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Serum Creatine Kinase and its Isoenzyme CK-MB in the Prediction of Tubal Ectopic Pregnancy

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Abstract

Background Ectopic pregnancy is a major cause of maternal morbidity and mortality. Creatine kinase is an enzyme that its increase reflects tissue injury and could be useful in the diagnosis of tubal pregnancy.

Objectives To evaluate the diagnostic value of total creatine kinase in women with ectopic pregnancy, tubal rupture ectopic pregnancy, spontaneous abortion, and normal pregnancy and to investigate the possible discriminatory ability of creatine kinase-MB for diagnosis of tubal rupture ectopic pregnancy.

Methods Forty women with ectopic pregnancy, 17 with intrauterine abortion and 24 women with normal gestation were studied. The diagnosis of ectopic pregnancy was based on clinical assessment and transvaginal ultrasonography. Serum human chorionic gonadotropin levels were measured by enzyme linked immuno-sorbent assay. Total serum creatine kinase and creatine kinase-MB values were determined by spectrophotometrical analysis.

Results Creatine kinase and creatine kinase-MB levels were significantly higher in tubal ectopic pregnancy compared with both intrauterine abortions and normal gestations. When using creatine kinase-MB of 4.55 IU/ml as a cut-off value for the diagnosis of tubal ectopic pregnancy from control groups, sensitivity 81.64%, specificity 84.3%, positive predictive value 88.5% and negative predictive value 71.4%. Creatine kinase level in the ruptured ectopic pregnancy group was significantly higher than in the unruptured ectopic pregnancy, and normal pregnancy. When using creatine kinase of 29.43 IU/ml as a cut-off value for the diagnosis of ruptured ectopic pregnancy from unruptured groups, sensitivity 92%, specificity 100%, positive predictive value 100%, negative predictive value 96% and efficiency 97.4%.

Conclusions Women with ectopic had a significantly higher levels of creatine kinase-MB compared with women with normal pregnancy or intrauterine abortion and it has a high discriminatory ability for diagnosis of tubal rupture ectopic pregnancy.

Keywords Ectopic pregnancy, creatine kinase-MB

Introduction

Ectopic pregnancy (EP) is a major cause of maternal morbidity and responsible for 6% of pregnancy deaths [1]. Distinguishing normal from abnormal pregnancies is a clinical challenge because there is no definitive noninvasive diagnostic test available before visualization on ultrasonography. Clinicians must therefore follow patients over the course of several days to weeks for diagnosis [1,2], a time in which there is some potential for the ectopic pregnancy to rupture and result in life-threatening intra-abdominal hemorrhage. Early treatment may also allow for tubal-conserving procedures to be used, which is important for a patient's future fertility [3,4]. Therefore,

development of a serum test to diagnose an ectopic pregnancy with high accuracy would be of great clinical significance.

Creatine kinase (CK) is an intracellular enzyme that catalyzes the formation of adenosine triphosphate (ATP) from creatine phosphate and adenosine diphosphate (ADP). It is therefore abundant in metabolically active tissues with significant energy demands, specifically skeletal and smooth muscle, myocardium, and brain [5].

Three distinct isoenzyme forms of CK have been identified, namely, CK-MM, MB, and BB (M: muscle, B: brain) [5]. Because an increase of CK plasma concentration always reflects injury to a tissue of high CK activity, CK measurements are particularly useful in the diagnosis of acute myocardial infarction, in which determination of CK-MB isoenzyme levels is much more specific than total CK [6,7].

In tubal pregnancy, the zygote penetrates the tubal epithelium and lies next to the muscular layer as the fallopian tube lacks a submucosal layer. This invasion into the muscle causes an increase in muscle cell creatine kinase (CK) in blood [8].

The extent of penetration into the muscle will depend upon the site of implantation. In 1993, Lavie *et al* [9] reported that an initial maternal serum CK was predictive of tubal pregnancy in first trimester. Subsequently, three studies [10-12], were able to reproduce their findings.

Another study by Kurzel *et al* [13] found an elevated mean CK level but with questionable clinical utility and four studies [14-17] reported no elevation in serum CK in tubal pregnancy.

The current study was designed: (a) to further evaluate the diagnostic value of total CK in women with EP, spontaneous abortion, and normal pregnancy; (b) To determine, whether serum CK level might be a marker for diagnosis of tubal rupture ectopic pregnancy; and (c) To measure CK-MB isoenzyme concentrations in the previously mentioned samples and to investigate the possible discriminatory ability of MB fraction.

Methods

Forty women with EP, 17 intrauterine abortive and 24 women with normal intrauterine gestation (controls) were followed up at Al-Kadhimyia Teaching Hospital, Baghdad, Iraq, between November 2010 and June 2011. Descriptive characteristics such as the age, height and weight of the patients were taken.

Diagnosis of EP was based on clinical assessment and transvaginal ultrasonography. All EP were treated by laparotomy and confirmed by histopathology. From all women, blood was drawn by routine venipuncture. Blood samples were centrifuged at 3000 rpm and sera were stored at -20 ^{o}C.

Exclusion criteria were the absence of any medical disorder that would raise the serum CK. Inclusion criteria were the confirmation of intrauterine pregnancies in the control group and abortive group with a positive serum human chorionic gonadotropin (hCG). For the cases with ectopic pregnancy; diagnosis should be confirmed by transvaginal ultrasound and a positive hCG.

Human chorionic gonadotropin (hCG) levels were measured by monoclonal antibody Enzyme Linked Immuno-Sorbent Assay (ELISA) techniques for follow up or confirmation of the diagnosis. Total serum creatine kinase and CK-MB values were determined by spectrophotometric analysis.

Values are presented as mean ± standard error for mean (S.E.M.). Comparison of means between different groups was performed with Student's t test.

Receiver Operator Characteristic (ROC) curves was constructed to plot sensitivity against specificity.

The areas under the ROC curves (AUC) were calculated and compared with the AUC (0.5) of the non-diagnostic test (the line with slope of 1). For cut-off values of significant sensitivity and specificity (> 70%), contingency tables (cross-tabs) were constructed for the calculation of positive and negative predictive values. Confidence intervals of sensitivity, specificity,

positive and negative predicted values were calculated.

Statistical analyses were performed by SPSS software (v. 11.5) and also Excel 2007. P value < 0.05 level of significance was considered statistically significant.

Results

The basic anthropometric and clinical parameters of the women studied are presented in table 1. In our study, there were no statistical significant differences between the groups regarding maternal age and body mass index (BMI). The age of tubal ectopic pregnancy (group A) was 28.97 ± 0.957 years, it was 32.05 ± 1.95 years in IU abortion (group B) while, in normal pregnancy (group C) was 25.8 ± 1.139 years. The BMI was 26.27 ± 0.735 Kg/m^2 in group (A) while it was (28.47 ± 1.08 and 24.98 ± 1.278 Kg/$m^{2)}$ in groups B and C, respectively. In gestational age there were no statistical significant differences between the groups between women with tubal EP and women with normal pregnancy, and there were statistical significant differences (P < 0.001) between tubal EP and women with IU abortive pregnancy. The gestational age was 6.425 ± 0.142, 10.394 ± 0.6 and 6.54 ± 0.26 in groups A, B and C, respectively.

Table 1. Basic anthropometric and clinical parameters of the studied women

Parameter	Ectopic pregnancy N = 40	IU abortion N = 17	IU normal N = 24
Age (yrs)	28.97 ± 0.957	32.05 ± 1.95	25.8 ± 1.139
BMI (kg/m2) Pregnancy	26.27 ± 0.735	28.47 ± 1.08	24.98 ± 1.278
period (weeks)	6.425 ± 0.142	10.394±0.6*	6.54±0.226

IU = intrauterine, * = P < 0.05 (ectopic pregnancy *Vs* IU abortion)

The ROC curves shown in table 2 and figure 2 demonstrated a significant discriminatory ability of increased total creatine kinase levels for the diagnosis of tubal ectopic pregnancy. The AUC for total creatine kinase was 0.903 (95%CI: 0.831–0.975). A significant difference was found in EP (P < 0.001).

When using total creatine kinase concentration of 22.22 IU/ml as a cut-off value for the diagnosis of ectopic pregnancy from control groups, sensitivity was 68.4%, specificity 100%, the positive predictive value was 100% and the negative predictive value 66.66%.

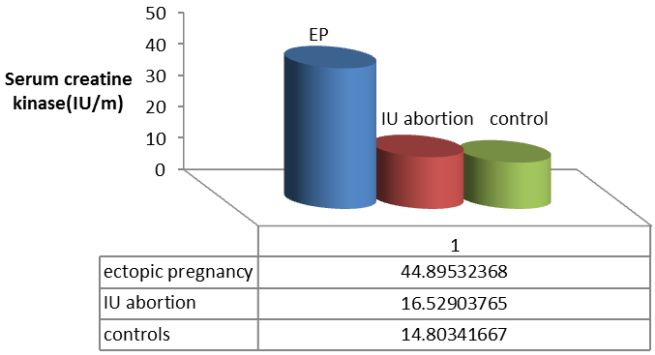
Table 2. AUC for ROC analysis of CK and CK-MB with testing for statistical differences

Enzyme	AUC ± SEM	95% CI*	*P value*
CK	0.903 ± 0.037	0.831 - 0.975	P < 0.001
CK-MB	0.938 ± 0.031	0.878 - 0.998	P < 0.001

* CI= Indicate to confidence interval.

Fig. 3 shows that the mean S.CK-MB levels were significantly higher in women with tubal EP compared with those of women with normal pregnancy (P < 0.0001) and (P < 0.001) IU abortion. A slight difference was also observed between women with abortive IU pregnancy and controls (P < 0.05).

The ROC curves demonstrated a significant discriminatory ability of increased CK-MB levels for the diagnosis of tubal ectopic pregnancy. The AUC for CK-MB was 0.983 (95%CI: 0.878–0.998). A significant difference was found in tubal EP (P < 0.001) as shown in fig. 4 and table 2.



	1
ectopic pregnancy	44.89532368
IU abortion	16.52903765
controls	14.80341667

Fig. 1. Levels of serum creatine kinase in tubal EP, IU abortion and control groups

When using CK-MB concentration of 4.55 IU/ml as a cut-off value for the diagnosis of tubal ectopic pregnancy from control groups, sensitivity was 81.64%, specificity 84.3%, the positive predictive value was 88.5% and the negative predictive value 71.4%.

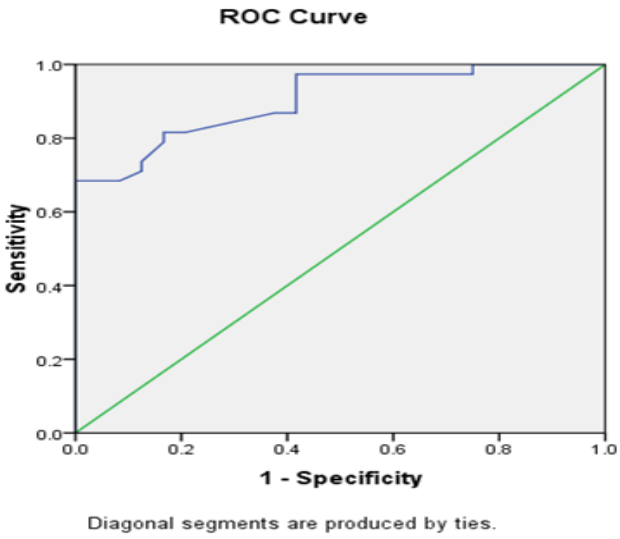
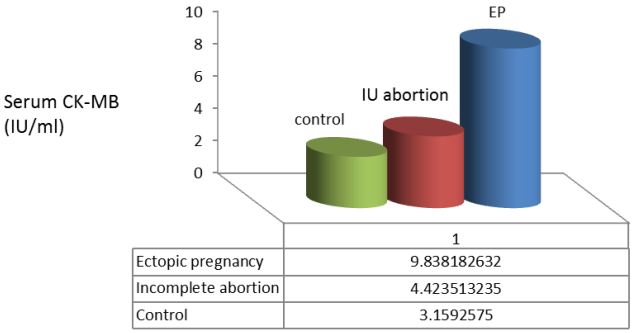


Fig. 2. Receiver Operator Characteristic (ROC) curves of increased total creatine kinase levels as diagnostic tests for ectopic pregnancy from control groups.

The concentration of creatine kinase (CK) and serum β-hCG levels in ruptured and unruptured of tubal ectopic pregnancy (EP) and control groups.

Fig. 5 shows the mean serum creatine kinase level in the ruptured ectopic pregnancy group was significantly higher than the levels in the unruptured ectopic pregnancy (P = 0.0001), and normal pregnancy (P < 0.0001) groups. No significant difference in β-hCG levels between ruptured and unruptured ectopic pregnancies.

The ROC curves demonstrated a significant discriminatory ability of increased CK levels in ruptured ectopic pregnancy from unruptured. The AUC for CK in ruptured was 0.974 (95% CI: 0.926–1.022). A significant difference was found in ruptured EP (P < 0.001).



	1
Ectopic pregnancy	9.838182632
Incomplete abortion	4.423513235
Control	3.1592575

Fig. 3. Levels of serum CK-MB in EP, IU abortion and control groups.

When using CK ruptured concentration of 29.43 IU/ml as a cut-off value for the diagnosis of ruptured ectopic pregnancy from unruptured groups, sensitivity was 92%, specificity 100%, the positive predictive value was 100%, the negative predictive value 96% and efficiency 97.4%.

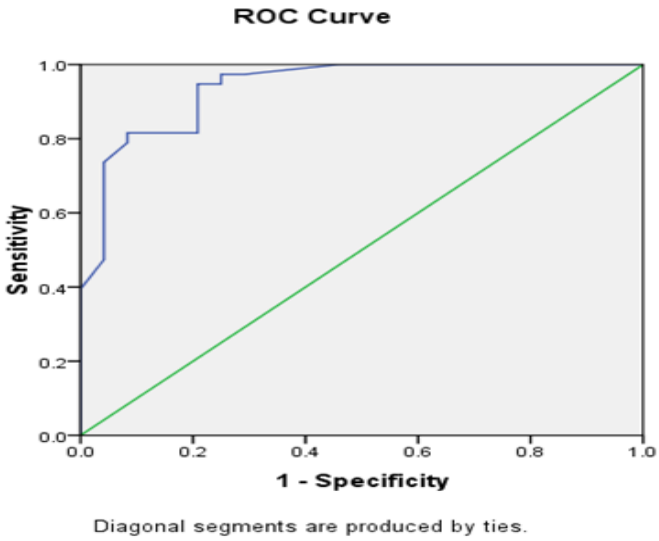
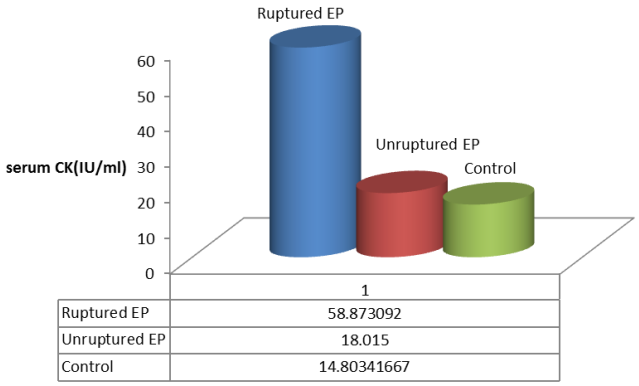


Fig. 4. Receiver Operator Characteristic (ROC) curves of increased CK-MB levels as diagnostic tests for tubal ectopic (EP) from control groups.

The ROC curves demonstrated a significant discriminatory ability of increased CK levels in ruptured ectopic pregnancy from control group. The AUC for CK in ruptured was 0.988 (95%CI: 0.964–1.013). A significant difference from the control was found in ruptured EP ($P < 0.001$).



	1
Ruptured EP	58.873092
Unruptured EP	18.015
Control	14.80341667

Fig. 5. Levels of serum CK in ruptured, unruptured EPs and control groups.

When using CK ruptured concentration of 23.3 IU/ml as a cut-off value for the diagnosis of ruptured ectopic pregnancy from unruptured groups, sensitivity was 96%, specificity 100%, the positive predictive value was 100%, the negative predictive value 96.9% and efficiency 97.9%.

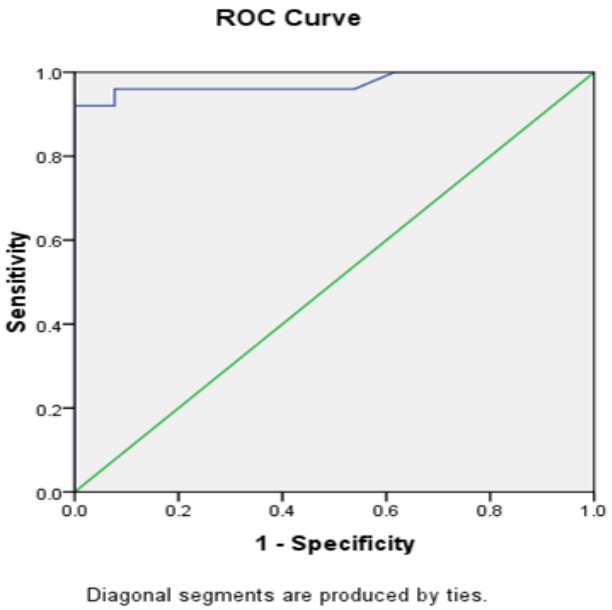


Fig. 6. Receiver Operator Characteristic (ROC) curves of increased CK in ruptured ectopic pregnancy levels from unruptured groups.

Discussion

Creatine kinase (CK) is an enzyme that is released when muscle becomes damaged [18]. The first study of CK as a marker of Fallopian tube damage produced some encouraging results [9].

In the current research, CK levels were significantly increased in women with tubal EP compared with both women with IU abortion ($P < 0.00$) and those with normal gestation ($P < 0.00$).

A rise in serum CK level is natural in tubal gestation, because the zygote penetrates the tubal epithelium and lies adjacent to the muscle layer which lacks a sub mucosa [8,10]. Due to invasion of the muscle layer by trophoblasts, the maternal blood vessels are eroded and blood leaks through the growing trophoblasts and damaged muscle layer giving a rise in muscle cell product like CK [8,10]. The pathology in missed abortions is different and there is no rise in serum creatine kinase [10], as is also evident from our results.

The present results are in agreement with Lavie *et al* [9], who studied the role of maternal serum CK levels as a predictor of tubal pregnancy. They found that serum CK levels were significantly higher in the tubal pregnancy group than in spontaneous abortion and normal pregnancy. Similar results were obtained by Saha *et al* [11] in their comparative study of 20 patients evaluated and endorsed the positive role of serum CK as a possible marker of tubal pregnancy. Develioglu and coworkers [19] conducted a comparative study on 32 cases and their results revealed that serum CK levels can be taken as an adjuvant tool in ruling out ectopic pregnancy, particularly if it was ruptured ectopic pregnancy. Yet another comparative study by Singh *et al* [8] on 15 patients revealed that CK levels were higher in tubal pregnancy than normal intra-uterine pregnancy. Several authors have, however, found conflicting results from Qasim *et al* and Vitoratos [14,17].

Also in this study serum CK concentrations were significantly higher in the patients with ruptured tubal ectopic pregnancy compared with

unruptured tubal ectopic pregnancy (P < 0.0001).

It is possible therefore that increased levels of creatine kinase associated with muscular damage may precede rupture of the tube. The positive correlation between gestational age and creatine kinase levels in ruptured tubal ectopic pregnancy is some evidence for this proposition as it shows that the increase in creatine kinase levels, and in the extent of tubal muscular damage that it marks, is a function of time, and not simply an end result of the eventual disruption of the tubal wall.

All the women, with ruptured tubal pregnancy had significant tubal damage and raised values of CK. These indicate that tubal rupture is associated with an increase in creatine kinase levels. These results are in agreement with Singh *et al* [8] in their study suggested that maternal CK levels are significantly higher in women with tubal pregnancy and are reliable in the diagnosis of a tubal pregnancy.

Because the differential expression of CK-MB isoenzymes varies significantly between different tissues [5,6], then it might be useful to estimate CK-MB levels separately. Notably, no previous studies on CK-MB fractions in tubal EP were found in the literature. Intriguingly, in present study women with EP were significantly higher CK-MB levels compared with the intrauterine abortion (P < 0.001) and normal intrauterine pregnancy (P < 0.0001).

Estimation of CK-MB with a cut-off value of 4.55 IU/L produces a good sensitivity 81.64%, specificity 84.3 % and 80.9 % efficiency, the positive predictive value was 88.5 % and the negative predictive value 71.4 % in diagnosis of tubal ectopic pregnancy.

Conclusively, the current study is the first to demonstrate that women with EP have a significantly higher of CK-MB levels compared with women with IU normal or abortive pregnancy. The exact reasons for the increase CK-MB relative level in tubal EP are at present unknown and remain to be elucidated.

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