Zagazig University Medical Journal
www.zumj.journals.ekb.eg

ORIGINAL ARTICLE

Assessment of Serum Copeptin in Poorly Controlled Type 1 Diabetes

Hossam Kamal Mustafa [1*], Ahmed Mohamed Gaballah[2], Mohamed Salah Fathalla [1]
[1] *Pediatrics Department, Faculty of Medicine, Zagazig university,*
[2] *Clinical Pathology Department, Faculty of Medicine, Zagazig university*

[*]Corresponding Author :
Hossam Kamal Mustafa
Pediatrics Department, Faculty of Medicine, Zagazig university
E mail :
hosamyousef90@yahoo.com

Submit Date	2019-07-10
Revise Date	2019-08-08
Accept Date	2019-08-13

ABSTRACT

Background: Children with type 1 diabetes are in risk of developing renal complications as a part of microvascular complications on the long run, early diagnosis of these complications can decrease morbidity and mortality of these complications. Copeptin has shown association with development of chronic kidney disease (CKD) in people with diabetes.**Methods:** This is a case - control study was conducted at Zagazig University Hospital of pediatrics from June 2017 to January 2018 on 40 cases suffering from T1DM. It also included 40 matched healthy controls who attend outpatient clinics for routine care.All cases were subjected to a complete clinical study (thorough history and physical examination) upon study inclusion, with emphasis on symptoms and signs of diabetes and renal affection.The following investigations were performed: HbA1c, Urine analysis, Albumin/creatinine ratio, renal function test (creatinine, BUN) Renal Ultrasound, Measure serum copeptin level by ELISA (Sandwich technique).**Results**: Our study showed that there is a significant increase in serum copeptin levels compared to healthy group. There is no significant difference between poorly controlled and well controlled children with T1DM regarding copeptin levels. Serum copeptin has also a positive correlation with albumin/creatinine ratio in children with T1DM. **Conclusion**: Serum copeptin is a potential prognostic biomarker in childhood diabetic renal affection.
Keywords: Copeptin; Type 1 diabetes; Diabetic Nephropathy.

INTRODUCTION

T1DM is one of multifactorial diseases, with high incidence worldwide. For example; the incidence of the disease has been increased in the last twenty years in Europe up to double in children below 5 years [1].

Despite the usage of insulin in the treatment of type 1 diabetes mellitus. this disease is still associated with increased morbidity due to the disease complications affecting the cardiovascular and renal and nervous system, which is affecting the quality of life and life expectancy of the children suffering from it[2].

Since the 1950s kidney disease has been recognized as one of the complications of diabetes mellitus [3].

Copeptin (CoPEP) is a peptide co-synthesized together with vasopressin in endothelial cells and pituitary gland respectively. This peptide has vaso-active, immune modulating, and metabolic properties. Copeptin increased in sepsis, has a short half-life. and is more stable and easier to measure than the active hormones [4].

Copeptin has shown association with development of chronic kidney disease (CKD) in people with diabetes. Early detection of individuals having the highest risk could help avoid this complication [5].

PATIENTS AND METHODS

This case control study was done in pediatric endocrinology outpatient clinics in Zagazig university hospital of pediatrics in the period between June 2017 to January 2018. It

was conducted on 40 patients age from 1 to 18 came for routine follow up in outpatient clinics. 25 of them were poorly controlled type 1 Diabetes mellitus, and 15 of them were well controlled type 1 Diabetes Mellitus and 40 healthy control who attend outpatient clinics for routine care.

Written informed consent was obtained from all participants' parents and the study was approved by the research ethical committee of Faculty of Medicine, Zagazig University. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Inclusion criteria

Children in pediatric department in Zagazig university hospitals between age of 1 to 18 years and diagnosed as Type 1 DM.

Healthy control: age and sex matched healthy children who attend outpatient clinics for routine care.

Exclusion criteria

- Cases with acute or chronic systemic disorders, Cases with any other renal pathology, Cases with any other endocrinal disease and Newly diagnosed cases with type 1 DM (less than one year) were excluded from our study

Methods:

All children were subjected to a detailed medical history which was taken from the parents of each child and symptoms analysis of clinical characteristics of diabetic group was done which included the age at onset and the duration of T1D, insulin dose, presence of microvascular complications, detailed clinical examination was also performed, All participants were subjected also to detailed pelvi-abdominal US .

A blood sample (5ml) was obtained from each child, and was collected into 2- and 3-ml tubes. A Urine Sample about (3ml) was obtained also from each child for routine urine analysis and albumin/creatinine ratio with precautions (First urine sample before any exercise),These samples were used to perform routine investigations which included urine analysis, albumin/creatinine ratio, renal function test (creatinine, BUN), HbA1c and measurement of serum Copeptin level by ELISA (Sandwich technique). eGFR was also calculated via Schwartz Formula

Statistical analysis of results

Data collected was coded, entered and analyzed using Microsoft Excel software. Continuous data were presented as Mean±SD if normally distributed or Median (Range) if not normally distributed. Categorical data were presented by the frequency and percentage. Normality was checked by Shapiro-Wilk test. Variables were compared using t-test, Mann-Whitney, u test, Chi-squared test of association, Spearman's correlation, Pearson's correlation, Multiple regression analysis. A receiver operating characteristic (ROC) curve was used to determine the threshold value for optimal sensitivity and specificity of a test. Threshold for significance P-value<0.05 indicates significant, P<0.01 indicates highly significant difference, P<0.001 indicates very highly significant difference while, P>0.05 indicates non-significant difference. All analysis was performed with Statistical Package for Social Sciences (SPSS) version 20.0 (SPSS Inc., Chicago, IL., USA)

RESULTS

Diabetic children had significant increases in serum creatinine, blood urea nitrogen, HbA1c, serum copeptin levels and urine albumin/creatinine ratio but a significant decrease in GFR compared with healthy children (P<0.05). (Table 1). on the other our study showed no statistically significant differences in all laboratory data in the diabetic children (P>0.05) except for HbA1c where a significant elevation was found in poorly controlled diabetic children compared with well controlled children (P<0.001). (Table 2).

Our study also showed a statistically significantly positive moderate correlation between age and serum copeptin level (P<0.05). Also, a very highly statistically significantly positive strong correlation between years since diagnosis and serum copeptin level was found (P<0.001) (Table 3).

The study also showed non-significant correlations between serum copeptin level and laboratory data (P>0.05) except for serum creatinine, blood urea nitrogen and urine albumin/creatinine ratio where a highly statistically significantly positive moderate correlation (P=0.005), a statistically significantly positive moderate correlation (P=0.021), a very highly statistically significantly positive strong correlation (P<0.001) respectively were found. (table 4).

Multiple stepwise regression analysis revealed that duration of diabetes (years since diagnosis of diabetes) is the independent determinant serum copeptin level in the diabetic children.

The regression model statistically significantly predicted the serum copeptin level, F=21.4, P<**0.001**, Coefficient of determination (R^2=36%). (table 5).

Receiver operating characteristic (ROC) curve analysis revealed that serum copeptin can fairly differentiate between diabetic children with mild to moderate impairment of GFR and diabetic children with normal glomerular filtration rate (GFR) with an area under the curve (AUC) of 0.71 for serum copeptin level (95% CI: 0.543 to 0.841, ***P*=0.019**). (Figure 1).

The optimal sensitivity and specificity were (68.7% and 79.2% respectively at a cutoff expression value>4.75). Impairment of GFR was dichotomized into normal GFR vs mild to moderate impairment.

ROC analysis also revealed that serum copeptin can well differentiate between diabetic children with normal urine albumin/creatinine ratio and diabetic children with microalbuminuria or macroalbuminuria with an area under the curve (AUC) of 0.83 for serum copeptin (95% CI: 0.680 to 0.931, ***P*<0.0001**). (figure 2).

The optimal sensitivity and specificity were (65.5% and 100% respectively at a cutoff expression value ≤4.5). Urine albumin/creatinine ratio was dichotomized into normal urine albumin/creatinine ratio and microalbuminuria or macroalbuminuria.

Table 1: Laboratory data of healthy controls vs diabetic children

Variables	**Healthy controls** *n=40*	**Diabetic children** *n=40*	**Mann-Whitney u test**	***P*-value**
Serum creatinine (mg/dL)			MW=252	**<0.001*** **
Median(Range)	0.5(0.3-0.8)	0.75(0.4-1.4)		
Mean rank	26.8	54.2		
Blood urea nitrogen (mg/dL)			MW=38.5	**<0.001*** **
Median(Range)	11(7-15)	18.5(14-47)		
Mean rank	21.5	59.5		
HbA1c (%)			MW=250	**<0.001*** **
Median(Range)	6(5.5-6.4)	8.99(4.73-14)		
Mean rank	26.8	54.3		
GFR (mL/min per 1.73 m^2)			MW=1.290	**<0.001*** **
Median(Range)	125(96.5-161)	103.75(53.3-163.6)		
Mean rank	52.8	28.3		
Serum copeptin (p*mol/L)*			MW=585.5	**0.039***
Median(Range)	3.95(2.6-5.3)	4.3(1.49-12)		
Mean rank	35.1	45.9		
Urine albumin/creatinine ratio (mg/g)			MW=417.5	**<0.001*** **
Median(Range)	6.7(5-14)	15(450-444)		
Mean rank	30.9	50.1		

*significant (P<0.05), ***=Very highly significant(P<0.001), MW, Mann-Whitney u test, GFR, glomerular filtration rate

Table 3: Correlations between serum copeptin level and baseline characteristics of the diabetic children

Variables		Serum copeptin (p*mol/L)*
Age (years)	***R***	0.37
	P	**0.035***
Years since diagnosis	r_s	0.539
	P	**<0.001*****

Table 4: Correlations between serum copeptin level and laboratory data of the diabetic children

Variables		Serum copeptin (p*mol/L)*
Serum creatinine (mg/dL)	***r***	0.439
	P	**0.005****
Blood urea nitrogen (mg/dL)	r_s	0.363
	P	**0.021***
HbA1c (%)	r_s	0.181
	P	0.26
GFR (mL/min per 1.73 m^2)	***r***	-0.257
	P	0.11
Urine lbumin/creatinine ratio (mg/g)	r_s	0.622
	P	**<0.001*****

r:Pearson correlation, r_s: Spearman's rho correlation, *significant (*P*<0.05), **= highly significant(*P*<0.01), ***=Very highly significant(*P*<0.001), *n*=40

Table 5: Summary of regression analysis for serum copeptin in the diabetic children

Variables	Univariable analysis			Multivariable stepwise regression		
	Unstandardized Coefficients		*P*-value	Unstandardized Coefficients		*P*-value
	β	$SE_β$		β	$SE_β$	
Age (years)	**0.23**	**0.13**	**0.035**			
Years since diagnosis	**0.85**	**0.18**	**<0.001**	**0.85**	**0.18**	**<0.001**
Height (cm)	0.04	0.02	0.071			
Sex (male=0, female=1)	-0.14	0.96	0.89			
Serum creatinine (mg/dL)	**5.26**	**1.75**	**0.005**			
Blood urea nitrogen (mg/dL)	0.11	0.08	0.16			
HbA1c (%)	0.13	0.15	0.4			
GFR (mL/min per 1.73 m^2)	-0.03	0.02	0.11			
Urine albumin/creatinine ratio (mg/g)	**0.018**	**0.005**	**0.002**			

Figure 1

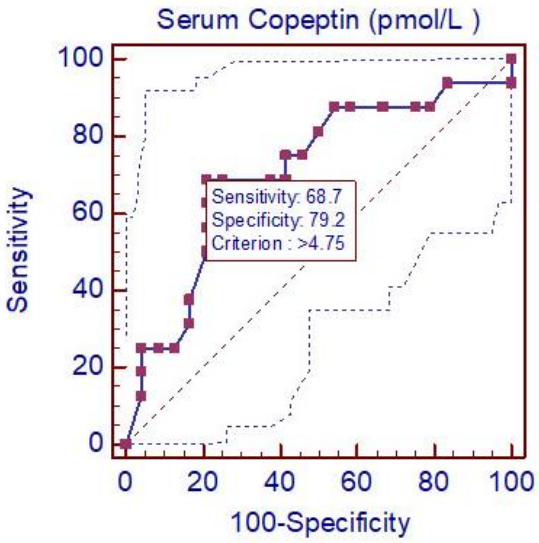
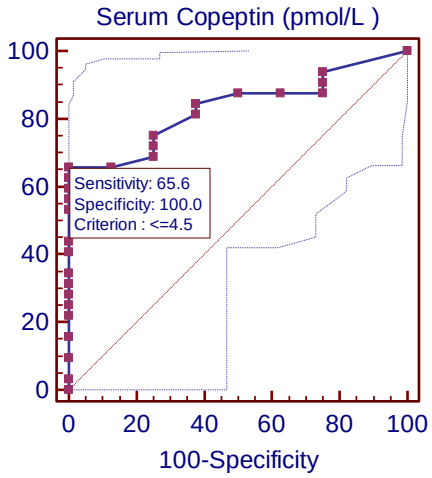


Figure 2



DISCUSSION

This case control study was conducted on 40 patients with T1DM with age range of 1 – 18 year who came for routine follow up in outpatient clinics or admitted on endocrinology department for concern of uncontrolled hyperglycemia. Twenty-five diabetic children were considered as poorly controlled (HBA1C ≤ 7.5%), and the other fifteen were well controlled (HBA1C > 7.5%). The aim of the present study was to investigate and compare the serum levels of copeptin in diabetic children vs. non diabetic healthy children and to clarify its relation to the degree of diabetic control to assess the usefulness of copeptin measurement in children in early diagnosis of DN in children with T1DM.

As regard to the personal criteria of the case group, age was ranging from 4 years to 17 years old, with median age of 9.5 years old. Female comprised 57.5 % of diabetic children included in our study. The average years since diagnosis of diabetes was 3.05±2.1 years. Serum copeptin level was not statistically different between males and females within the case group, an assumptive expected finding that are matching with others like ***Abbasi et al.*** [6] .

Regarding serum copeptin, diabetic children has significant higher values of serum copeptin levels (levels ranging from 4.73 to 14 p*mol/L*) median (4.3 p*mol/L)* compared to healthy controls with serum copeptin (levels ranging from 5.5 to 6.4 p*mol/L) median* (3.95 p*mol/L*) (P = 0.039**)**. These finding were matching with that reported by ***Bjornstad et al.*** [7] in their study on adults with T1DM. They mentioned that the mean serum copeptin level in diabetic group was 3.5 p*mol/L (levels ranging from* 2.3 to 3.8 p*mol/L)* which is significantly higher compared to serum copeptin level in the healthy group; 2.8 p*mol/L* (levels ranging from 2.7 to 3.1 p*mol/L*) (p = 0.003). However ***Schiel et al.*** [8] in their study on children and adolescents with T1DM, disagrees with our data, as serum copeptin levels in diabetic group was lower than the healthy group with mean serum copeptin level were 4.75 p*mol/L* and 5.56 p*mol/L respectively with non-significant statistical difference.* Similar findings like our study were found in adult patients with T2DM as reported by

Zellweger et al. [9] who found significantly higher serum copeptin levels in diabetic group compared to healthy non diabetic group; 9.4 p*mol*/*L and* 4.1 respectively.

In our study, conventional renal markers including serum creatinine, blood urea nitrogen and albumin/creatinine ratio had significant higher values in diabetic children compared to healthy children. On the other hand, eGFR was significantly lower in the former group (eGFR ranging from 53.3 to163.6 mL/min per 1.73 m^2 in diabetic group compared to 96.5 to 161 mL/min per 1.73 m^2 in healthy controls) (median 103.75 mL/min per 1.73 m^2 in diabetic group compared to 125 mL/min per 1.73 m^2 in healthy controls) (P <0.001). This is agreed with ***Bamanikar et al.*** [10]who reached a similar conclusion for the same parameters in their study which focused on patients with T1DM particularly regarding values of BUN; which in addition to creatinine are related to duration since diagnosis of diabetes. Also, ***Faulkner et al.*** [11] and ***de Boer et al.*** [12] agreed with our study as regard to Albumin/creatinine ratio which was stated in their reports to be higher in diabetic patients; both T1DM and T2DM, compared to healthy controls.

Boertien et al. [13] focused on patients with T2DM and found that copeptin concentration is significantly associated with higher baseline albumin/creatinine ratio and lower eGFR values and with a decline in eGFR during follow-up in diabetic patients. This last association is independent of, and stronger than, most traditional risk factors for kidney function decline. Additionally, ***Yalta et al.*** [14] reported higher plasma copeptin concentrations, higher albuminuria and a lower decrease in eGFR The similarity of the above with our findings on children with T1DM, clarify that the decline in eGFR and the higher albumin / creatinine ratio is strongly related to duration since diagnosis of diabetes rather than the type of diabetes.

On the other hand, ***Schiel et al.*** [8]found no significant differences in respect of serum creatinine, BUN or eGFR between patients with type 1 diabetes mellitus and the healthy controls, the findings that disagreed with our results. Different DM management strategies and different levels of patient/family compliance; particularly related to the parental education, in addition to small number of participants in both studies, may explain this difference. Worth mentioning in this aspect, the higher mean of HbA1C level in our study (8.99 %) compared to 7.85 % in the study of ***Schiel et al.*** [8].

In our study and within the diabetic group, non-statistically significant differences were found between the two subgroups, well controlled and poorly controlled children with T1DM regarding in serum copeptin (P = 0.62). The same speaking could be said regarding renal markers; serum creatinine, BUN and albumin/creatinine ratio (P = 0.062, 0.76, and 0.069 respectively). These data are agreed with the study of ***Schiel et al.*** [8] . Again, this may be attributed to the small sample size of the diabetic group and subgroups.

On the other hand, and on correlation analysis within the diabetic group, our study showed a statistically significantly positive correlation between serum copeptin levels and the age (P = 0.035), diabetes duration (P = 0.001), serum creatinine (P = 0.005), blood urea nitrogen (P = 0.021), and urine albumin/creatinine ratio (P <0.001). Comparing with our results, ***Schiel et al.*** [8], found a strong inverse correlation between copeptin and GFR, a finding which is not matching with ours which showed lack of significant correlation to serum copeptin with GFR. Findings of ***Schiel et al.*** were also contrary to our data regarding correlation with serum creatinine, albuminuria, HbA1c, blood glucose, age, height and BMI. The same authors, noted that patients with T1DM with a diabetes duration of >7 years had higher serum copeptin than those with diabetes duration <7 years, though the serum copeptin not significantly correlated to diabetes duration in the same study.

In our study, Multivariate analysis showed that duration of diabetes is the independent determinant of serum copeptin

level in the diabetic children (P = 0.001). However, ***Schiel et al.*** [8] and ***Pikkemaat et al.*** [15] disagreed with our study as they reported that only the GFR could be identified as a parameter correlated with copeptin. They suggested that the copeptin concentration is also a surrogate parameter for kidney function and a decline of GFR in children.

Compared to above data, ***Abbasi et al.*** [6] in their study on patients with T2DM, demonstrated that plasma copeptin is a reliable surrogate parameter for the prediction of T2DM mellitus and perhaps for diabetic nephropathy. Thus, it can be surmised that the serum copeptin concentration is an independent risk factor for the decline in renal function in patients with T2DM. Moreover, ***Artunc et al.*** [16] concluded in their study on patients with T2DM, that copeptin in combination with the measurement of urine albumin excretion rate is very helpful for the early diagnosis of diabetic nephropathy.

Receiver operating characteristic (ROC) curve analysis confirmed the ability of copeptin to diagnose decline of eGFR. Furthermore, when we classified the patients according to eGFR to patients with normal, mild, moderate and severe impairment of eGFR, Serum copeptin was able to fairly distinguish between them with optimal sensitivity and specificity were (68.7% and 79.2% respectively at a cutoff expression value>4.75) (P<0.0001). In a study by ***Zittema et al.*** [17], copeptin showed sensitivity and specificity of 63% and 83%, respectively; the figures that are comparable to our findings.

ROC curve analysis confirmed the ability of serum copeptin to diagnose albuminuria. Furthermore, serum copeptin can differentiate between microalbuminuria and macroalbuminuria and normal urine with optimal sensitivity and specificity were (65.5% and 100% respectively at a cutoff expression value ≤4.5) (P<0.0001). In ***Hu et al.*** [18] study, Copeptin showed sensitivity and specificity (78.9% and 88.9%, respectively). This is conceptually matching with matched cross-sectional case–control study of **Bjornstad et al.** [7], who reported that copeptin was significantly higher in men with T1DM and albuminuria compared to those with normoalbuminuria. Furthermore, higher copeptin concentrations conferred greater odds of impaired GFR, independent of other important risk factors.

CONCLUSION

serum copeptin is a potential prognostic biomarker in childhood renal affection due to T1DM. Further large-scale studies with larger number of participates should be conducted to confirm the value of copeptin in renal affection specially in children with T1DM.

REFERENCES

1. Patterson C C, Dahlquist G G, Gyürüs E, Green A, Soltész G, & EURODIAB Study Group. Incidence trends for childhood type 1 diabetes in Europe during 1989–2003 and predicted new cases 2005–20: a multicentre prospective registration study. The Lancet 2009; 373(9680): 2027-2033
2. Huxley R R, Peters S A, Mishra G D & Woodward M. Risk of all-cause mortality and vascular events in women versus men with type 1 diabetes: a systematic review and meta-analysis. The lancet Diabetes & endocrinology 2015; 3(3): 198-206.
3. Chowdhury I A. Prevalence of epidemiological influence, risk factors of type-2 Diabetes Mellitus and analysis of hypertension as a complication among the relatively newly diagnosed patients from BIRDEM hospital. Diss. BRAC University, 2017.
4. Alcoba G, Manzano S, Lacroix L, Galetto-Lacour A & Gervaix A. Proadrenomedullin and copeptin in pediatric pneumonia: a prospective diagnostic accuracy study. BMC infectious diseases 2015;15(1): 347
5. Tasevska I, Enhörning S, Christensson A, Persson M & Nilsson P M. Increased levels of copeptin, a surrogate marker of arginine vasopressin, are associated with an increased risk of chronic kidney disease in a general population. American journal of nephrology 2016; 44(1): 22-28.
6. Abbasi A, Corpeleijn E, Meijer E, Postmus D, Gansevoort RT, Gans RO, et al. Sex differences in the association between plasma copeptin and incident type 2 diabetes: the Prevention of Renal

and Vascular Endstage Disease (PREVEND) study. Diabetologia. 2012;55(7):1963-70.
7. Bjornstad P, Maahs DM, Jensen T, Lanaspa MA, Johnson RJ, Rewers M, et al. Elevated copeptin is associated with atherosclerosis and diabetic kidney disease in adults with type 1 diabetes. Journal of Diabetes and its Complications. 2016;30(6):1093-6.
8. Schiel R, Perenthaler TJ, Steveling A, Stein G. Plasma copeptin in children and adolescents with type 1 diabetes mellitus in comparison to healthy controls. diabetes research and clinical practice. 2016; 118:156-61.
9. Zellweger C, Wildi K, Twerenbold R, Reichlin T, Naduvilekoot A, Neuhaus J, et al. Use of copeptin and high-sensitive cardiac troponin T for diagnosis and prognosis in patients with diabetes mellitus and suspected acute myocardial infarction. International journal of cardiology. 2015; 190:190-7.
10. Bamanikar S, Bamanikar A, Arora A. Study of Serum urea and Creatinine in Diabetic and non-diabetic patients in in a tertiary teaching hospital. The Journal of Medical Research. 2016;2(1):12-5.
11. Faulkner MS, Quinn L, Fritschi C. Microalbuminuria and heart rate variability in adolescents with diabetes. Journal of Pediatric Health Care. 2010;24(1):34-41.
12. de Boer IH, Gao X, Cleary PA, Bebu I, Lachin JM, Molitch ME, et al. Albuminuria changes and cardiovascular and renal outcomes in type 1 diabetes: the DCCT/EDIC study. Clinical Journal of the American Society of Nephrology. 2016:CJN. 02870316.
13. Boertien W, Riphagen I, Drion I, Alkhalaf A, Bakker S, Groenier K, et al. Copeptin, a surrogate marker for arginine vasopressin, is associated with declining glomerular filtration in patients with diabetes mellitus (ZODIAC-33). Diabetologia. 2013;56(8):1680-8
14. Yalta K, Yalta T, Sivri N, Yetkin E. Copeptin and cardiovascular disease: a review of a novel neurohormone. International journal of cardiology. 2013;167(5):1750-9.
15. Pikkemaat M, Melander O, Boström KB. Association between copeptin and declining glomerular filtration rate in people with newly diagnosed diabetes. The Skaraborg Diabetes Register. Journal of Diabetes and its Complications. 2015;29(8):1062-5
16. Artunc F, Nowak A, Mueller C, Breidthardt T, Twerenbold R, Wagner R, et al. Plasma concentrations of the vasoactive peptide fragments mid-regional pro-adrenomedullin, C-terminal pro-endothelin 1 and copeptin in hemodialysis patients: associated factors and prediction of mortality. PloS one. 2014;9(1):e86148.
17. Zittema D, van den Brand JA, Bakker SJ, Wetzels JF, Gansevoort RT. Copeptin, a surrogate marker for arginine vasopressin, is associated with disease severity and progression in IgA nephropathy patients. Nephrology Dialysis Transplantation. 2017;32(suppl_1):i146-i53.
18. Hu W, Ni Y-J, Ma L, Hao H-R, Chen L, et al. Serum copeptin as a new biomarker in the early diagnosis of decline in renal function of type 2 diabetes mellitus patients. International journal of clinical and experimental medicine. 2015;8(6):9730.

To Cite This Article: Mustafa HK, Gaballah AM, Fathalla MS. Assessment of Serum Copeptin in Poorly Controlled Type 1 Diabetes . Zumj May. 2020(26) No.3,397-404. DOI: 10.21608/zumj.2019.14517.1322