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Creatine Kinase Activity in Human Seminal Fluid

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Introduction: Male infertility is an important factor of infertility in couples. In most cases it is caused by defects in sperm cell function; whether low sperm cell count or reduced motility along with altered morphology. Defects in sperm cells development are influencing enzyme activities in the seminal fluid. **Aim:** This research tried to determine can activity of creatine kinase (CK) in seminal fluid be used as a marker of fertilizing ability in infertile the region of the plane Dukagjini in Kosovo, and to determine the link between that activity and the quality of sperm cells.**Materials and methods:** Seminal fluid and blood samples were taken from 200 patients, 150 of whom were infertile with confirmed oligozoospermia, oligoasthenozoospermia and azoospermia. Sperm cells number, motility and morphology, CK activity in seminal fluid and concentrations of gondaotropic hormones were analysed in comparison to values obtained from 50 normozoospermic subjects used as a control.
Results: CK activity in seminal fluid of patients with primary and secondary infertility is significantly higher than that of the control group ($p<0,005$). CK activity depended on sperm cells number, motility and morphology. Higher ratio of abnormal sperm cells parameters correlated with higher CK activity in seminal fluid.
Conlusion: Enzymatic activity of CK in seminal fluid is a valuable biochemical marker in determining fertilizing ability of sperm cells. This biochemical marker is used in clinical assesment of sperm cells fertilizing potential and represents an important diagnostic feature.
Keywords: infertility, creatine kinase (CK), oligozoospermia, oligoasthenozoospermia, azoospermia, normozoospermia

Introduction

The ability of sperm cells to fertilize an egg cell is of utmost importance in cases of inexplicable infertility in men, when analysis of ejaculate does not detect any changes in spermiogram parameters. It is therefore necessary to recognize cell markers for sperm cell quality which, used with other dignostic methods, make identifying specific deficits linked with sperm cell function easier. One of those markers is the enzyme creatine kinase (CK).

Sperm cells require high energy intake for their active movement. CK is the key enzyme in providing that energy. The role of CK in mitochondria is to catalyze phosphorilation of creatine into creatine phosphate [Wallimann T, Hemmer W.1994, Huszar G. 1990]. CK in sperm cell neck catalyzes rephosphorilation of adenosine diphosphate (ADP) into adenosine trifphosphate (ATP).

Sperm cell CK is composed of two subunits: CK-B and CK-M. There are also mitochondrial forms of the enzyme called mi-CK which differ in respect to serum CK originating from cytosol. The difference is seen in electrophoregram obtained separating the CK isoenzymes in a gel (Huszar G, Corrales M, Vigue L. 1988).

Previous study has shown a negative correlation between sperm cell number and CK activity. Metabolic properties of sperm cells in men with oligozoospermia are different than in patients with normozoospermia. Cytochemical study (Huszar G, Vigue L. 1993) has shown higher values of CK in ejaculate samples proven to contain cytoplasmic residues, ie immature sperm cells (Celic-Ozenci C et al. 2002).

Normally, mature sperm cells are formed with complete lack of cytoplasm in the neck region and a properly developed flagellum. This form is associated with low levels of CK (Huszar G, Vigue L. 1990). Research show that immature sperm cells with cytoplasmic residue do not attach to human egg cells (Huszar G, Vigue L, Oehninger S. 1994 Aitken J, Krausz C, Buckingham D, 1994. Gomez E, 1996).

In the light of the new findings about factors determining male infertility, the aim of this study was to determine the significance of CK activity in seminal fluid of infertile men compared to normally fertile men, to confirm the activity of CK as a marker for infertility.

Materials and Methods

This study involved the total of 200 patients. 150 of them, aged 25-40, were treated for primary and secondary infertility at the *BIOLAB-ZAFI* polyclinic in Klina, Republic of Kosovo.

Seminal fluid analysis, spermiogram, biochemical analysis, serum hormonal levels and CK activity in seminal fluid assesments have been done in the biochemistry lab within the polyclinic. For this research, seminal fluid samples were taken from all 200 patients. Patients were divided into four groups, according to the results of ejaculate analysis: normozoospermia (group A), oligozoospermia (group B), oligoasthenozoospermia (group C) and azoospermia (group D).

Activity of CK in seminal fluid has been determined by photometric analyzer *Reflotron* (Roche, Germany).

Results were processed using a statistical software SPSS, ver. 15. Value of $p<0,05$ was considered to be statistically significant.

Results

This investigation is the first of its kind done on the region of the plane Dukagjini in Kosovo.

Subjects were grouped according to age and duration of infertility: fertile subjects (n=50) as a control group with normozoospermia parameters seen in ejaculate samples (group A), and infertile subjects (n=150) with reduced spermiogram parameters.

Table 1. Subjects grouped by age and duration of infertility.

	Normozoospermia group A	**Oligozoospermia** group B	**Oligoasthenozoospermia** group C	**Azoospermia** group D	
	n=50	n=68	n=60	n=22	
Age (years)	20-40 (35,5±10.5)	25-30 (25,8±2.6)	30-35 (30,5±2.7)	25-40 (37,6±8.1)	p<0,002
Duration of infertility (years)	1,7±0,5	3, 5±0,9	5,5±1,5	8,0± 2,4	p<0,001

Table 1 shows grouping of patients according to age and duration of infertility. Significant difference has been found in regard to age ($p<0,002$) and infertility period ($p<0,001$) between the subject groups. Mean values and standard deviations are given in brackets for every patients group.

Table 2. Spermiogram results for involved subjects (n=200)

	Normozoospermia n=50 group A	**Oligozoospermia** n= 68 group B	**Oligoasthenozoospermia** n=60 group C	**Azoospermia** n=22 group D	
Duration of apstinence (days)	2-5	2-4	2-5	2-5	NS
Volume (ml)	3,41± 0,08	3,17± 0,05	3,00 ± 0,08	2,68 ± 0,05	<0,001
Duration of liquefaction (minutes)	<30	30-60	30-60	<10	<0,005
Viability (%)	80±1,63	73±0,82	78±1,63	-	NS
Number of sperm cells ($x10^6$/ml)	78,3±5,18	11.95±0,26	13.5±0,54	-	<0,005
Total number of sperm cells ($x10^6$)	266,3±6.99	38,42±0,63	40,52±0,85	-	<0,005

Motility (%)	58,2±0,90	42±0,61	38±0,56	-	<0,005
Progressive motility (%)	35±0,47	24±0,82	18±0,52	-	<0,005
Normal morphology (%)	50,6±2,09	32,21±0,67	23,2±0,60	-	<0,005

NS = not statistically significant

Spermiogram results for infertile patients (n=150) – groups B-D, showed significant differences (p<0,005) compared to control group A (n=50).
Significant differences have been found between groups in sperm cell count per 1 ml of seminal fluid (p<0,005) and seminal fluid volume (p<0,001). Differences between groups are seen in the other spermiogram parameters as well: total motility and progressively mobile sperm cells number. Sperm cells morphology shows significant difference between the group A and the groups involving infertile men (p<0,005). Decreased number of sperm cells with normal morphology is characteristically seen in infertile subjects within groups B and C, suffering from oligozoospermia and oligoasthenozoospermia. Significant differences in the set apstinence time and sperm cells viability have not been observed.

Table 3: Results obtained by determining the catalytic activity of CK in seminal fluid of tested subjects

	Normozoospermia n=50 Group A	**Oligozoospermia** n= 68 Group B	**Oligoasthenozoospermia** n=60 Group C	**Azoospermia** n=22 Group D	
CK activity IU/10^8 sperm cells	108-238 (175,0±65,0)	295-558 (435,0±184,6)	304-618 (452,2±157,1)	450-638 (557,1±94,3)	p<0,005

CK activity in seminal fluid of tested patients, expressed as IU/10^8 sperm cells, is shown in Table 3. Mean values and standard deviations of measurements are given in brackets.

CK activity in seminal fluid from fertile patients (normozoospermia) is in range of 108 - 238 IU/10^8 sperm cells. Results indicate statistically significant difference (p<0,005) between fertile and infertile patients groups.

Table 4: CK activity in subjects according to severity of oligozoospermia.

	Mild oligozoospermia n=22	**Moderate oligozoospermia** n=10	**Severe oligozoospermia** n=36	
Number of sperm cells (x10^6/ml)	10,8 ±1,62	8,9± 0,27	4,2 ± 0,26	p<0,005
CK activity IU/10^8 sperm cells	295-315 (305,3±10,0)	402-498 (446,6±48,0)	451-558 (505,4±53,5)	p<0,005

CK activity in patients with oligozoospermia (expressed in IU/10^8 sperm cells) is given in Table 4. Mean values and standard deviations of measurements are given in brackets.

Statistically significant difference has been observed in CK activity in seminal fluid samples from subjects with oligozospermia depending on the severity of oligozoospermia. CK activity in

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seminal fluid is higher with increasing severity of oligozoospermia (p<0,005).

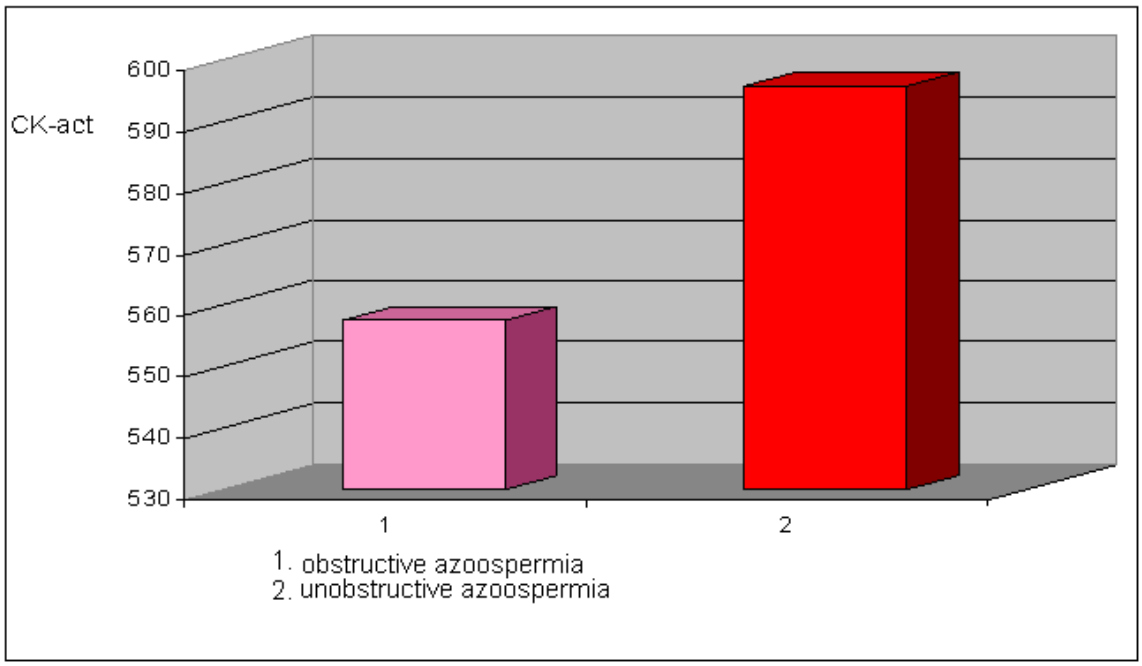

Figure ***1. CK activity in seminal fluid from infertile men with azoospermia (n=22)***
CK-act. = CK activity, IU/10^8 sperm cells

CK activity in seminal fluid from patients suffering from two types of azoospermia is given in Figure 1. CK activity is increased in azoospermia compared to all other subject groups (p<0,005). Two types of azoospermia show significant difference in CK activity as well.

Table 5. Serum hormone values and CK activity in seminal fluid from both fertile and infertile subjects (n=200).

	Normozoospermia n=50 Group A	**Oligozoospermia** n=68 Group B	**Oligoasthenozoospermia** n= 60 Group C	**Azoospermia** n= 22 Group D	
FSH (mIU/ml)	4,0-5,12 (4,55±0,56)	5,1-6,7 (5,78±0,46)	4,96-6,52 (5,12±0,85)	7,8-32,0 (10,8±13,19)	p<0,002
LH (mIU/ml)	2,67-3,6 (2,88±0,48)	4,7-6,76 (5,46±1,04)	4,12-6,26 (5,02±1,07)	6,8-12,31 (10,8±2,78)	p<0,005
CK activity IU/10^8 sperm cells	108-238 (175,0±65,1)	295-558 (435,0±131,6)	304-618 (452.,2±157,8)	450-638 (557,4±94,3)	p<0,005

Table 5 shows the correlation between CK activity and hormone concentrations in the patients blood. CK activity is expressed in IU/10^8 sperm cells. Mean values and standard deviations of measurements are given in brackets.

Results indicate that serum concentration of gonadotropins (FSH, LH) are significantly higher (p<0,005) in infertile men with azoospermia. CK activity is in range of 295-638 IU/10^8 sperm cells for groups B, C and D, in contrast to gonadotropin values elevated only in group D, azoospermic men.

Table 6. CK activity in subjects with primary and secondary infertility

	Primary infertility	**Secondary infertility**	
Number of subjects	125	25	p<0,005
CK activity IU/10^8 sperm cells	304-638 (625,8±189,4)	295-618 (445,2±161,3)	p<0,005

Table 6 shows CK activity depending on the type of infertility. CK activity is expressed in IU/10^8 sperm cells. Mean values and standard deviations of measurements are given in brackets.
Significant differences are observed in CK enzimatic activity in seminal fluid samples from patients with primary and secondary infertility (p<0,005).

Discussion

Sperm cells are male reproductive cells. The ultimate test for fertilizing ability of sperm cells is by all means pregnancy (WHO 2010). It is therefore important to find quality markers for sperm cells which, taken together with other diagnostic tools, can help discover specific disorders linked to sperm cells function (Huszar et al. 2006).
According to WHO data from 2010, a male with less than 15 million sperm cells per 1 ml of ejaculate is considered to be potentially infertile. Various factors affect the fertilizing ability of the sperm cells; morphology, motility and genetic defects of the sperm cells, mechanical trauma, various infections and other testicular factors (Cooper et al. 2010). Although rapid and easily done, seminal fluid analysis is not the only test in determining the fertilizing ability of a male patient.
Besides the routine seminal fluid analysis, further testing is required in assessing sperm cells functional status (Huszar & Vigue 1994). Various studies indicate how sperm cells function is often linked to increased activity of key enzymes, one of those enzymes being CK (Huszar, Vigue, Morshedi 1992, Geraci & Giudice 2005, Menkveld 2007).
Enzymes in seminal fluid are not directly responsible for loss of sperm cells function, but act as biochemical markers for normal differentiation of sperm cells. Ezymes present in the seminal fluid are therefore good indicator of functional metabolic activity of the sperm cells (Guerin et al. 1979).
From our study it is clear how CK activity in seminal fluid does represent a valid biochemical marker for decreased sperm cell count, decreased sperm cell motility and decreased fertilizing potential in men. Higher CK activity in human seminal fluid is a marker for cytoplasmic residues in immature sperm cells. Higher CK activity in seminal fluid has been previously found in sperm cells with cytoplasmic residues (Huszar & Vigue 1993, Dokras et al. 1999). CK activity in seminal fluid is several times higher than that of serum, which indicates CK is locally created by gonads also (Huszar & Vigue 1993).
Our results obtained with 200 subjects divided by age show how patients aged 25-30 years are mostly oligozoospermic, representing the largest group of infertile subjects. As the results of this study relate only to patients of Albanian ethnicity, results differ from previously published (Jale 2008). Previously published results showed infertility in oligozoospermic men is highest at 20 to 29 years of age.
There is a prolonged period of infertility in men with lower spermiogram parameters. The lowest those parameters are, infertility period is longer and the condition is harder to treat. Subjects age and duration of infertility are positively correlated with changes in spermiogram parameters. Results linked to infertiliy period are given in Table 1, and are in accordance with the results of other authors (Geraci i Giudice, 2005).
Results from ejaculate analysis are in full accordance with 2010 WHO criteria describing normal parameters for spermiogram analysis of normospermic ejaculate samples. Other subject groups; B, C, and D; are categorized as infertile based on the same criteria, with reduced sperm cell count (oligozoospermia), reduced sperm

cells number and motility (oligoasthenozoospermia) and absence of sperm cells in seminal fluid (azoospermia).

Statistically significant difference is observed between seminal fluid samples from above mentioned subject groups of infertile patients based on sperm cells number, seminal fluid volume and sperm cells motility and morphology.

Results of this study regarding the CK activity in seminal fluid indicate there is a correlation between reduced spermiogram parameters and the measured CK activity in seminal fluid of fertile and infertile subjects. As the normal values of CK activity in seminal fluid of fertile subjects, values of 108-238 IU/10^8 sperm cells are taken. Results show statistically significant differences ($p<0,005$) between groups of fertile and infertile subjects. CK activity in samples of azoospermic patients is highest compared to the other infertile subject groups, which may be caused by CK locally synthesized by gonads, mostly prostate, so the CK activity in seminal fluid could be used as a prostate fuction marker. These results are similar to those published by Hallak et al.

With lower sperm cells number, CK activity in seminal fluid is higher ($p<0,005$). In our study a difference in CK activity is observed between patients with mild, moderate and severe oligozoospermia. Results confirming high CK activity in different stages of oligozoospermia are also published by Huszar G, Corrales M. and Vigue L. (1988).

Azoospermia represents an absence of sperm cells in ejaculate. Based on etiology, it can be obstructive and unobstructive (WHO, 2010). We have analyzed the CK activity in seminal fluid of patients with azoospermia in regards to the type, and we found both the higher CK activity in azoospermia compared to other groups of infertile patients and the statistically significant difference in CK activity between different types of azoospermia ($p<0,005$). The measured CK activity is significantly higher in cases of unobstructivne azoospermia (582,0±29,5 IU/10^8 sperm cells), compared to cases with obstructive azoospermia (557,4±18,8 IU/10^8 sperm cells). Similar results are published by Hallak et al. (2001).

Normal values for gonadotropic hormone concentrations have been observed in fertile subjects with normozoospermic seminal fluid samples, and with oligozoospermia and asthenozoospermia those values are higher compared to fertile subjects group. The highest FSH and LH concentrations were found in patients with azoospermia. Contrary to hormone concentrations, CK activity was found to be increased in all three groups of infertile subjects, in accordance to previously published data by Jalala (2008).

Infertility as defined by WHO is classified as primary or secondary infertility. Most infertile subjects involved in this research suffered from the primary infertility. They have been unable to conceive a pregnancy after two years of regular intercourses (Sigman et al. 1997, WHO 1999, WHO 2010). Significant differences have been observed in CK activity in seminal fluid samples between patients with primary and secondary infertility. Subjects with primary infertility had increased CK activity in the seminal fluid, compared to those with secondary infertility. Primary infertility patients included patients with azoospermia, oligozoospermia and oligoasthenozoospermia. Patients with secondary infertility included patients with oligozoospermia and oligoasthenozoospermia. This is in accordance with the results published previously by Hallak et al.

Biochemical method used in this research measures CK enzymatic activity in human seminal fluid and is done using dry chemistry principles. The method is simple, gives results in short period of time, is cost effective and reliable. Therefore, assessing the CK activity in seminal fluid constitutes a useful additional biochemical marker in determining the patient's fertility potential.

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