Elemental Analysis of Human Semen

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Abstract:

Survival of any population is depends on fertility in the past, present or in the future. While, fertility partially depends on normal healthy semen. The study was conducted on 63 men at the age 25 to 35 years, 43 infertile patients & 20 control subjects. The study was carried out in Baghdad from sep 1999 to July 2000. Complete medical history was recorded for patients & control subjects. The sample of semen was collected directly into sterile clean dry glass container by masturbation in the hospital after 3 days of sexual abstinence.

Analysis of semen parameters was carried out in accord with standard procedures. Zinc content of seminal fluid of control men is higher than sub-fertile men (group1) and infertile men (group11). Magnesium content of seminal fluid of control men is higher than sub-fertile men (group1) and infertile men (group11). Also, iron content in seminal fluid of infertile men is lower than controls. Moreover, The seminal fluid values for Cu and Mn did not vary significantly between healthy control men & patients of both groups even though their values in control group is higher than patients. In addition to there are no significant differences in regard to potassium contents of seminal fluid between control group & patients. While calcium content of patient's seminal fluid is higher than controls.

Introduction:

Survival of any population is depends on fertility in the past, present or in the future. While, fertility partially depends on normal healthy semen (1,2,3).

The survival of the society as a whole was highly dependent on fertility of its people & also on the land that fed them. In ancient Egypt, religion & magic were at the center of most fertility & regeneration rights (1).

Infertility has been intensively investigated at present time (4). Infertility is defined as the inability to conceive or to fertilize the ovum and it is due to various socio-biological factors. A couple is considered to be infertile after unsuccessfully attempting pregnancy for 1 year. Infertility is termed primary when it occurs without any prior pregnancy and secondary when it follows a previous conception (5).

During the last few years, the relationship between biochemical metabolism of trace & major elements & pathological processes in medicine has received particular attentions in Iraq (4,5). There is much interest at present in the studying the potential biological roles of many trace elements in biological function in human (6,7).

The aim of the present study is to determine relationship between the elements in seminal fluid & infertility in men.

While the objectives of the study are: -

1-Determination of the normal concentration of zinc, iron, copper, manganese, magnesium, calcium & potassium in normal semen.

2-Comparison the concentration of zinc, iron, copper, manganese, magnesium, calcium & potassium in normal fertile & sub-fertile men.

3-Correlate between semen elements & semen parameters (sperm count, motility & morphology).

Patients & methods:

The study was conducted on 63 men at the age 25 to 35 years, 43 infertile patients & 20 control subjects. The study was carried out in Baghdad from Sep 1999 to July

2000. Complete medical history was recorded for patients & control subjects.

Patients were excluded from the study to eliminate other factors affecting the aim of the study likely to cause spermatogenic dysfunction such as undescended testicles at any age, drug treatment to improve fertility, varicocele, soft testis, orchitis, alcoholism, drug that affect semen quality & any abnormal pathological conditions.

The sample of semen was collected directly into sterile clean dry glass container by masturbation in the hospital after 3 days of sexual abstinence.

Analysis of semen parameters was carried out in accord with standard procedures .

Using student t- test did statistical analysis. All data were presented as a mean & standard deviation (SD). A probability value < 0.05 was considered statistically significant.

Results:

Sixty-three subjects (males) were participated in this study. The subjects distributed into three groups: -

1-Control Group; consist of 20 healthy married men having children recently.

2-All patients are married and suffered from infertility & on the basis of semen analysis, these 43 patients divided into two subgroups:-

Group 1 consists of 22 patients after semen analysis they found that having accepted normal semen parameters.

Group 11 consists of 21 patients having abnormal semen parameters.

Table (1) shows the sperm count for all patients & control, 20 patients having a sperm count less than 20 million/ml in group 11, while 10 patients have a sperm count 20-40 million /ml (group 1 & 11). 5 patients 40.1-60 million/ml in-group 1,and 7 subjects have sperm count 60.1-80 million /ml (3 control & 4 Patients), & 21 subjects have 80.1-100 million / ml (17 control & 4 patients from group 1).

Table (2) shows the morphology of semen, the highest normal ratio in group 11 patients was in 80.1 - 90 % (one patient only), while the normal ratio of morphology of 50.1 to 60 % in group 11 patients was found at 4 patients only) which is considered normal morphology. In group 1, all patients have a normal ratio in 60.1 to 80% (22 patients). While the distribution of normal morphology of semen in control subjects distributed from 60.1 to 70% (7 patients) and 6 subjects below 80%, 5 subjects below 90% and only two subjects below 100%.

The mean and S.D of semen count, active motile ratios of sperm and normal morphology are presented in table 3. The mean & S.D for sperm count in control group are 99.5 ± 14.5 million / ml (m/ml), while the mean sperm count for group 1 patient is 55 ± 25 (m/ml), and for the group 11 patient is 10 ± 9.4 (m/ml) (Table 3).

There is significant differences between control men & group 1 patients in regard to sperm count ($t= 31$, $p<0.01$, d.f 31). Moreover, also there is significant differences between control men & group II patients in regard to sperm count ($t= 48$, $p<0.01$, d.f 30).

The mean & S.D of normal morphology of semen for control, patients group1 and group 11 is $78 \pm 10\%$, $64.4 \pm 5.6\%$ and 37.7 ± 22.5 % (Table 3). There is significant differences between control & patient 1 regarding ratio of normal morphology ($t= 12.5$, $p<0.01$, d.f 31). Also, there is significant differences between control & patient 11 regarding ratio of normal morphology ($t= 27.9$, $p<0.01$, d.f 30). The control men have a higher ratio of normal morphology than patients of group 1 & 11 (table 3).

In regard to active motility of sperm, The mean values of active motility of sperm in control, patient group 1 & patient group 11 are 74.9 ± 6.3, 61.1 ± 7.5, and 29.6 ± 23 % respectively. There are significant differences between control & patient 1 regarding ratio of active motility of sperm ($t= 14.3$, $p<0.01$), & group 11 patients ($t= 29.4$, $p<0.01$).

The mean & S.D of zinc, magnesium, manganese, iron, copper, calcium, & potassium of control and patients (group 1& II) are presented in table 4.

The concentration of zinc in semen of healthy control individuals was higher (64.2 ± 44.2 ppm) than patients group 1 (56.9 ± 28.5 ppm) & group 11 patients (23.5 ± 9.4). There is significant differences between control

men & group 1 patients in regard to zinc concentration (t= 3.75, p<0.05), in addition to that, there is significant differences between control men & group II patients (t= 18.6, p<0.01). Also, there is reduction in zinc content of patient seminal fluid of – 26.3% comparing with control.

Moreover, there is significant differences between patients group 1 men & group II patients in regard to zinc concentration (t= 25.3, p<0.01, d.f 41). This result indicates clearly that zinc content of seminal fluid of patients is lower than control group (Table 4).

While, The concentration of Mg in semen of healthy control individuals was higher (46.9 ± 26.2 ppm) than patients of group 1 (17.2 ± 8.9) & group 11 patients (16.8 ± 8.2 ppm). There is significant differences between control men & group 1 patients in regard to Mg concentration in seminal fluid (t= 15.5, p<0.01), also, there is significant differences between control men & group II patients (t= 15.8, p<0.01). (Table 4).

Moreover, there is reduction in Mg content of patient seminal fluid of – 26.1% comparing with control. And, there is a positive correlation between semen Mg & sperm count (r=0.61, p<0.01), percent of normal formed sperm (r=0.41, p<0.05), and sperm active motility (r=0.65, p<0.01).

Also, the concentration of Calcium in seminal fluid of healthy control individuals was lower (55.8 ± 25.8 ppm) than patients group 1 (124.9 ± 35.7) & group 11 patients (198.1 ± 26.4). Group 11 patients seminal fluid contains high calcium concentration than group 1 patients (see table 4).

There is significant differences between control men & group 1 patients in regard to Ca concentration (t= 35.6, p<0.05), and, there is significant differences between control men & group II patients (t= 29.8, p<0.01). Moreover, there is significant differences between group 1 patients & group II patients regarding Ca content of seminal fluid (t= 19.5, p<0.01).

In addition to, the mean value for Mn in seminal fluid of control men is 0.062 ± 0.017 ppm. While, the mean values for patients are 0.049 ± 0.019 for group 1 and 0.052 ± 0.02 ppm for group 11. Even though, the seminal fluid Mn content of control is higher than patients, there is no significant differences between control & patients (see table 4).

The mean value for Iron (Fe) in seminal fluid of control men is 0.329 ± 0.22 ppm. While, the mean value for patients is 0.098 ± 0.017 for group 1 and 0.068 ± 0.045 ppm for group 11 (Table 4). A decrement percentage of seminal iron in patients is – 22.2% comparing with control. The iron content of seminal fluid of control is higher than patients of both groups, but it reach the significant level between control & patients group 11 only (t=1.096, p<0.05).

The mean value of copper in seminal fluid of control men is 0.1665 ± 0.08 ppm, while, the mean values for patients are 0.079 ± 0.011 ppm for group 1 and 0.069 ± 0.04 ppm for group 11 (Table 4). However, the copper content of seminal fluid of control is higher than patients, but it does not reach the significant level between control & patients (t= 0.69).

The mean value of K in seminal fluid of control men is 0.695 ± 0.093 ppm, while, the mean values for patients are 0.63 + 0.14 ppm for group 1 and 0.67 ± 0.13 ppm for group 11 (Table 4). However, there is no significant difference between control & patients of both groups.

Table (1): Sperm count of control subjects & patients.

Sperm count range (million/ml)	**Control subjects**	**Patients**	
	Control	Group 1	Group 11
2 – 20	-	-	20
20.1 – 40	-	9	1
40.1 – 60	-	5	-

60.1 – 80	3	4	-
80.1 – 100	17	4	-
Total	20	22	21

Table (2): Normal morphology of semen of control subjects & patients.

Percentage %	**Control**	**Group 1**	**Group 11**
10 – 20	-	-	4
20.1 – 30	-	-	3
30.1 – 40	-	-	4
40.1 – 50	-	-	3
50.1 – 60	-	5	4
60.1 – 70	7	14	2
70.1 – 80	6	3	1
80.1 – 90	5	-	-
90.1 - 100	2	-	-
Total	20	22	21

Table (3): The mean & S.D of sperm count, sperm normal morphology and percent of active motility of sperm.

	Control subjects	**Patients**	
Mean & S.D	Control	Group 1	Group 11
Sperm count (million/ml)	99.5 ± 14.5	55 ± 25.8	10 ± 9.4
Normal morphology %	78.8 ± 10	64.4 ± 5.6	37.7 ± 22.5
Active motility %	74.9 ± 6.3	61.1 ± 7.5	29.6 ± 23.7

Table (4) : The mean & S.D of zinc, Mg, Mn, Fe, Cu, Ca, & K concentrations (ppm) in semen of controls & patients.

	Control men	**Patients**	
Mean & S.D	Control	Group 1	Group 11
Zinc	64.2 + 44.2	56.9 ± 28.5	23.5 ± 9.4
Magnesium	46.9 ± 26.2	17.2 ± 8.9	16.8 ± 8.2
Manganese	0.062 ± 0.017	0.049 ± 0.019	0.052 ± 0.02
Iron	0.329 ± 0.22	0.098 ± 0.017	0.068 ± 0.045
Copper	0.1665 ± 0.08	0.079 ± 0.011	0.069 ± 0.04
Calcium	55.8 ± 25.8	124.9 ± 35.7	198.1 ± 26.4
Potassium	0.695 ± 0.093	0.63 ± 0.14	0.67 ± 0.13

Discussion:

In the present study, semen parameters were measured in addition to measurement of zinc, copper, manganese, magnesium, iron and calcium. So, this study concerns with relationship between elements in seminal fluid & infertility in men. As expected, in this study, abnormality in semen parameters (sperm count, sperm motility and sperm morphology) was found lower in infertile patients.

Infertility in men can result from a wide range of various causes (8,9,10). Man should be evaluated by taking detailed history, complete physical examination, and examination of seminal fluid (1).

In the present study, seminal zinc concentration was found low in infertile patients comparing with control. The effect of pathological conditions on biochemical elements metabolism could not be considered to be the cause, until the elements themselves were measured (11,12). The role of zinc in human seminal fluid is not well understood & rather little known about the relationship between subnormal semen zinc concentration & abnormal semen parameters.

Zinc is responsible for chromatin stability & for chromatin motility, normal morphology & seminal plasma volume (13,14).

Beisel (15) reported that leukocyte endogenous mediator is released from white blood cells (WBC) in semen during illness. It plays a role in metabolism of trace elements by its direct action on the liver and mediate alteration of trace elements. Previous reports suggest that men whose prostate inflammation, prostate fluid contains increased number of WBC, were found to have low concentration of zinc & acid phosphatase (16).

Previous works on semen analysis, state on the presence of zinc in seminal fluid (14,15), also Noack-Fuller (1993) found there is no significant differences in zinc concentration between semen and seminal plasma (16).

This findings may be explain as increased demand and use of zinc incase of inflammation when there is a high number of WBCs involved in the inflammation process (14, 15).

Previously Laidpo (17) found no differences in the concentration of zinc in seminal fluid of infertile men. The author did not mention more information about the control men. However, in this study group 1 patient regarded as a control for group 11 patient but this is not enough because both of the groups consist of men complaining of infertility. For this reason a control group of healthy married men having children recently included in this study.

Zinc concentration in Laidpo study (17) was measured by calorimetric methods, however, lack of specificity is a major disadvantage of this method, and many metal ions especially at higher concentration are known to interfere with the analysis. The metal ions include Hg, Bi, and Cd. For this reason colorometeric & fluorometric methods are no longer recommended for zinc analysis (18).

In the present study, the concentration of Mg in semen of healthy control individuals was higher than patients of group 1 and group 11. Also, there is reduction in Mg content of patient seminal fluid of – 26.1% comparing with control. As previously mentioned in introduction, Mg act as an enzyme activator involve in the hydrolysis & transfer of phosphate group from ATP & other compounds containing high energy phosphate (11).

Sperm motility depends directly on energy supply and a low Mg content is associated with low energy output at cellular level and subsequently with low sperm motility. This findings was noticed in-patient's seminal fluid of low Mg content have a high percentage of inactive sperm.

Moreover, In the present study, a low Mg in patients seminal fluid is also related with low number of sperm count, because Mg is important in the synthesis of new tissue & binding of RNA with ribosome for protein synthesis (18). All of these Mg functions are necessary for spermatogenesis.

In the present study, the seminal fluid content of Cu, Mn, & K was not differ significantly between control & patients. All these three elements found in very low level. Potassium is present mainly in intracellular fluid where its concentration exceeds that of ECF by a factor of about 40 times (11). For this reason K may be found in seminal fluid in very low concentration.

It is surpassingly that seminal copper is very low because body contains only 80 mg (5,6). Also, 95% of serum copper is associated with ceruloplasmin, while the remaining 5% is loosely bound to albumin (12). Even so, copper & iron are related to the activity of

oxidation-reduction activity of tissue such as cytochrome system (11,19, 20). This system involves in generation of energy from glucose, which is necessary for sperm motility. In the present study seminal copper of patients is lower than control group but does not reach the significant level.

Although, men regarded as infertile when semen quality fall below the followings: semen volume 2-5 ml, sperm count more than 2 m/ml, sperm motility greater than 50%, and normal form greater than 60% (1,2,3). All these are characteristics of group 1 patients who are complains as infertile but by semen analysis, the results of these patients reveal that the have accept normal values. While, the elemental analysis of semen reveal this group have a low zinc, low magnesium and high calcium in their seminal fluid.

These findings support the idea of measurement of elements as diagnostic markers for infertility in men. According to the result, it is advisable to estimate the level of these elements in the semen of subfertile men in order to use these elements in the management of infertility.

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