Assessment of some minerals in seminal fluid of Asthenospermic patients

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Abstract

Objective :

The aim of present study was to estimate the levels of some minerals (Zn, Se , Ca , Mg , Na and K).Also relationships between mentioned components and some sperm parameters and the infertility period were studied as well.

Material and Methods :

This study was performed on human semen specimens obtained from Asthenospermic patients (101 specimens) and Normospermic males (61 specimens) who were attending to the laboratories of Fertility center in ALSader Hospital of ALNajaf ALAshraf city,Iraq during the period extended from December 2009 to September 2010.

Results :

The results revealed significant decrease ($P < 0.05$) in the concentrations Zn, Se and Ca. A significant increase ($P < 0.05$) of Na while insignificant difference of Mg and K values were found in Asthenospermic specimens compared with those of Normospermic specimens. Correlation study showed negative relationship between infertility period and sperm motility percent, normal sperm morphology percent and Grad activity A, and positive relationship with Grade activity D.

Conclusion :

The results showed negative relationship between the abnormal sperm morphology percent and concentration of Se, Na and Ca in seminal plasma . Sperm motility percent correlated positively with Zn and Ca

concentrations. The current study concluded that the change in the concentration of some of the elements lead to change parameters and sperm quality in terms of movement.

Keyword : Asthenospermia (AS), Normospermia(NO), Minerals

Introduction

Infertility is defined as the inability to conceive after one year of unprotected sexual intercourse[(1)]. Approximately 15% of couples are infertile and half of these couples are diagnosed with male factor infertility[(2)]. Many studies on male infertility have been focused on oxidative stress-related mechanisms of sperm damage[(3),(4)].Asthenospermia(AS) and Normospermia(NO) were reflected that unexplained infertility because most possible reasons for reduced fertility ,delay in marriage due to social or economic reasons and further delay in childbearing to build academic or professional career gives an impression that fertility is declining ,more couples seek help for infertility because of the delay in childbearing ,the aging process makes conception more difficult ,modern life style , more women joining work force ,environmental pollutants and stress of modern life has modified the reproductive behavior[(5),(6),(1)]. AS is defined as a sperm motility of less than 50 percent with forward progression (categories a & b) or less than 25 per cent with rapid progressive motility (category a movement)[(7)]. NO is defined as a Total number of spermatozoa ,and percentages of progressively and morphologically normal spermatozoa, equal or above $39x10^6$ per ejaculate,32% &4% respectively[(8)]. Virtually every human ejaculate is contaminated with potential sources of oxidative stress(OS) , such as peroxidase-positive leukocytes and morphologically abnormal spermatozoa, it follows that some of the sperm cells will incur oxidative damage and a concomitant loss of function in every ejaculate, thus, the

impact of OS on male fertility is a question of degree rather than the presence or absence of the pathology[9]. All cellular components, lipids, proteins, nucleic acids, and sugars are potential targets for reactive oxigen species (ROS) , the extent of damage caused by ROS depends not only on type and the amount of ROS involved but also on the moment and duration of ROS exposure and on extra-cellular factors such as temperature, oxygen tension, and the composition of the surrounding environment, including ions, minerals ,proteins, and ROS scavengers[10]. Sodium or sodium components were used in many studies as an activation the fertility or media used in artificial insemination. In[11] 2001 were used sodium /hydrogen exchanger -3 to activation the fluid reabsorption in reproductive tract function with fertility male mice ;while sodium fluoride has been experienced in fertility studies in several species of laboratory animals . In almost potassium utilizing was activated spermatozoa on artificial media. Burkman[12] was acted activation to the human spermatozoa by using several concentrations to pyruvate with and without different various concentration of potassium in the culture media, however, they were improved the utilizing potassium in the culture was very good .Recently researches were evaluated about using various minerals and the potassium its one of them to determinate the sex ratio on offspring birth [13] . The requirements of horses for calcium, phosphorus, and magnesium change in pregnancy owing to increased needs of these elements by the developing fetus[14] . Utilizing magnesium with calcium to determinate sex ratio was more activated than sodium with potassium in offspring birth by sex ratio[13]. Calcium is one of importance minerals that using for reproductive functions. In[15] 2001 were studied the calcium concentration and other minerals in the cervical mucus of bovine during normal oestrus and oestrus induced by progesterone and/or $PGF_{2\alpha}$, they found positive correlation between

calcium (and other minerals) and viscosity fertile cows. Zinc in human semen seems to play an important role in the physiology of spermatozoa [16] .It appears to be a potent scavenger of excessive superoxide anions produced by defective spermatozoa and/or leukocytes in human semen after ejaculation[17],[18] .Nearly half of a male's total selenium is concentrated in the testicles and seminal ducts. This antioxidant mineral is vital for healthy sperm formation, particularly motility. It also protects against toxic metal contamination.[19]

Calcium, sodium, and other minerals in spermatozoa and seminal plasma are effected on homeostasis between extracellular and intracellular of semen, therefore the aim of this study was to explain some problems of idiopathic infertility in NO compared with AS men semen and the steps for achievement this aim were: first ; To investigate some minerals (zinc, selenium, calcium, magnesium, sodium, and potassium) concentrations in AS and NO Second; To investigate relationship between above minerals concentrations and some parameters of semen analysis (Grad activity percentage, motility percentage, and abnormal morphology percentage of sperms) on AS patients. Third , To investigate effecting of some risk infertile men factors (age and infertility period) and correlated with parameters of AS patients.

Material and Methods

This study involved 2221 infertile men attendance to the Fertility center/ Al-Sadder Medical City/Al-Najaf Health Directorate/Ministry of Health/Iraq. The study duration from December, 2009 to September, 2010. The mean of infertile age to AS and NO men were 34.92 ± 0.86, 30.56 ± 0.85 respectively years old that were selected from donor (101 men as AS & 61 men NO) .The clinical assessment was evaluated by a specialist urologist for detecting varicocele, hydrocele, cryptorchidism, hernia and other congenital abnormalities. Seminal fluid speciemens were

collected after 3-5 days of abstinence directly in a clean, dry and sterile disposable container by masturbation in a private and quite room adjacent to the semen analysis laboratory. The specimens were placed in an incubator at 37ºC for 30 minutes to allow liquefaction. The liquefied semen was carefully mixed for few seconds, and then the specimen was examined in details by microscopic and macroscopic examinations. The standard form WHO[7],[8] are used to estimate the results of seminal fluid analysis. The seminal fluid specimens of AS (101 samples) and NO (61 samples) were centrifuged at 3000 rpm for 5 minutes to semen specimens for obtaining the seminal plasma. And diluted 1:10 in deionized water, they were run in 1,2,3,4,and 5 ppm to obtaining stock stander for minerals (Zn,Se,Mg,Ca,K, and Na) and assayed with an atomic absorption spectrophotometer (AAS) – Varian model spectra AA 300/400, Germany). All the trace element stock standers (of concentration 1000 ppm) were obtained from Fluka Chemica Switzerland , and the results were calculated as mmol/l[20] . Analysis of data was performed by using SPSS (Version 10) in the home computer. Results are expressed as mean ± S.E. Statistical differences were determined by Least Significance Differences (LSD) test for multiple comparisons between different groups after performing Fisher (F-test) and ANOVA making. The data analysis was done using t-test with mean between parameters of Asthenospermia specimens and Normospermia specimens and r value for correlation. P-value < 0.05 was used as a level of statistically significant.

Result

The seminal fluid analysis of semen specimens were showed a significant differences ($P < 0.05$) in most parameters except ejaculation volume , liquefaction time and round cell concentration (table 1) . The results showed a significant decrease ($P < 0.05$) of Zn , Se and Ca and

significant increase (P < 0.05) of Na in AS specimens compared to NO specimens ,while there were non significant differences (P > 0.05) of Mg and K between two groups (figure 1).

Table (1) comparison between AS and NO in seminal fluid parameters.

Parameter of macro and microscopic examination		Asthenospermic(AS) men semen n=101		Normospermic(NO) men semen n=61`		P value
		Mean	SE	Mean	SE	
Volume ejaculation (ml)		2.93	± 0.156	2.9	± 0.151	P> 0.05
Liquefaction time (minute)		32.1	± 0.696	31.4	± 0.982	P> 0.05
Sperm Concentration (x 10^6 sperm/ml)		44.7	± 2.39	76.5	± 2.51	*,P<0.05
Sperm motility (%)		21.85	± 1.53	59.3	± 1.12	*,P<0.05
Sperm grad activity (%)	A	7.7	±0.66	29.2	±1.54	*,P<0.05
	B	14	± 0.959	30.5	± 0.945	*,P<0.05
	C	14.02	± 2.12	8.97	± 3.27	*,P<0.05
	D	64.8	± 4.46	31.9	± 1.19	*,P<0.05
Abnormal sperm morphology (%)		80.97	± 1.32	61.21	± 1.47	*,P<0.05
Round cells x 10^6 cell		1.73	± 0.201	1.74	± 0.181	P> 0.05

* Significant difference on AS men semen comparison with NO men semen (p < 0.05).

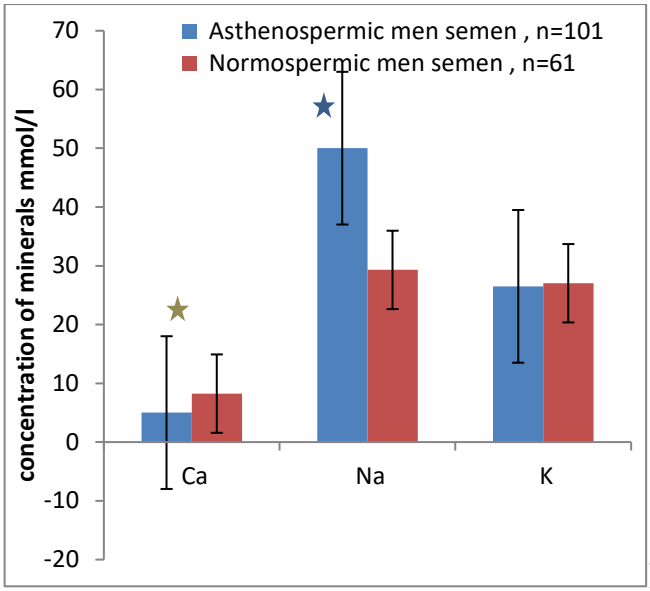


Figure 1 (B)

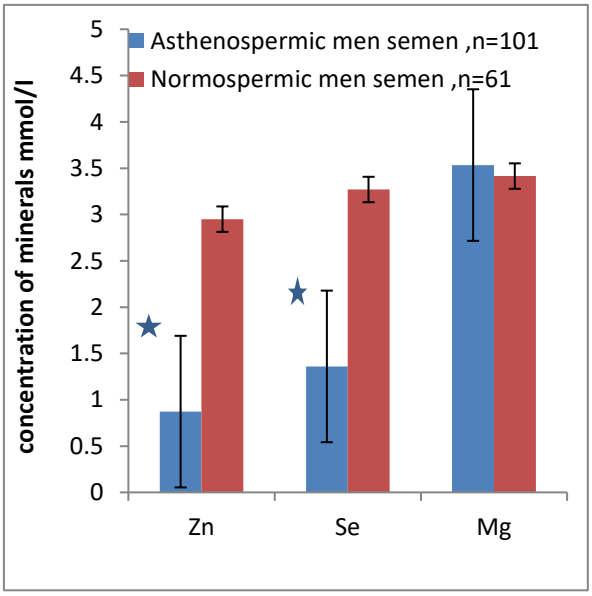


Figure 1 (A)

Figure (1) The comparison of minerals {B (Zn, Se, Mg) & A (,Ca ,Na, and K)} concentration between AS and NO men semen.

significant difference ($p < 0.05$).

The comparison of seminal parameters between the age groups in both NS and AS revealed insignificant differences ($P > 0.05$) in abstinence period, ejaculation volume, liquefaction time and round cells concentration.

In NS specimens, the results showed significant increase ($P < 0.05$) in sperm concentration and significant decrease ($P < 0.05$) in sperm motility percent in ≥ 40 years age group compared to other groups (20-29 years & 30-39 years) .Also it was observed a significant increase ($P < 0.05$) in grad activity B and D and a significant decrease ($P < 0.05$) in grad activity A in age group ≥ 40 years compared to other age groups, while for grad activity C and abnormal sperm morphology percent was noticed a significant increase ($P<0.05$) in both 30-39 years and ≥ 40 years age groups compared to age group 20-29 years .

In AS specimens, the results showed a significant increase ($P < 0.05$) in sperm concentration in both age groups 30-39 and ≥ 40 years compared to age group 20-29 years ,also a significant increase ($P < 0.05$) in sperm motility percent in age group ≥ 40 years compared to other age groups .The grad activity A & B was significantly increase ($P < 0.05$) in age group ≥ 40 years compared to age group 20-29 years , while non significant differences ($P > 0.05$) between 20- 29 years and 30-39 years age groups and between age groups 30-39 and ≥ 40 years . Grad activity C was significant increase ($P < 0.05$) in age groups 30-39 years and ≥ 40 years compared to 20-29 years age group, while a significant decrease ($P< 0.05$) in grad activity D and abnormal sperm morphology percent of age groups ≥ 40 & 30-39 years compared to age group 20-29 years (table 2) .

Comparison minerals values between AS and NS due to age groups ,figure 2showed a significant decrease ($P< 0.05$) of Zn concentration in AS compared to NS in all age group , while Se decreasing in 30-39 and ≥ 40 years from age groups. Group ≥ 40 years only observed the significant effect of high calcium concentration and low concentration of sodium while non significant differences ($P> 0.05$) in other age groups for Mg and K concentrations (Figures 2, 3) .

Table (2) Comparison of age groups for LSD values between AS patients and NO .

	Normospermia (NO) (n=61)			LSD value	Asthenospermia(AS) (n=101)			LSD value
Age (years)	20 – 29 (n=24)	30 – 39 (n=31)	≥ 40 (n=6)		20 – 29 (n=25)	30 – 39 (n=50)	≥ 40 (n=26)	
Ejaculation volume (ml)	3.1 ±0.23	2.9 ± 0.23	2.7 ±0.25	0.6	3 ± 0.225	2.82 ± 0.199	2.87 ±0.224	0.63
Liquefaction time (minute)	32.1 ±1.88	31.1 ± 1.22	31.7 ±2.47	2.82	32.6 ± 2.0	31.32 ± 0.8	32.31 ± 0.21	1.94
Sperm Concentration (x 10^6 sperm/ml)	74.4 ±4.23 a	74.35 ±3.63 a	80.8 ±3.52 b	6.2	36.6 ± 4.02 ac	47.7 ± 3.63 abc	49.78 ±4.76 abc	6.58
Sperm motility (%)	61.7 ±2.01 a	62.1 ±1.43 a	54.2 ±2.39 b	7.2	20.1 ± 3.92 ac	21.6 ± 1.98 ac	24 ± 2.7 bc	2.29

Sperm grad activity (%)	A	33.5 ±2.6 a	34 ± 1.93 a	20 ±3.87 b	4.16	6.8 ± 1.56 ac	7.8 ±0.87 ac	8.6 ±1.27 abc	1.75
	B	28.3 ±1.3 a	28.1 ±1.46 a	35 ±1.83 b	2.6	12.44 ±2.33 ac	13.8 ±1.28 ac	15.35 ±1.73 abc	2.68
	C	7.2 ±0.95 a	9.7 ±0.86 ab	10 ±1.83 ab	1.53	12.36 ±2.59 ac	14.96 ±2.15 abc	14.73 ±2.49 abc	1.9
	D	31.4 ±2.41 a	28.5 ±1.2 a	35.8 ±3.27 b	3.3	67.56 ±4.34 ac	63.72 ±2.66 abc	63.19 ±3.53 abc	4.2
Abnormal sperms morphology (%)		57.8 ±2.23 a	62.7 ±6.01 ab	63.1 ±1.95 ab	3.1	84.3 ±2.41 ac	78.7 ±1.88 abc	79.92 ±2.72 abc	3.66
Round cells concentration (x 10^6 cell)		1.63 ±0.43	1.79 ±0.16	1.81 ±0.23	0.63	1.84 ±0.46	1.8 ±0.24	1.54 ±0.22	0.49

Different letters refers to significant differences between groups

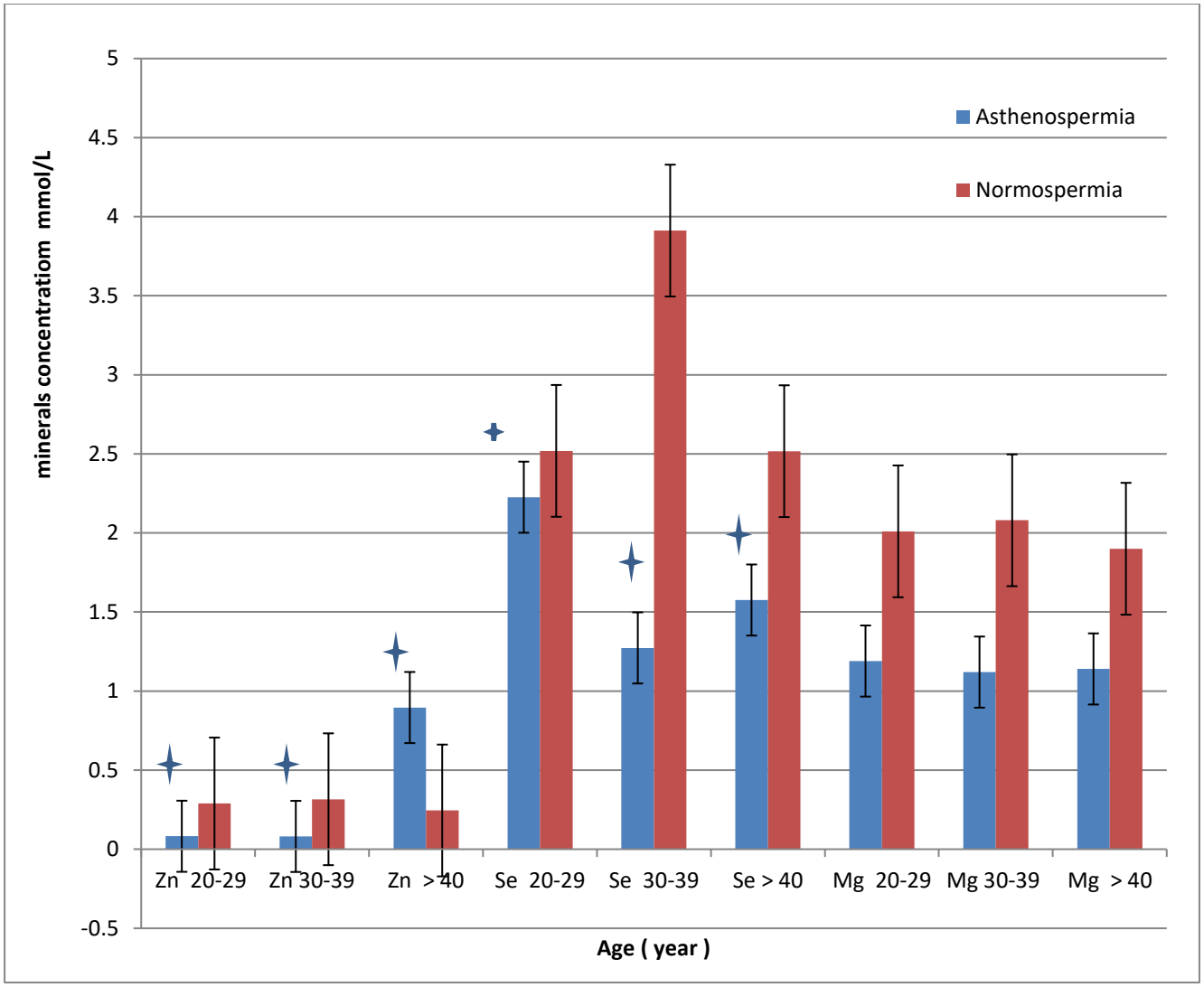


Figure (2) Comparison of age groups between Asthenospermic and Normospermic men semen in minerals (Zn, Se,& Mg)concentration. Significant difference ($p < 0.05$).

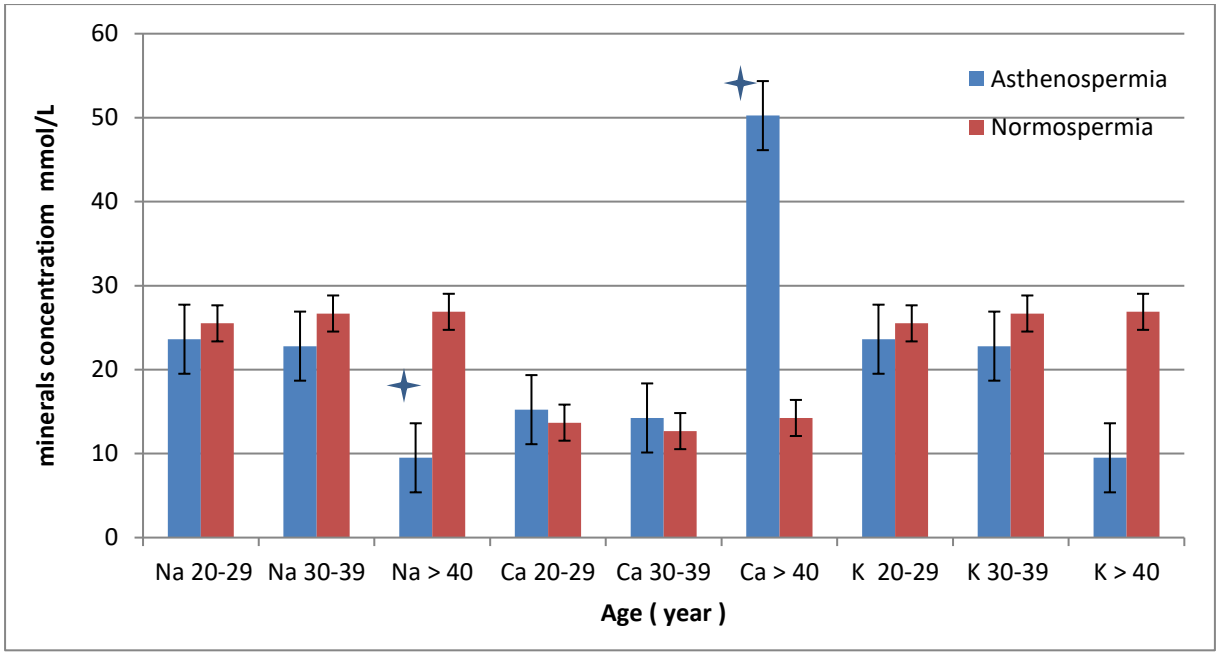


Figure (3) Comparison of age groups between Asthenospermic and Normospermic men semen in minerals (Na, Ca ,& K)concentration. Significant difference ($p < 0.05$).

A number of positive and negative relationships observed in this study. The relationship between the clear negative effect of the length of infertility and selenium concentration , the percentage of sperm motility , normal sperm morphology , and grad A sperm activity percentage while it was positive with grad D percentage (figure 4: number 1,2,3 ,4 and 5). The percentage of abnormal sperm morphology was a negative relationship with the concentrations of minerls sodium, selenium and calcium of AS patients (figure 4: number 6, 7,and 8).

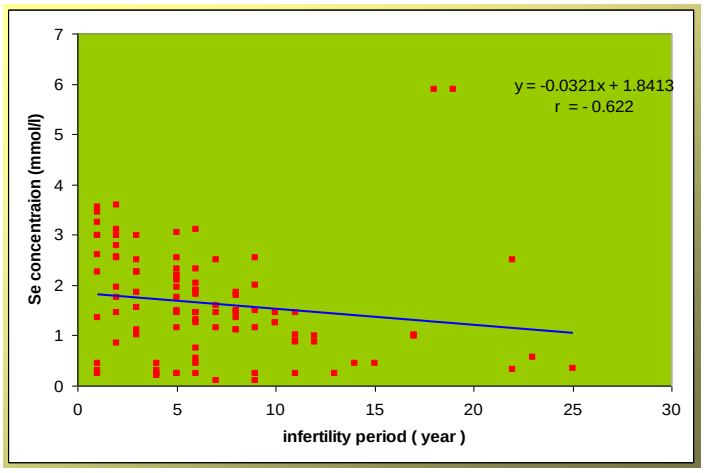


Figure (4 – 1) The correlation of infertility period (years) with Se concentrations (mmol/l) of Asthenospermia patients. n =101, p<0.05

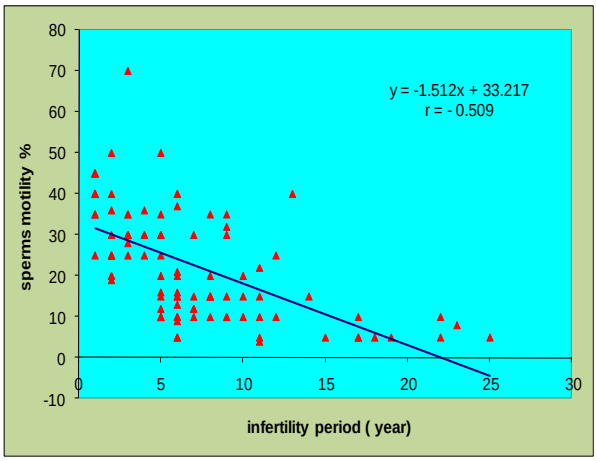


Figure (4 – 2) The correlation of infertility period (years) with sperms motility percentage of Asthenospermia patients. n =101, p<0.05

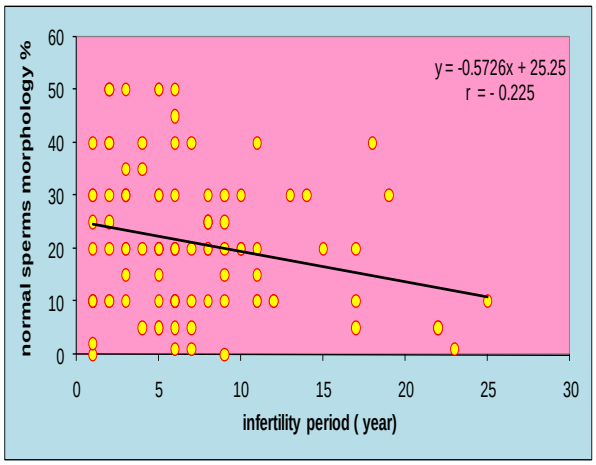


Figure (4 – 3) The correlation of infertility period (years) with normal sperms morphology percentage of Asthenospermia patients. n =101, p<0.05

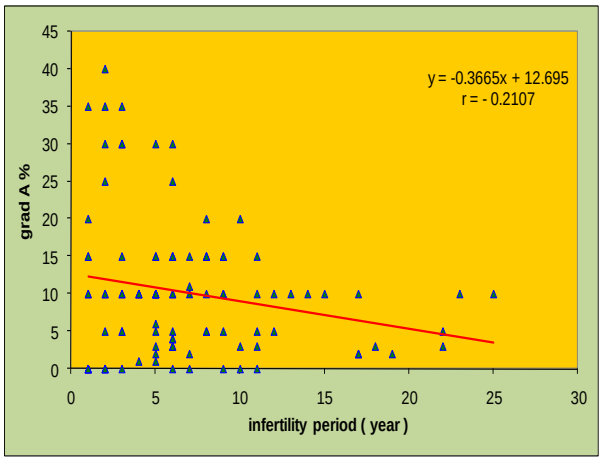


Figure (4 – 4) The correlation of infertility period (years) with with grad A {Linear and rapid sperms progressive

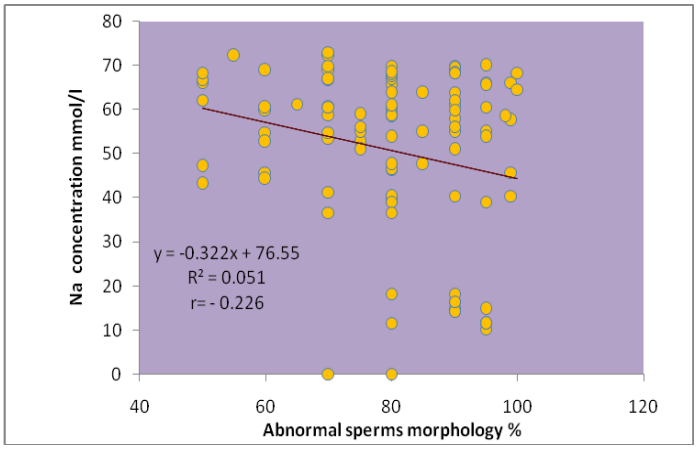


Figure (4 – 6) The correlation of infertility period (years) with abnormal sperms morphology percentage of Asthenospermia patients. n =101, p<0.05

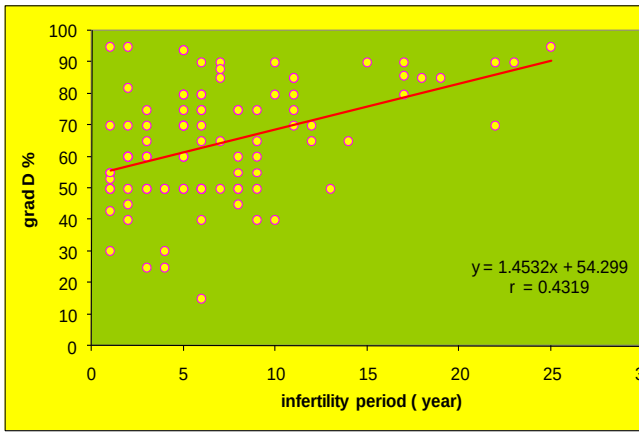
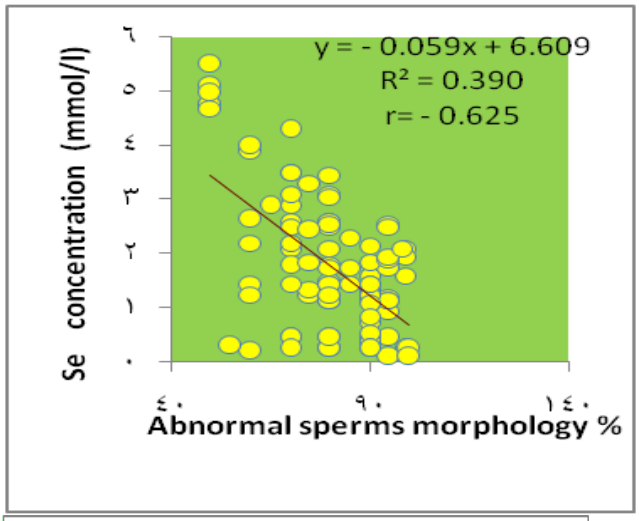


Figure (4 – 5) The correlation of infertility period (years) with with grad D{ sperms Immotile (%) } (%) of Asthenospermia patients. n =101, p<0.05



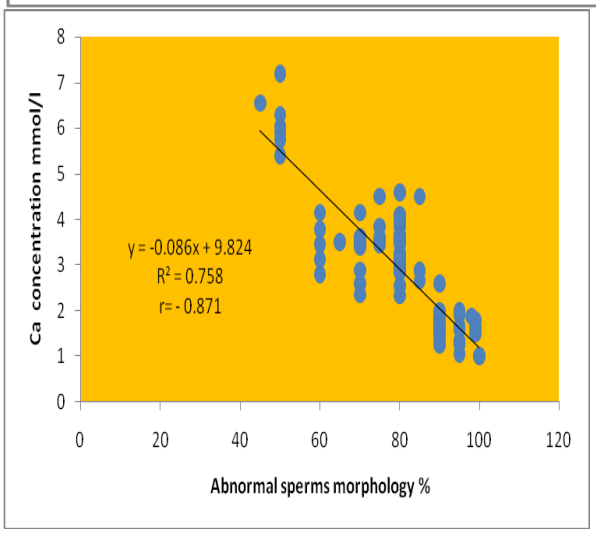


Figure (4 – 7) the correlation of abnormal sperms morphology percentage with Selenium concentrations (mmol/l) of Asthenospermia patients. n =101, p<0.05

Figure (4 – 8) the correlation of abnormal sperms morphology percentage with Calcium concentrations (mmol/l) of Asthenospermia patients. n =101, p<0.05

Discussion

The results of present study revealed a significant decrease in the concentrations cat-ions all of zinc, selenium and calcium while the significantly increased on sodium concentration of the AS specimens

compared to NS. A significant difference noted strongly in 30-39 and ≥ 40 age groups. These results mean the decline of some minerals may affect on the quality of semen .In fact minerals involved calcium ,magnesium selenium and zinc play very vital role in effecting various parameters of semen ,among minerals increasing evidence of a direct relationship of zinc was found with seminal parameters [21] .Zinc deficiency leads to gonadal dysfunction ,decreases testicular weight ,and causes shrinkage of seminiferous tubules .The gonads are the most rapidly growing tissues in the body , and vital enzymes involved in nucleic acid and protein synthesis are zinc metalloenzymes [16] .Zinc appears to be a potent scavenger of excessive superoxide anions produced by defective spermatozoa and/or leukocytes in human semen after ejaculation[17],[18] .Thus ,it seems that seminal plasma, because of its high content of zinc, will exert protective, antioxidant-like activity sufficient to cope with the excessive amount of superoxide anions [22] .The positive correlation between zinc concentration and catalase activity and reflected on the important of association mineral - zinc – with the functions of many enzymes (for example in the present study is Catalas) and reflected on the quality of the semen [23] .Therefore, the importance of Zn concentration to enhancing sperms motility by positive correlation between them. Any reason may increase oxidative stress that lead to decreasing of zinc concentration. Semenologists[24] were suggested that high seminal zinc concentrations have a suppressing effect on progressive motility of the spermatozoa (quality of movement) but not on percentage of motile spermatozoa (quantity of movement). The result of present study do not agreement with Eliasson & Lindholmer [25] study which are noted no correlation between zinc concentration and sperm density ,motility or morphology .While , agreement with studies of Chia *et al* [26] and Colagar *et al*. [27] which are revealed that seminal plasma zinc

concentration significantly positive correlated with sperms density ,motility and viability.

Among many other concepts, zinc deficiency is characterized by decreased testosterone levels and sperm counts. Zinc levels are typically much lower in infertile men with low sperm counts indicating that low zinc may be a contributing factor to infertility .[28] Selenium is an essential element required for normal animal growth and reproduction, selenium is a very important minerals that association with many antioxidant of the seminal plasma. Se has been demonstrated to be a constituent of spermatozoa and an essential element for spermatogenesis, both low and high concentrations of seminal plasma selenium may be harmful to male fertility [29],[30],[31] . The present study showed significant decrease of selenium concentration in AS specimens compared to NS and noted inverse correlation between infertility period and selenium concentration and that is mean any oxidative stress may decrease the minerals and reflecting on sperms motility, therefore the positive correlation between selenium and non enzymatic antioxidant vitamin E and negative correlation between Se concentration and abnormal sperm morphology percent evidenced the deficiency of those vital biochemical markers of decline of semen quality due to AS infertile men [23]. Calcium concentration in the present study was significantly decreased while the sodium concentration was significantly increased in AS compared to NS. These concentrations of the minerals that may inverse homoeostasis to the functions minerals seminal fluid. Calcium is very important for the cell function .it exist in a high concentration in some body fluids. The calcium concentration in seminal plasma is 3-4 `old high with respect to blood serum level, the calcium in semen is originated from semen [32]. Calcium is important for sperm physiology including motility , metabolism , acrosome reaction and fertilization[33],[34] . The encouraging

correlation between calcium concentration and sperms motility percentage and inverse correlation between Ca and abnormal sperm morphology percent may be refer to associated the calcium ions of many enzymes function for example ATPase enzyme of sperms mitochondria that its important to release energy supporting to movement of sperms. In mammals, a successful fertilization requires that sperm accomplish specialized functions that involve intracellular calcium [Ca^{+2}] changes. Indeed, processes such as the hyperactivation of motility, the so-called ‘‘capacitation’’ and the acrosome reaction (AR), require concerted changes in ion permeability leading to intracellular calcium increases[35]. In mouse [36] and human [37],[38] sperm, the studied species, calcium removal from the medium depolarizes sperm. Despite the clear positive relationship between Ca^{2+} decrease and Na^{+} dependent depolarization, evidence is strong that depolarization is not controlled by Ca^{2+}; instead, calcium removal from a putative external site triggers it. Calcium restoration produces a rapid Ca transient increase that peaks above resting and then decreases to basal values [39]. Concomitantly, Na dependent depolarization is detained and hyperpolarization occurs, inhibited by ouabain or by the absence of potassium in the medium, suggesting that this hyperpolarization is produced by stimulated Na,K -ATPase activity.[40],[39]. In human sperm, removal of external calcium produces a fast Na+-dependent depolarization that is presumably due to sodium permeation through calcium channels. Calcium restoration produces an ouabain-sensitive hyperpolarization that brings the membrane potential to values frequently more negative than resting. Torres-Flores *et al*.[41] had been showed evidence indicating that external calcium removal induces an increase in the intracellular sodium [Na+] and that this phenomenon is related to the Na+-dependent depolarization.

It was concluded that several imbalance in minerals concentrations can share the diagnosis the causes of Asthenospermia patients .

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