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PYRUVATE KINASE ISOFORM M2 AS A DIAGNOSTIC TOOL FOR RENAL CELL CARCINOMA

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ABSTRACT

Renal cell carcinoma (RCC) is the most common cancer in the kidney. Pyruvate kinase is an enzyme that catalyzes the conversion of phosphoenol pyruvate and ADP to pyruvate and ATP in glycolysis. **Aim of the study:** The current study was conducted to evaluate pyruvate kinase M2 (PKM2) level in serum as a tumor marker in renal cell carcinoma. **Subjects and Methods:** serum PKM2 level of 120 patients with RCC and 80 healthy volunteers was detected by ELISA, and correlated with tumor stage and grade. **Results:** serum PKM2 level was significantly higher in RCC group than in control group**s.** There was a significant positive correlation between PKM2 and both of tumor stage and grade. ROC curve revealed that, at a cut -off point of (2.27 ng/ml), the sensitivity of PKM2 in diagnosis of renal cancer was 70%, the specificity was 68.27%, and accuracy of the test was 66.46%. **Conclusions**: Serum PKM2 could be considered as a diagnostic marker for RCC.

Highlights

- Hypertension and diabetes are risk factors for development of RCC
- Serum PKM2 is significantly increased in RCC group when compared to controls.
- Serum PKM2 may have a role in RCC staging , helping in clinical evaluation.

KEYWORDS: ELISA-PKM2-RCC-tumor.

ABBREVIATIONS

(RCC)Renal cell carcinoma
(PKM2) pyruvate kinase M2
(ADP) adenosine di-phosphate
(ATP) adenosine triphosphate
(BMI) body mass index
(ELISA) enzyme linked immunosorbent assay
(SEER) surveillance, epidomology and end result
(DM) diabetes mellitus
(HTN) hypertension
AUC= Area under the Curve
PPV= Positive predictive value.
NPV= Negative predictive value.

INTRODUCTION

Renal cell carcinoma (RCC) is the second common and the most lethal cancer in urinary system. It accounts for about 2-3% of all cancers. RCC originates within the renal cortex, is responsible for 80 - 85% of all primary renal neoplasms. The incidence of RCC varies widely (by as much as 10-fold) across the world with the highest rates reported in Europe and North America and the lowest rates in Asia and Africa. In USA, there are approximately 64,000 new cases and almost 14,000 deaths/ year from RCC.[1] In the European Union, there were about 84,000 cases of RCC and 35,000 deaths/ year due to renal cancer.[2] In Egypt, National Cancer Institute reported that RCC represent 0.8% of all newly diagnosed cancers and 6% of the newly diagnosed cancers in genitourinary system, the estimated number of RCC in 2015 was about 1528 patients and 1438 in 2013, the Incidence rates/100,000 population of kidney cancer in Upper Egypt 0.95% and 0.64%, Middle Egypt 1.53% and 1.25%, and lower Egypt 1.61% and 0.87% in males and females respectively.[3] [4] RCC with different types have specific genetic and histopathological characteristics. There is a 1.5:1male predominance, with a highest incidence between 60 and 70 years. Risk factors for RCC include smoking, obesity[5], hypertension, acetaminophen and non-aspirin non-steroidal anti-inflammatory drugs[6], and viral hepatitis.[7] Increased body mass index (BMI) is associated with a higher risk of RCC.[8] Pyruvate kinase is an enzyme that catalyzes the conversion of phosphoenolpyruvate and ADP to pyruvate and ATP in glycolysis and plays a role in regulating cell metabolism.[9] Inhibition of the pyruvate kinase step in glycolysis is necessary for channeling of metabolites into the pentose phosphate

pathway to support nucleotide biosynthesis required by a rapidly dividing cell.[10] Attention was increased because PKM2 is expressed in essentially all human cancers, and efforts have been made to use PKM2 as a cancer biomarker.[11] A direct connection between PKM2 and oncogenic signaling was made when it was shown that PKM2 interacts with peptides and proteins phosphorylated on tyrosine residues in the context of a src-like motif, and that these interactions facilitate tumor growth by inhibiting the enzyme to promote anabolic metabolism.[12] PKM2 is expressed in some differentiated tissues, such as lung, fat tissue, retina, and pancreatic islets, as well as in all cells with a high rate of nucleic acid synthesis, such as normal proliferating cells, embryonic cells, and especially tumor cell.[13]

The aim of work is to evaluate serum levels of pyruvate kinase (PKM2) as diagnostic marker in renal cell carcinoma.

Subjects and Methods

I- Subjects

This study was carried out by cooperation between Clinical Oncology & Nuclear Medicine, Medical Biochemistry Departments, Faculty of Medicine, Menoufia University in the period from January 2016 to March 2018. It included 200 subjects. They were classified into two groups, **group I**: It included 120 patients with RCC,(64 males and 56 females),. Their ages ranged between (29 and 55) years. **group II**: It included 80 healthy subject served as acontrol group. They were 32 males and 48 females. Their ages ranged between 29 and 55 years. RCC patients' inclusion criteria were: 1-histological diagnosis of RCC the following subtypes: clear cell, papillary, chromophobe and unclassified RCC, all disease stages, 2-all patients received standard treatment; complete surgical resection for stage I, II, III and multi-tyrosine kinase inhibitor oral drug Sunitinib 50 mg for four weeks every 6 weeks per treatment cycle as first line in stage IV disease clear and non clear cell histology. We excluded patients with poor initial performance status; we also excluded patients with incomplete clinical and survival data, and patients with who refused treatment and patients with sarcomatoid histopathological features, collecting duct and medullary histological subtypes as they have different treatment options.

II- Methods

Prior to collection of blood samples, written informed consent (approved from Committee of Ethics and Human Rights in Research at Faculty of Medicine, Menoufia University) was obtained from all subjects enrolled in this study. They were subjected to the following: history taking, physical examination including anthropometric measurements. Weight was evaluated in kilograms and height was measured in meters. Body mass index (BMI) is the ratio of weight (in kilograms) divided by the squared height (in meters). Patients were classified according to BMI by the World Health Organization into underweight (BMI< 18.5 kg/m^2), normal weight (BMI= 18.5 - 25 kg/m^2), overweight (BMI= 25 - 30 kg/m^2), and obese (BMI> 30 kg/m^2).[14] Staging was done according to the American Joint Committee on Cancer (7th edition).[15] Data of histopathological subtypes, grade and stage were collected.

Specimen Collection: 5 ml of venous blood were withdrawn from every subject. the blood were transferred into plain tube, left at 37ºC for 30 min to clot then centrifuged for 10 min at 4000 r.p.m. The serum obtained was kept frozen at - 20ºC divided into two fractions:

a--3ml of serum used for detection of serum urea colorimetric method using Diamond urea kits, Germany[16] and serum creatinine by kinetic method using international serum creatinine kits, England[17] ***(Bowers and Wong., 1980),*** b--2 ml were kept in collecting tubes for PKM2assay. It was done in steps using a double-antibody sandwich enzyme-linked immune sorbent assay. The chroma of color and the concentration of the Human Substance PKM2 sample were positively correlated.[18]

Statistical analysis

Results were collected, tabulated, statistically analyzed by IBM personal computer and statistical package SPSS version 20. Chi-square test is used to study association between two qualitative variables and whenever one of the expected cells is less than 5, Fisher's Exact test was used. The student t- test is used to assess the statistical significance of difference between two groups having quantitative variables. Mann-Whitney test (nonparametric test) is used for comparison between two groups not normally distributed having quantitative variables. Kruskal-Wallis test (nonparametric test): is used for comparison between three or more groups not normally distributed having quantitative variables. The ROC curve is a graphic representation of the relationship between sensitivity and specificity at different cut-off points for a diagnostic test. Pearson correlation was used for normally distributed quantitative variables, while Spearman correlation was used for not normally distributed quantitative variables or when one of the variables is qualitative. $P < 0.05$ is considered significant.

RESULTS

Our result revealed that, there was no significant difference between the two study groups regarding age & gender (Table 1).

There was significant statistical difference between the two studied groups as regards history of DM & hypertension while there was no significant statistical difference between the two groups regarding weight, height, BMI, smoking and family history of cancer (Table 2).

The distribution of different grades in patients with renal cell carcinoma revealed that 30.8% of RCC were of grade I, 46.2% of RCC were of grade II and 23% RCC were of grade III(fig 1).

The distribution of different stage in patients with renal cell carcinoma (n=120) was showed the highest percent of RCC patients are of stage IV 53,85% followed by stage II 23,08%, followed by stage I 15.38, and the lowest percent was of stage III 7.69% (Fig 2).

There was significant statistical increase in serum urea & creatinine levels in RCC group when compared to controls (Table 3).

There was significant statistical increase in serum levels of PKM2 in RCC group when compared to controls (Table 3 & fig 3).

There was a significant statistical positive correlation between the serum PKM2 level and BMI (P value <0.05) (Table 4 & fig 4), while there was no significant correlation between serum PKM2 level and each of age, sex, weight and height in patient with RCC.(Table4).

There was a significant statistical positive correlation between serum PKM2 level and tumor staging (P value <0.05) (Table5).

There was significant positive correlation between serum PKM2 and tumor stage (r=0.427) in RCC Patients (n=120) was showed in fig (5).

At cut of point of (2.27 ng/ml), the sensitivity of serum PKM2 in diagnosis of RCC is (70%), the specificity is (68.27%), the negative predictive value is (61.6), the positive predictive value is (75%) and the accuracy of the test is (66.46) (Table 6).

ROC (Receiver Operating characteristic) Curve of cut of point for serum PKM2 in diagnosis of renal cell carcinoma (n=200), AUC = 0.738was showed in fig (6).

Table (1): Comparison of demographic data between the two studied groups (n=200).

Groups	**Group I (Cases) n=120**		**Group II (Control) n=80**		x^2	**P value**
Variables	**No**	**%**	**No**	**%**		
Age (years) (mean±SD)	45.41±12.41		58.45±9.45		1.956	0.086
Gender:						
Female	56	46.2	48	56.4	0.404	0.376
Male	64	53.8	32	43.6		

X2: chi squre
P value <0.005 is (significant)

Table (2): Comparison of anthropometric and general characteristics between the two studied groups (n=200).

Groups	**Group I (Cases)n= 120**		**GroupI1 (Control) n=80**		x^2	**P value**
Variables	**No**	**%**	**No**	**%**		
Weight(Kg) (mean±SD)	80.41±19.41		77.14±18.41		.535	.600
Height (cm): (mean±SD)	165.74±8.45		165.41±6.75		.076	.940
BMI (Kg/m2):						
<18.5	0	0	0	0		
18.5-24.9	44	38.5	32	43.2	.309	0.857
25-29.9	20	15.4	20	18.4		
>30	56	46.1	28	37.4		
History of DM						
Yes	52	42.5	0	0	9.142	0.002
No	68	57.5	80	100		
History of HTN						
Yes	32	23.4	0	0	4.250	0.044
No	88	76.6	80	100		
Smoking						
Yes	64	53.8	36	43.8	0.404	0.376
No	56	46.2	44	56.3		
Family history of cancer						
Yes	36	26.9	16	12.5	1.245	0.224
No	84	73.1	64	87.5		

DM= diabetes mellitus
HTN=hypertension
P value <0.005 is (significant)
BMI: Body Mass Index

Table (3): Comparison of laboratory (biochemical) parameters between the studied groups (n=200).

Groups **Lab tests**	**Group I (n=120)**	**Group II (n=80)**	**T- test**	**P value**
Serum urea (mg/dl) (mean±SD)	45.2.2±6.74	31.33±9.41	8.726	<0.005
Serum creatinine (mg/dl) (mean±SD)	1.42±.65	0.75±.32	6.904	<0.005
Serum PKM2: (ng/ml) (mean±SD)	8.78±8.45	2.12±.64	4.046	<0.005

PKM2: Pyruvate kinase marker isoform (m2).

Table (4): Pearson correlation between TUMPK and different parameters in renal cell carcinoma group (diseased) (n=120).

Parameter	**r**	**P value**
Age	0.277	0.171
***Sex**	0.100	0.304
Weight	0.373	0.061
Height	0.100	0.627
BMI	0.421	<0.05

* Spearman correlation

Table (5): Pearson correlation between serum TUMPK & each of tumor stage & grade in renal cell carcinoma group (group 1) (n=120).

Parameter	**r**	**P value**
Tumor stage	0.427	<0.01
Tumor grade	0.279	0.077

Table (6): Validity test of serum TUMPK in diagnosis of RCC (n=200).

AUC	**P value**	**Cutoff point**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy**
0.738	0.010	2.27	70%	68.27%	75%	61.6%	66.46%

- AUC= Area under the Curve.
- PPV= Positive predictive value.
- NPV= Negative predictive value.

Figure legends

Figure 1: Tumor grading in RCC patients.
Figure 2: Tumor staging in RCC patients.
Figure 3: Comparison of serum PKM2 levels between two studied groups (n=200).
Figure 4: Pearson correlation between serumTUMPK and BMI
Figure 5: Pearson correlation between Serum TUMPK and Tumor stage of renal cell carcinoma patients.
Figure 6: ROC (Receiver Operating characteristic) curve of Serum TUMPK level.

Figures

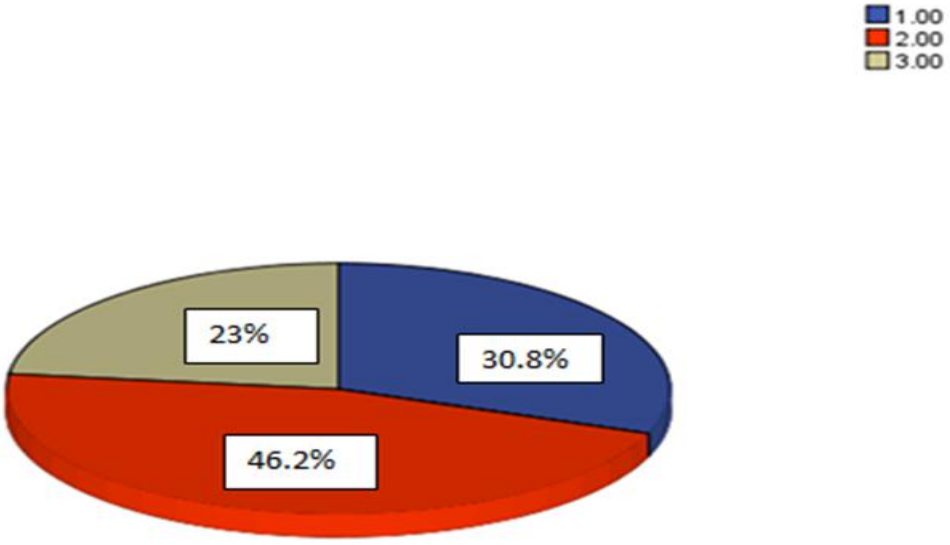

Figure 1: Tumor grading in RCC patients.

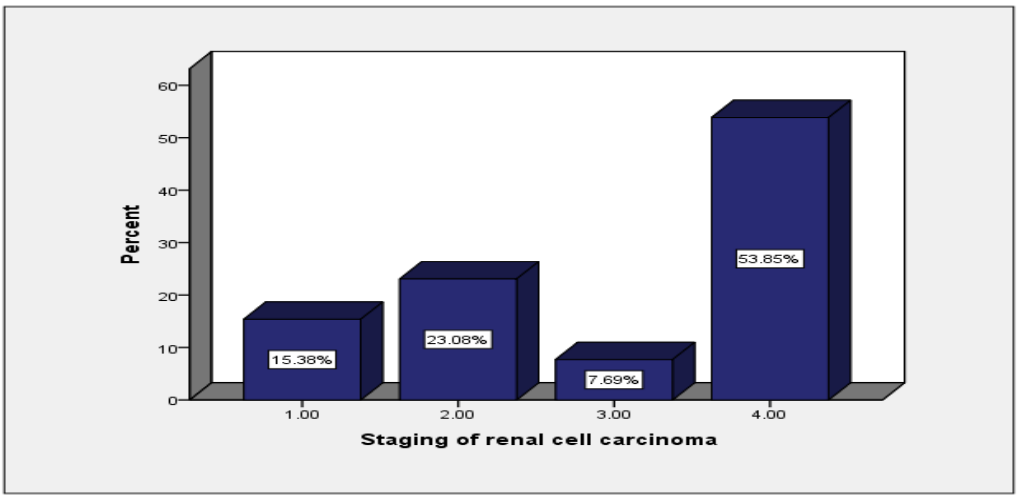

Figure 2: Tumor staging in RCC patients.

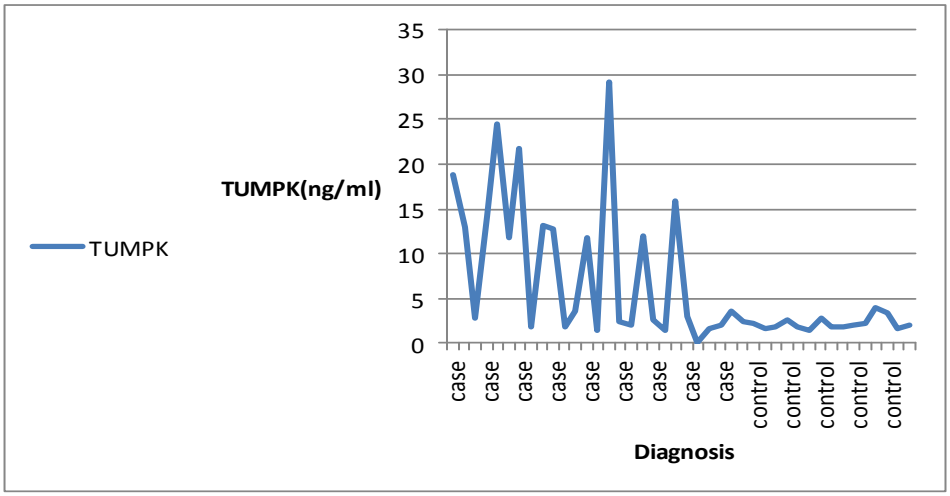

Figure 3: Comparison of serum PKM2 levels between two studied groups (n=200).

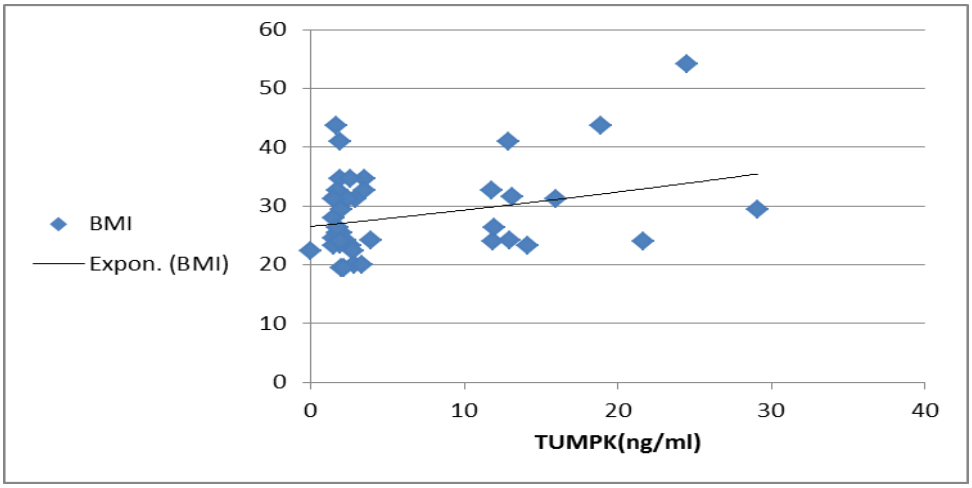

Figure 4: Pearson correlation between serumTUMPK and BMI.

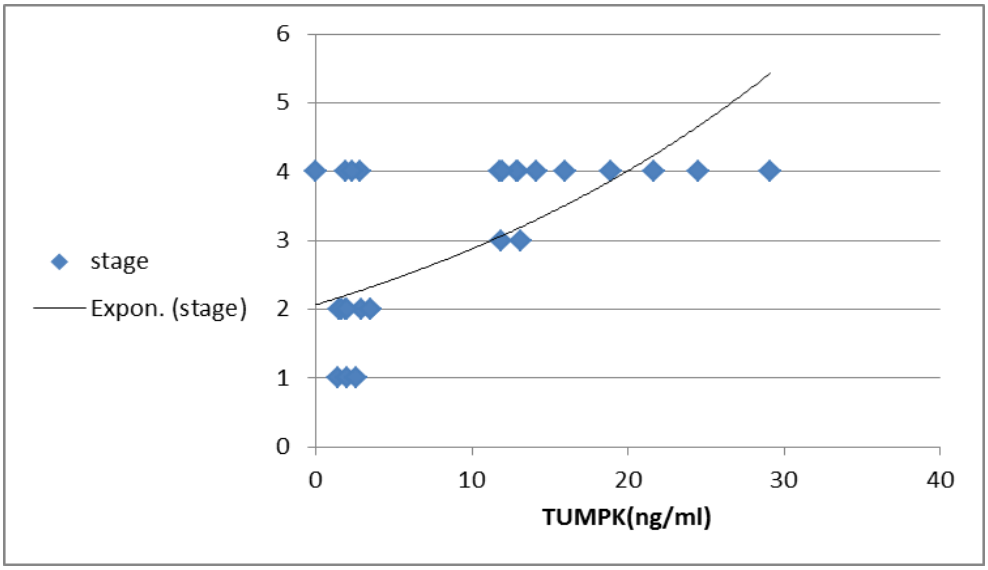

Figure 5: Pearson correlation between Serum TUMPK and Tumor stage of renal cell carcinoma patients.

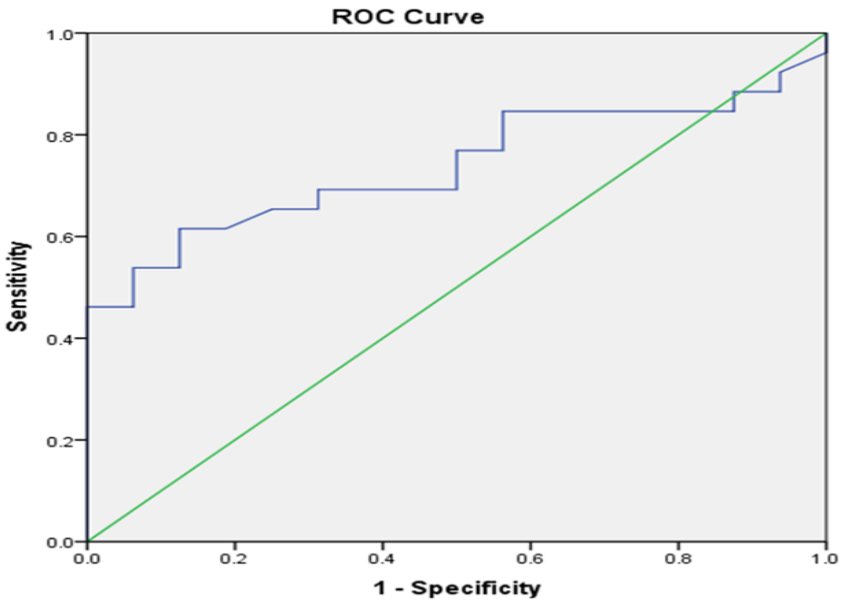

Figure 6: ROC (Receiver Operating characteristic) curve of Serum TUMPK level.

DISCUSSION

Renal cell carcinoma (RCC) is the most common cancer in the kidney, accounting for approximately 85% of all renal neoplasms. Most RCCs are of clear cell type and they harbor somatic mutations in the Von Hippel Lindau (VHL) gene.[19] Renal cell carcinoma is a kidney cancer that originates in the lining of the proximal convoluted tubule, a part of the very small tubes in the kidney that transport primary urine. Pyruvatekinase catalyzes the last step within glycolysis, the dephosphorylation of phosphoenolpyruvate to pyruvate, and is responsible for net ATP production within the glycolytic sequence.[21] PKM2 is an isoenzyme of the glycolytic enzyme pyruvate kinase. Depending upon the different metabolic functions of the tissues, different isoenzymes of pyruvate kinase are expressed. PKM2 is expressed in some differentiated tissues, such as lung, fat tissue, retina, and pancreatic islets, as well as in all cells with a high rate of nucleic acid synthesis, such as normal proliferating cells, embryonic cells, and especially tumor cells.[22] PKM2 is an ubiquitous prototype enzyme present in all tissues during the embryonic stage and is gradually replaced by other isozymic forms in specific tissues during development. Although the primary function of PKM2 is to catabolize glucose, it is possibly involved in many other nonglycolytic functions too.[23] PKM2 has recently been found to translocate into the nucleus upon mitogenic and oncogenic stimulation In the nucleus, PKM2 functions as a transcriptional co-activator and a protein kinase that phosphorylates histones, highlighting the crucial role of PKM2 in the epigenetic regulation of gene transcription that is important for the G1-S phase transition and the Warburg effect (which states that most cancer cells produce energy by a high level of glycolysis followed by lactic acid fermentation).[24]

The aim of work is to study serum level of tumor marker M2 purvate kinase (TUM2PK) in patients with renal cell carcinoma.

In this study, there is no significant statistical difference between the two studied groups regarding age; this is due to selection of the control group and patients to be age matched with renal cell cancer group.

In the current study, the mean age of renal cell cancer patient was (45.41±12.41) years, which is approximate to the results of Weikert et al.,*[25]* SEER (surveillance, epidomology and end result) data indicate that RCC incidence rates increase with age for all racial groups until the age of 70 years. *Howlader et al.,*[26] and Ferlay et al.,[27] found that, RCC incidence indicates that men are at an increased risk of developing RCC and in the present study, male to female ratio was 1.14.

In this study; male predominance among renal cancer patients is manifested as 53.8% of the malignant cases are of male gender. These results are in accordance with Aron et al.,[28] who reported that**,** RCC is approximately twice as common in men than in women. Chen et al.,[29] also reported that, the ratio of male to female is 1.7 in the United States of America, 1.37 in Europe and 1.90 in China.

The current study reported that, there was significant statistical difference between studied groups regarding history of DM (42.5%). This high percentage of history of DM in renal cancer patients is in agreement with the results obtained by Robert, et al[30] who reported that, (40%) of renal cancer patients were diabetics. Joh et al.,[31] stated that**,** history of diabetes mellitus is reported to be associated with increased risk of RCC in some North American, Asian, and European studies.

Kim et al.,[32] stated several mechanisms implicated in the development of renal cancer in diabetes have included increased growth factors and/or their receptors, hyperinsulinemia and glucose availability.

The current study reported that, there was significant increase of history of hypertension in RCC group when compared to control group these result come in agreement with the results conducted on the USA and in the People's Republic of China by Macleod et al,*[33]* who reported that hhypertension is a significant risk factor for RCC.

Haase et al.,[34] and Weikert et al.,[35] reported that,. The biological mechanism underlying the relationship between elevated blood pressure and increased risk of RCC remains unknown. One theory suggests that the chronic renal hypoxia accompanying hypertension promotes tumor cell proliferation and angiogenesis by a transcription factor known as hypoxia inducible factor. Gago-Dominguez et al.,[36] reported that, individuals with elevated BMI, patients with essential hypertension also exhibit increased lipid peroxidation, which has been implicated in the pathogenesis of RCC.

The present study reported that there was a significant increase of serum urea/creatinine levels in RCC group when compared to control. Kamal et al.,[37] & Suresh et al.,[38] reported that, creatinine tests diagnose impaired renal function and measure the amount of creatinine phosphate in blood. Urea and creatinine are good indicators of a normal functioning kidney and increase in the serum are indications of kidney function… Blood tests for Blood Urea Nitrogen (BUN) which is a major nitrogenous end product of protein and amino acid catabolism and creatinine which is a breakdown product of creatine phosphate in muscle are excreted by kidneys. BUN is an indirect and rough measurement of renal function that measures the amount of urea nitrogen in blood and is directly related to excretory function of kidney Gowda et al.[39]

In this study, there was significant statistical difference among studied groups regarding TUMPK level. It is significantly increased in renal cell cancer group when

compared with the control group. These results are in accordance with Wechsel et al.,[40] & Nisman et al.,[41] and Roigas et al.,[42] who found. Serum level of TuM2PK were significantly elevated in patients with RCC than healthy patients.

Wechsel et al.,[43] found that,, The isoenzyme TuM2Pk could be demonstrated in RCC and their metastasis by immunohistochemistry with a monoclonal antibody specific for pyruvate kinase type M2. In normal kidney cells pyruvate kinase type M2is not detectable. The stability of TuM2Pk was studied in the serum within 30 minutes. No circadian rhythm was found. Most serum TuM2Pk comes from tumor. Serum evaluation in healthy persons was used to determine normal values, with an upper concentration of 28 U/ml of TuM2Pk. Serum evaluation in RCC showed a significant difference to healthy persons and a positive correlation with Robson stage and grading.

Nisman et al.,[44] reported that significantly higher levels of TuM2-PK were found in patients with RCC compared with healthy participants TuM2-PK was significantly associated with tumor grade. The presence of extensive tumor necrosis (> 50%) was associated with high TuM2-PK. The 5-year recurrence-free survival for patients with elevated TuM2-PK was significantly lower compared with those for patients with normal marker levels. Christofk.,[45] stated that, TuM2PK has been implicated as a driver of aerobic glycolysis, and shown to be a marker of malignancy in several neoplasms potentially useful urinary marker. The current study showed that, there is significant increase TUMPK level in different stages and grade of renal cell cancer group. These results come in line with Gayed et al.,[46] who stated that, in renal cell carcinoma, elevated preoperative levels of TuM2PK significantly correlated with increased tumor size and advanced grade. Nisman et al.,[47] stated that elevated levels of TuM2Pk were significantly associated with worse pathological features, including grade and tumor necrosis and revealed that patients with elevated circulating TuM2PK had worse 5-year RFS than patients with normal marker levels.

In an attempt to evaluate TUMPK level in serum as tumor marker for RCC, the current study found, a cutoff value for TUMPK level of (2.27 ng/ml) that gives a sensitivity of 70% and a specificity of 68.27% for diagnosis of renal cell cancer. The area under ROC curve was 0.738., the negative predictive value is (61.6%), the positive predictive value is (75%) and the accuracy of the test is (66.46%).

Weinberger et al.,[48] *and* Gayed et al.,[49]**,** reported that, the benefit of using Tu M2-PK as a tumor marker for primary detection of RCC by (ROC) analysis. The area under the curve was 0.674, and the sensitivity, specificity and positive predictive value (PPV) were 44.4%, 87.5% and 88%, respectively, at the ROC optimal cut-off of 28.2 kU/L. Roigas et al.,[50] found that, only patients with RCC (non metastatic and metastatic) showed significantly increased concentrations of TU M2-PK compared to normal individuals. In metastatic RCC, TU M2-PK levels were highest and were also significantly enhanced compared to non-metastatic RCC. The sensitivity for non metastatic RCC was 27.5% and for metastatic RCC 66.7% at the 95% reference value of the control group. These results indicate that PKM2 concentrations in serum may be a potential biomarker of advanced RCC.

CONCLUSION

Serum pyruvate kinase M2 could be consider a powerful, none invasive, rapid, sensitive approach for diagnosis of renal cell carcinomas. Serum PKM2 can aid in tumor staging and help in clinical evaluation of RCC patients.

Notes on contributors

Ibrahim T. Esay planned the study.
Sally M. El-Hefnway performed the laboratory tests and submitted the study.
Alshimaa M. Alhanafy collected blood samples and data from patients.
Khadiga M.Sallam analyzed the data statistically.

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Conflict of Interest: No conflict of interest was reported by the authors.

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