Effect of Men Infertility on Serum Creatine Kinase Activity

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Abstract

The present study was carried out on 60 patients with male factor infertility compared with 60 healthy controls, their ages ranged between 20-56 years in Maternity and Childhood Teaching Hilla Hospital and in a biochemistry laboratory of Pharmacy college of Babylon university, between June 2014 – Junuary 2015. The purpose of this study is to investigate CK activity, serum creatine, creatinine levels and semen parameters in infertile men only ,infertile with DM, smoking ifertile, and hypertention with infertile patients. The present results showed a statistically significant deferences ($p<0.05$) in creatine kinase activity, creatine and seminal characteristics while non significant in creatinine and in Abnormal sperm morphology % .

There were statistically significant deferences between the biochemical and seminal parameters in diabetes , hypertension and smoking patients and to control group at a p value ($P<0.05$). There were no observed significant differences in creatinine and Abnormal sperm morphology % of smokers compared to control group ($P>0.05$). The same results were obtained in diabetic and hypertension when compared to control group ($P<0.05$). Our results indicated that the diabetes , hypertension and smoking reduce serum CK creatinine and semen parameters in male infertility . Enzymetic activity of CK in serum is a biochemical marker in determining infertility and this biochemical marker will represents an important diagnostic feature with seminal parameters in the future.

Keywords: Creatine kinase **,** infertility, sperm and semen analysis.

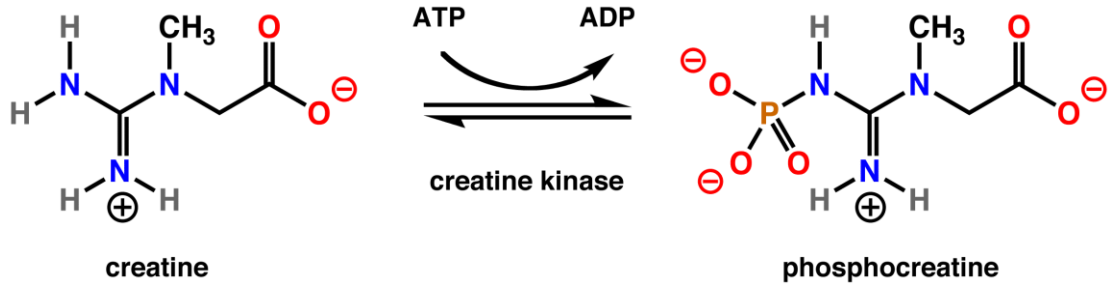
الخلاصة

العقم هو عدم قدرة الرجال على التسبب في الحمل في الإناث الخصبة. اجريت هذه الد راسة على 60مريضاً من الذكور ممن يعانون من العقم مقارنة مع 60 من الاصحاء, تتراوح اعمارهم بين 20–56 سنة في وحدة علاج العقم في مستشفى الحلة للولادة والاطفال وفي مختبر الكيمياء الحيوية في كلية الصيدلة بجامعة بابل وللفترة من حزيران 2014 – كانون اول 2015. الغرض من هذه الدراسة هو للتحقيق في نشاط انزيم كرياتين كاينيز، ومستويات الكرياتين، الكرياتينين في السيرم و نتائج فحص السائل المنوي في الرجال المشخصين بالعقم مع تحديد معاناتهم من بعض الامراض المزمنه و كذلك المدخنين . بينت هذه الد راسة وجود اختلافات ذات دلالة إحصائية ($P <0.05$) في فعالية انزيم كرياتين كاينيز والكرياتين و نتائج فحص السائل المنوي في الاشخاص الذين يعانون من مرض السكري,والتدخين , وارتفاع ضغط الدم. بينت التنائج في هذه الد راسة لم يكن هناك فروق معنوية ملحوظة في الكرياتينين وشذوذ الحيوانات المنوية الشاذة٪ في مرضى السكري,والمدخنين , وارتفاع ضغط الدم مقارنة مع مجموعة السيطرةعند ($P <0.05$) . هناك انخفاض في مستوى كرياتين كاينيز في مصل الدم في الاشخاص الذين يعانون من العقم و لم تكن هناك فروق معنوية ملحوظة في الكرياتينين وشذوذ الحيوانات المنوية الشاذة٪ من المدخنين مقارنة بمجموعة السيطرة ($P> 0.05$).و تم الحصول على نفس النتائج في مرضى السكري وارتفاع ضغط الدم عند المقارنة مع مجموعة السيطرة ($P <0.05$). وأظهرت نتائجنا أن مرضى السكري، وارتفاع ضغط الدم والتدخين يقلل المصل كرياتينين والمعلمات السائل المنوي في العقم عند الذكور. النشاط الإنزيمي من الكرياتنين كاينيز في المصل هو علامة البيوكيميائية في تحديد العقم وهذه علامة البيوكيميائية تمثل ميزة التشخيص الهامة مع المعلمات المنوية في المستقبل.

الكلمات المفتاحية: الكرياتين كيناز، العقم، الحيوانات المنوية وتحليل السائل المنوي

Introduction

Infertility is a medical condition characterized by an inability men to cause pregnancy in a fertile women. Approximately 50% of infertile couples are related to male factor. Infertility is common among couples with childbearing age (Sidhu *et al.*, 1998). Infertility is a growing problem in the world. In 2010, an estimated 48.5 million couples worldwide were infertile (Mascarenhas *et al.*, 2012). The etiology of male factor infertility is poorly understood. Male factor infertility is a common condition with unknown etiology in most of the cases . One of the reasons that lead to infertility in men is the abnormality of Sperm , as well as a number of diseases and lifestyle-related and the effect on fertility are linked to and the effect on fertility in men, such as obesity, diabetes and smoking (Hirsh , 2003; SANDRO *et al.*, 2012; Gaur *et al.*, 2007). Creatine kinase (CK) is a mitochondrial and cytosolic enzyme. This enzyme catalyzes the conversion of creatine to phosphocreatine and consumes adenosine triphosphate (ATP) and adenosine diphosphate (ADP) as follows: (Oda *et al.*,2010)



The CK enzyme is distributed in various organs and cell types such as: brain, spermatozoa, skeletal, heart muscle, retina , hair cell of the inner ear, smooth muscle, and nervous systems (Maysoon, 2012). CK is an important enzyme in tissue cell that consumes ATP rapidly. This enzyme supplies ATP to the sperm. Its biological role is to provide an ATP buffering system for tissues that require large amounts of energy (Ghassan and Hedef, 2009). Many Studies show that the phosphoryl creatine and ATP shuttle are important energy sources for sperm (Miyaji *et al.*, 2001). Thus CK is an important enzyme in sperm. Serum creatinine is an important indicator of renal health. Creatinine is produced via a biological system involving creatine, phosphocreatine , and adenosine triphosphate. It is removed from the blood by the kidneys (Allen *et al.*, 2012). Creatine is synthesized primarily in the liver and then transported through blood to the other organs, muscle, and brain, where, through phosphorylation, it becomes the high-energy compound phosphocreatine (Taylor, 1989). Creatine conversion to phosphocreatine is catalyzed by creatine kinase; spontaneous formation of creatinine occurs during the reaction (Mcleish and Kenyon, 2005).

A central compound in the energy metabolism of cells in tissues with a highly fluctuating energy demand is Creatine. The non-enzymatic conversion of creatine to creatinine, which is finally excreted in urine, the creatine body pool must be maintained by de novo synthesis and nutritional intake. The de novo synthesis is mainly localized to liver, kidney, and pancreas (Wyss and Kaddurah-Daouk, 2000). The creatine/phosphocreatine system is an essential part for cellular phosphate coupled energy storage and production, especially in tissues subject to high metabolic demands. This system is important to transfer energy from mitochondria

to the flagellum, which is essentially for the swimming of sperms. Therefore, we propose that CK has an important role in sperm movement. The aim of this study was to determine the mean concentration of serum CK and semen parameters of infertile males and to compare the result of serum CK concentration with semen parameters between infertile males and healthy normal fertile volunteers (control group). This research was trying to examine differences in serum CK , creatine and creatinine levels between normal healthy donors and infertile patients with various diagnoses , to determine the link between these levels and the quality of sperm in DM , smoking and hypertention infertile males.

Materials and Methods

Subjuct selection

The study was done during the period from June 2014 to Junuary 2015. All mesurments were done in a clinical biochemistry laboratory of college Pharmacy, university of Babylon. The study sample include 60 infertile patients aged from 20 to 56 years , and 60 apperantly healthy volunteers in the same ages range as control group. This study involved patients and healthy subjects were invistigated for the enzymes activity of CK , creatine, creatinine and semen analysis .

CK was determined according to Biolabo manufacture kit [CK-NAC].The principle of kit is enzymetic method described by Oliver and modified by Rosalki and later by Szasz(Szasz *et al.*, 1976). Creatinine was determined according to Biolabo manufacture kit (Jaffe's reaction , colorimetric reaction) and involving the alkaline sodium picrate method, is the widely accepted for creatinine measurement (Tietz, 1999). Creatine was determined by The non-enzymatic method genemlly employed in biological samples lack specificity, because of interference from sever& compounds normally present. The determination of creatinine and creatine in serum is based on the Jaffh reaction after conversion of creatine into creatinine.The mathmaticall relation between creatine and creatinine is described by ratio of molecular weight of creatine to molecular weight of creatinine.

Semen collection and preparation

Samples of semen ejaculate were collected from all married patients and volunteers in laboratory of Maternity and Childhood Teaching Hilla Hospital, and brought within 20 minuts into a clean aseptic vails. After ejaculation the specimens were allowed to liquefy at 37°C for 30 minutes before the sperm characteristics (concentration, motility, and morphology) were evaluated. seminal fluid analysis was performed to measure sperm concentration, sperm morphology, sperm motility in accordance with the recommendations of the World Health Organization (WHO) (World Health Organization, 1999). Seminal plasma was separated by centrifugation at 2000 x g for 10 minutes at room temperature. The supernatant was removed immediately and kept in 20ºC. The specimen and all microscopic and macroscopic examinations were examined according to WHO criteria.

The semen samples were liquefied after 30 minuts at room temperature. Semen samples were placed at 37°C for liquefaction, followed by routine semen analysis, and the remaining semen samples were centrifuged at 500xg for 30 minutes. The upper layer seminal plasma was collected for the determinations of biochemical markers. The supernatant was measured by a centrifuge test tube and it was used for enzymatic measurements (Dandekar and Parker ,1999).

Blood samples were centrifuged at 3 000 ×g for 5 minutes to isolate serum for the same analyses as for seminal plasma. For each measurement a 5µL aliquot was loaded on a 20- µm counting chamber (MicroCell, Conception Technologies, Inc La Jolla, CA) and analyzed for motility , sperm count , Sperm concentration and Motility were verified manually by Olympus BH2-S microscope Olympus; Tokyo, with a X20 positive phase-contrast objective. The WHO criteria for sperm normality used were as follows: sperm concentration ≥20 millions/mL of ejaculate, percentage of sperm motility ≥50% and normal sperm morphology ≥30%.

Statistical analysis.

All data collected from patients and control groups were analysed using SPSS program version 15. The data were analysed as mean and standard deviation (SD), also the significant value was examined as $p<0.05$.

Results and Discussion:

The analysed data for serum of infertile patients show a decrease in both CK activity and creatine concentration with respect to control group, while there is no change in creatinine conc. , as shown in table (1). It seems that the CK activity decrease from 98.45±.20.595 in control group to 70.5 + 6.128 IU/L in patients group, at the same time creatine conc.was decreased from 0.908±0.123 to 0.76 ± 0 .157 in both control and patient groups respectively ,The results in table(1) indicate significant difference at ($P<0.05$) .

Table (1): The levels of CK activity, creatine and creatinine in serum of infertile patients compared with healthy controls .

	Ck activity(IU/L)	Creatine(mg/dl)	Creatinine(mg/dl)
Control	**98.45±.20.595**	**0. 908 ± 0.123**	**0. 827±.432**
Patients	**70.5 ± 6.128***	**0.76 ± 0 .157***	**0.862 ± .0118**

*** Significant value less than P < 0.05.**

No available data are present to compare our results with other studies. The activity of creatine kinase (CK) in serum has been observed in a variety of clinical conditions. These results may be found as a consequence of diminished efflux of the muscle enzyme in serum from reduced physical activity caused by illness or advanced age or may result from reduced muscle mass accompanying muscle wasting or cachectic states(Sidney ,1998).

The present results in Table (2) showed a comparison between seminal characteristics in infertile male and control groups .The present data revealed that there was a statistically significant differences ($P<0.05$) of sperm cell count per 1 ml of seminal fluid and seminal fluid volume when compared with control group. Also differences are seen in the other seminal parameters as well: Sperm active motility, Sperm sluggish motility, Normal sperm morphology % and sperm concentration per 1 ml when compared with control group.There are no significant differences in Abnormal sperm morphology percentage when compared with control group. semen analysis is an essential of the laboratory evalution of the infertile men and it still

provides the fundamental information on which clinicians base their initial diagnosis , so it is imperative that it is performed as accurately as possible.

The results in the table (2) showed a comparison between seminal characteristics in infertile male and control groups . Sperm concentration, sperm cell count, percentage of Sperm active motility , Sperm sluggish motility and seminal fluid volume in sperm decreased differences at ($P<0.05$), and Normal sperm morphology percentage was significantly higer than control group. There are no significant differences in abnormal sperm morphology percentage when compared with control group. The seminal analysis and that CK activity in serum have help to define the severity of the male factor. A low sperm count and the decreased in thep perm motility (movement) which indicates the Sperm abnormalities. our results agreed with other studies (Sallmen *et al.*, 2006; Magnusdottir *et al.*, 2005; Ahmed *et al.*, 2012). There is no significant effect about the sperm abnormality, so we suggest for more future study using other parameters . They are a critical factor in male infertility.

More than 90% of male infertility cases are due to low sperm counts, poor sperm quality, or both. The remaining cases of male infertility can be caused by many conditions, including anatomical problems, hormonal imbalances, and genetic defects. Aging also can reduce sperm counts and motility. If less than 40% of sperm are able to move in a straight line, the condition is considered abnormal. Sperm that move sluggishly, these results may be due to genetic or other defects that render them incapable of fertilizing the egg. Poor sperm motility may be associated with DNA fragmentation and may increase the risk of passing on genetic diseases.

Table(2)*: seminal characteristics in infertile male and control groups.*

Sperm variables	Infertile male Mean ± Sd.	Control group Mean ± Sd.
Total count $x10^6$/ml	41± 3.58*	60.33±5.13
Sperm active motility %	23.5± 15.37*	65.61±1.85
Sperm sluggish motility %	11.667±6.416*	30± 2.8
Normal sperm morphology %	48.46+2.96*	37.38± 2.5
Abnormal sperm morphology %	36.53±2.96	34.61±2.56
Volume(ml)	2.02± 1.23*	4.1± 0.08
Conc 10^6/semen volume	26.6±19.39*	67. 4±25.9

*** Significant value less than P < 0.05**

Abnormal Sperm Morphology refers to shape and structure. Abnormally shaped sperm cannot fertilize an egg. In our findings , there are no significant differences in Abnormal sperm morphology % when compared with control group, that's mean no Abnormally shaped sperm . Lower amounts of volume and concentration can be a sign of prostate problems, blockage, or retrograde ejaculation. Abnormal results may suggest prostate gland problems or lack of sperm. The volume of the semen sample, approximate number of total sperm cells, sperm motility, and % of sperm with normal morphology are measured. This is the most common type of fertility testing

Table(3)*:* Biochemical and seminal parameters in diabetes , hypertension and smoking patients compared with healthy controls.

Variables	Smoking infertile male Mean ± Sd.	Hypertension infertile male Mean ± Sd.	Diabetic infertile male Mean ± Sd.	Control group Mean ± Sd.
Total count $x10^6$/ml	35.5±3.06*	43.4+3.11*	48.2±3.4*	60.33±5.13
Sperm active motility %	24.416±17.33	22.23±14.85*	23.4286±15.06*	65.61±1.85
Sperm sluggish motility %	12.0833±6.5*	10.88± 5.66*	11.42± 5.34*	30± 2.8
Normal sperm morphology %	48.5± 2.1*	47. 6+2. 5*	48. 6+2. 6*	37.38± 2.5
Abnormal sperm morphology %	35.1±2. 4	36.2±2. 5	36.5±2. 6	34.61±2.56
Volume(ml)	2.4231+ 1.15*	1. 7±0.9*	1.607±0.9441*	4.1± 0.08
Conc 10^6/semen volume	25.15±14.51*	27.4+18.97*	31.75+18.34*	67. 4±25.9
Ck activity (IU/L)	50.23±10.52*	72.82+6.22*	64.357±5.676*	98.45+.20.595
Creatine(mg/dl)	0.687±0.036*	0.75±0.16*	0.725±0.131*	0. 908+0.123
Creatinine (mg/dl)	0.831±0.083	0.857±0.12	0.827±0.091	0. 827+.432

* **Significant value less than P < 0.05**

Table(3) showed there were significant deferences observed between the biochemical and seminal parameters in diabetic , hypertention and smoking patients with compared to control group at p value (P < 0.05). There were no observed significant differences in creatinine and abnormal sperm morphology % of smokers, diabetic and hypertention with compared to control group (P < 0.05). The main cause of male infertility is low semen quality. In men who have infertility can be caused by low sperm count due to endocrine problems, drugs, radiation, or infection. There may be testicular malformations, hormone imbalance, or blockage of the man's duct system (Mishail *et al.,* 2009).

Today diabetes mellitus has emerged as a major healthcare problem throughout the world. Diabetes mellitus (DM) is known to cause many systemic complications including male reproductive dysfunctions and infertility. Several clinical studies have focused on the molecular mechanism responsible for the alterations induced by DM in male reproductive potential including endocrine disorders, neuropathy, and increased oxidative stress (Thompson and Bannigan, 2008).

Our results showed that diabetes can lead to reduced sperm quality due to deficiencies in the semen quality is used as an important measure of male infertility. Our findings were agree with Garcia-Diez et al.was stated that type 1 diabetes mellitus (insulin-dependent) lowers seminal fluid volume, the concentration,

motility, and the proportion of normal shape spermatozoa (Garcia-Diez and Corrales-Hernandes, 1991). Sexual dysfunction in all its forms (reduced erection, impotence, and other libido dissociations) is an accompanying phenomenon of the diabetic disease. Testicular dysfunction, impotence, decreased fertility potential and retrograde ejaculations are conditions that have been described in diabetic males. Diabetes is also the most common cause of erectile dysfunction in men.

Poor semen quality has also been reported in diabetic men, including decreased sperm motility and total count. Because sexuality and fertility are important aspects in the lives of individuals and couples, and considering that over 177 million individuals worldwide suffer from Dia betes (Agbaje *et al.*, 2007). This study highlighted the diabetes cause implications for sexual problems. Diabetes is a well-recognized cause of male sexual dysfunction , which in itself may contribute to subfertility. The results of this study showed a decrease in semen volume, sperm count, and sperm motility in smokers infertility patients compared with non-smokers. Smoking has been caused of death in our society and the most important public health issue of our time and Tobacco smoking is killing 1 in 10 adults in worldwide (Ng *et al.*, 2014).

Lifestyle factors such as smoking and substance abuse can lead to problems with fertility in men. Chemicals such as : nicotine, cyanide, and carbon monoxide, in cigarette smoke effect on the rate of sperm. Male smokers can suffer decreased sperm quality with lower counts (numbers of sperm) and motility (sperm's ability to move) . Smoking might also decrease the sperm's ability to fertilize eggs that's mean may cause infertility (Dai *et al.*, 2015).

A number of studies have shown that the harmful products in tobacco damage the testicles and kill sperm (Thompson and Bannigan, 2008 ; Agarwal *et al.*, 2005; Robbins *et al.*, 2005). Many studies were similar to our study in that smoking reduces semen quality (Zhang *et al.*, 2000; Gaur *et al.*, 2007). The findings of this study shown that CK activity in serum , sperm cells and total semen significantly decreased with smoking. The present study has been suggested that harmful components of tobacco smoke are able to pass through the blood- testis barrier and damage the sperm. Ghaffari et al. (Ghaffari *et al.*, 2008) have suggested that some cigarette components such as nicotine, cotinine and cadmium can decrease human sperm CK activity in an *in vitro* model but in this study ,we have demonstrated that ck activity in serum was decreased . The our study showed that the smoking affect CK activity in serum, sperm count cells, volume ,creatine, concentration and normal morphology.

These resuls indicated that exposure to smoke can diminish sperm motility via inhibition of CK activity and creatine. As sperm motility depends on intact mitochondrial function and energy levels. Thus reduced intracellular creatine stores may contribute to decreased sperm motility leading to male infertility. The findings in our study showed that the sinificant deferences of CK activity in serum, sperm count cells, volume ,creatine, concentration and normal morphology in patient with hypertension (high blood pressure) and DM , but abnormal sperm morphology % and creatinine showed no deferences in those patients. Several studies demonstrated that the hypertension in men could be associated with impaired reproductive potential(Fogari *et al.*, 2002).

Finally , from this viewpoint , the present study suggests that it is necessary tofocus on the possible effects of DM , smoking and hypertention as an etiology of male infertility in men and the cause of reduced fertility may accompanied in a fact of existing to the decrease concentration of serum CK and semen quality .

Conclusion

Enzymatic activity of CK in serum is an important biochemical marker in determining nfertility and this biochemical marker is represents an important diagnostic feature. We found from the biochemical and seminal parameters in the diabetic , hypertention and smoking cause impaired sperm quality and CK levels in male. As a consequence, this effect may be one of the several important factors that possibly cause infertility in male. This research was performed to discuss the relation between diabetes, hypertension and smoking with male infertility. In this study we found that there was a decrease in the sperm quality and CK levels in serum.

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