase in boar inhibited by Theophylline, Caffeine and Pentoxifylline which lead to the improvement of sperm parameters (11) but the same research added that this result may be different when studied on human sperm.

This study aimed to study the correlation between the level of Alkaline Phosphatase and male infertility

2. Materials and Methods

Semen specimens

Semen specimens were collected from Normospermic (NS), Asthenospermic (AS), Oligoasthenospermic (OAS) and Azoospermic patients by masturbation after three days of sexual abstinence. The specimens were allowed to liquefy at 37C°, and then seminal fluid analysis was performed to determine the sperm parameters include: sperm concentration, sperm motility percent, grade activity and abnormal sperm morphology percent.

Preparation of Seminal Fluid specimens for estimation of Alkaline Phosphatase

One ml of each specimen was centrifuged (3000 rpm for 10 minutes) after the Seminal Fluid Analysis was performed to obtain the seminal plasma as supernatant and pellet. The supernatant (Seminal Plasma) was transferred to the other tube and then used for estimation the level of ALP enzyme. The pellet was washed by using one ml of Normal Saline and mixed well , then re-centrifuged again (3000 rpm for 5 minutes), then the supernatant was removed completely. The ALP enzyme connected with the sperm plasma membrane, so that the sperm should be crushed by wood stick, then 0.5 ml of distilled water was added to the specimens contain the sperms for disruption the cell membrane due to the different of osmotic pressure . The seminal plasma and sperm suspension was froze until the test.

Alkaline phosphatase concentration:

The ALP enzyme level in the seminal plasma and sperms was estimated by colorimetric method by using a kit manufactured by Biolabo: France Company.

Statistical analysis:

Analysis of data was performed by using Statistical Package for (SPSS) (Version). Results are expressed as mean ± S.E. Statistical differences were determined by Least Significance Differences (LSD) test for multiple comparisons between different groups.

3. Results

The results showed significant decrease in sperm concentration in (OAS) group ($p<0.01$), and ($p<0.05$) in (AS) group compared to (NS). The sperm motility percent and grade activity in (AS) and (OAS) groups were significant decrease ($p<0.01$) compared to (NS), also there was a significant increase ($p<0.01$) in abnormal sperm morphology percent in all infertile groups compared to normospermia.

The comparison between the ALP concentration in seminal plasma and sperms showed significant increase ($p<0.01$) of ALP level in the seminal plasma compared to sperm in (AS), (OAS) and (NS) specimens. Also the results revealed a significant decrease ($p<0.01$) in seminal ALP concentration in (OAS) and azoospermia compared to (NS), while this decreasment is non significant ($p>0.05$) in (AS). Also there was a significant decrease ($p<0.01$) of sperm ALP level in (OAS) compared to (NS) Table-1-.

Seminal fluid parameters Mean ± SE	Normospermia Mean ± SE	Asthenospermia Mean ± SE	OligoAsthenospermia Mean ± SE	Azoospermia Mean ± SE
Sperm concentration (million/ml)	74.285 ± 4.257	57.826 ± 5.258 *	7.700 ± 1.955 **	---
Sperm motility percent	65.00 ± 2.182	28.913 ± 2.981**	25.227 ± 2.662**	---
Grade activity	3.714 ± 0.106	1.913 ± 0.168**	1.500 ± 0.177**	---
Abnormal sperm morphology percent	59.761 ± 3.423	76.956 ± 2.82**	87.391 ± 2.492**	---
ALP Level in seminal plasma (IU/L)	273.6 ± 29.9	218.5± 26.8	179.2± 13.0 * *	156.7± 15.0 *
ALP Level in sperms (IU/L)	68.6 ± 6.2	54.4 ±3.1	45.1 ±26.8* *	-----

* Significant difference ($p<0.05$)
** Significant difference ($p<0.01$)

Table 1. Seminal Fluid Parameters and Alkaline Phosphatase (ALP) Level in Infertile groups and Normospermia.

4. Discussion

The results of the current study showed, the parameters of semen and sperm for patients with infertility significant decrease ($p < 0.05$) in the sperm concentration for (AS) and (OAS) patients compared to (NS) men, this result is agree with other studies (12; 13). The concentration of sperm in the (NS) is higher than the other infertility groups due to several factors, including: a defect in the action of hormones or bacterial infections that affect the male reproductive system, or varicocele.

Also the results revealed significant increase in abnormal sperm morphology percent in (AS) and (OAS) patients compared to (NS), this result agree with the study of (14), that the normospermic men have 75.67% normal sperm morphology as compared to other groups of infertility.

The results showed significant increase ($p < 0.01$) in the concentration of the alkaline phosphates (ALP) enzyme in the seminal plasma of AS and OAS patients compared to the concentration of ALP enzyme in sperm. The place and the amount of secretion of the ALP enzyme different according to the type of organism varies from one species to another (1). The studies conducted with human have indicated that the secretion of ALP enzyme from the prostate and testis (3), while the another study revealed that this enzyme in rabbits and dogs is secreted by the epididymis (2). There is another study conducted on one of the cattle breeds showed an inverse relationship between the ALP concentration and sperm concentration (15), so this variation may be due to the presence more than one source of the ALP enzyme secretion (16;17).

The comparison study of the concentration of seminal plasma ALP between the different infertility groups and normospermia showed non significant decrease of ALP in (AS) and significant decrease in (OAS) and Azoospermia. The significant differences of ALP concentration refer to sperm number only not for the sperm motility percent or grade activity, this result agree with the other study (3), which are revealed to the existence of a positive relationship between sperm counts and concentration of this enzyme in the semen samples. Another study found that the concentration of spermatozoa increase with the reduction of ALP, while the sperm concentration increase with the increasing of ALP, and the same study showed that the lowest activity of ALP enzyme in azoospermic patients compared to another groups of infertility (16). The levels of ALP enzyme increased in the first split ejaculate compared to the second split is because the largest amount of the first split of ejaculate secreted from prostate (18).

The ALP enzyme in the sperm showed a significant decrease in sperm of AS and (OAS) patients compared to (NS), this significant decrease in (OAS) may be refer to the decline sperm count in this group of patients. Low and Saltiel; 1988 (19) revealed, that the ALP enzyme linked to the sperm cell membrane by Phosphatidylinositolglycan located on the outer surface of the sperm. The site of ALP enzyme is in the plasma membrane in addition to cytoplasmic droplet and acrosome body of the sperm (20; 21). The ALP enzyme act through hydrolysis of phosphate ester of the nucleotide, sugars and ATP and has a potential role in removing phosphorus from Adenosine Monophosphate (AMP), also works to prevent the addition carbohydrates groups to glycoproteins in the surface of sperm (22). The another study indicated that the ALP enzyme present in the chloride channels in the sperm (23), and that have a role in the acrosome reaction (24), and thus there is a positive linear relationship between the level of this enzyme and sperm concentration in the semen samples of NS, AS and OAS.

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