

COMPARATIVE STUDY OF CREATININE AND UREA IN THE CASE OF WELL AND POORLY CONTROLLED DIABETES

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Abstract: The glycosylated hemoglobin is useful in monitoring diabetes regardless of its type allowing a retroactive evaluation over a period of 45 days according to the last studies, being a suggestive mirror for the glycemic status. The protein metabolism is evaluated through biochemical determinations of creatinine and urea. The medical practice is interested in how ensuring a good control or a less good control, of diabetes, will generate changes in the case of these biochemical parameters.

Keywords: HbA1c, urea, creatinine, nonenzymatic glycosylation, protein catabolism.

Introduction

Diabetes can be considered a complex metabolic disease, that implies besides hyperglycemia as an essential element of diagnosis important modifications of lipid, protein, carbohydrate, electrolyte, acid-base metabolism.

Hyperglycemia is the one that stimulates the appearance of insulin

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independent paths of glucose: the polyol path and the glicuronic acid path.¹ The glicuronic acid path leads to the glycosilation of serum and glomerular proteins with the perturbation of glomerular selectivity for proteins, that will cross easier the basal glomerulus membrane. In these conditions a decrease of catabolism proteins that are glycoproteins takes place.

An indicator of nonenzymatic glycosilation is just the serum level of HbA1c, the glucose having a reaction with the circulating or structural proteins proportional to the serum level.²

The A1c component of HbA1c is the most important from a quantitative point of view, but also of the significance in the control of diabetes.³ The increasing level of urea and serum creatinine may be due to a functional renal insufficiency, secondary to hypoxia (glycosilated hemoglobin affinity for oxygen explaining the hypoxia tissue) and to dehydration, and in the case of urea and as a result of the intensification of protein catabolism.⁴ At the same time, in decompensated diabetes, also a decrease of the protein synthesis can exist, what could also lead to a decrease of urea synthesis. When an organic renal lesion exists, the values of urea and creatinine can increase significantly.⁵ In diabetes an excessive degradation of the lipids, proteins, glycogen, takes place in the organism's attempt to compensate the lack of energy, which, normally is released from glucose metabolism.⁶

The present study aims to show the way in which the existence of a well controlled or decompensated diabetes can influence biochemical parameters linked to protein metabolism such as urea and creatinine.

Experimental

There have been obtained human serum samples in October 2014 - February 2015 from people that came to sanitary units from urban areas

servicing both the urban place and the subordinated villages respectively. The study was conducted on two groups of patients with diabetes, a group being represented by patients with a well controlled diabetes, and the other being a group with poorly controlled diabetes. For both groups urea and creatinine were determined. Detailed information regarding gender, age and the living places of the pattern people are available for each test and are given in Table 1.

Table 1. Distribution on age, gender and the living places of the patients.

Hbglic	Age	Residence	Gender- F	Gender- M	Hbglic	Age	Residence	Gender- F	Gender- M
norm	01-10	Rural	0	0	norm	51-60	Rural	16	13
norm	01-10	Urban	0	0	norm	51-60	Urban	12	9
big	01-10	Rural	1	0	big	51-60	Rural	14	6
big	01-10	Urban	0	0	big	51-60	Urban	13	5
norm	21-30	Rural	0	0	norm	61-70	Rural	11	10
norm	21-30	Urban	1	0	norm	61-70	Urban	3	3
big	21-30	Rural	0	0	big	61-70	Rural	15	7
big	21-30	Urban	0	0	big	61-70	Urban	9	4
norm	31-40	Rural	0	0	norm	71-80	Rural	13	3
norm	31-40	Urban	2	0	norm	71-80	Urban	1	1
big	31-40	Rural	0	0	big	71-80	Rural	7	0
big	31-40	Urban	0	1	big	71-80	Urban	4	6
norm	41-50	Rural	3	5	norm	81-90	Rural	2	0
norm	41-50	Urban	2	1	norm	81-90	Urban	0	0
big	41-50	Rural	1	4	big	81-90	Rural	1	1
big	41-50	Urban	2	3	big	81-90	Urban	1	0
norm	Total	Rural	5	9	norm	Total	Rural	79	40
	01-50					51-90			
big	Total	Urban	7	5	big	Total	Urban	43	28
	01-50					51-90			

Total Residence *Gender: Rural (F) = 84; Urban (F) = 50; Rural (M) = 49; Urban (M) = 33; Total sample = 216

Total Residence: Rural 133; Urban = 83; Total sample = 216;

Total Gender: Female = 134; Male = 82; Total sample = 216.

big - poorly controlled; norm - well controlled

The blood samples were collected in BD vacutainers with anticoagulant. The serum was separated through centrifugation at 2700 rpm for 5 minutes.

The glycosylated hemoglobin was determined by chromatographic method, using ion exchanger resins. The HbA1c values were determined with a SPEKOL 11 Carl Zeiss Jena spectrophotometer at a wavelength of 540 nm, using as a reference distilled water with quartz cuvettes of 1 cm. For creatinine, a BioSystems BTS – 3500 semi-automatic device was used, and for urea, the “end point” method was applied to the ClinDiag FA/200 automatic biochemistry device.

Working procedure for HbA1c

The working procedure for the glycosylated hemoglobin consists of a chromatographic method to obtain the glycosylated fractions and the total HbA1c was spectrophotometrically determined at 415 nm.

HbA1c was obtained in several stages which involve obtaining the hemolysate, preparation of the column with resin ion exchangers, separation and reading of HbA1c and then the reading of total HbA1c.

The percent of HbA1c was calculated according to:

$$\%HbA1c = \frac{A_{HbA1c} \cdot V_{HbA1c}}{A_{HbTOTAL} \cdot V_{HbTOTAL}} \cdot 100$$

where: A_{HbA1c} is the absorbance of the glycosylated hemoglobin;

V_{HbA1c} is the volume of the glycosylated hemoglobin (mL);

$A_{HbTOTAL}$ - the absorbance of the total glycosylated hemoglobin;

$V_{HbTOTAL}$ = the volume of the total glycosylated hemoglobin (mL).

Working procedures for creatinine and urea

For creatinine, a test tube containing 3 mL picric acid and 1 mL non-hemolyzed serum, was gently shaken and then subjected to

centrifugation for 5 minutes at 2500 rpm. From the sample to 2 mL of supernatant 0.1 NaOH was added, let it to rest for about 20 minutes, and after that the absorbance at 530 nm was read.⁷

For urea, 1500 μ L working reagent and 10 μ L non-hemolyzed serum were pipetted in a reaction cuvettes. The absorbance at 340 nm was read after 30 minutes (A1) and after 90 minutes (A2).

The concentration of urea in the sample that is to be analysed was calculated using the following formula:

$$C_{sample} = \frac{\Delta A_{sample}}{\Delta A_{standard}} \cdot C_{standard}$$

where: C_{sample} is the sample concentration;

ΔA_{sample} - sample absorbance;

$\Delta A_{standard}$ – the absorbance for the standard;

$C_{standard}$ = standard concentration.

Results and Discussion

Two groups of patients with diabetes were studied, one poorly controlled at which HbA1c is higher than 8% named conventional “increased HbA1c”, and the other well controlled having HbA1c lower than 8%, named conventional “normal HbA1c”, for which urea and creatinine were determined.

The glycosylated hemoglobin is different from the native hemoglobin through total electric charge having affinity for grouping the fructose and antigenic properties. The criteria based on which patients were selected were the elevated values and/or normal values of the glycosylated hemoglobin. The glycosylated hemoglobin values exceeding 8 means poorly controlled diabetes. (Figure 1 and Figure 2)

The percentage of high values is in this case higher for the individuals with poorly controlled diabetes (50.7%) compared to the other group (46.8%).

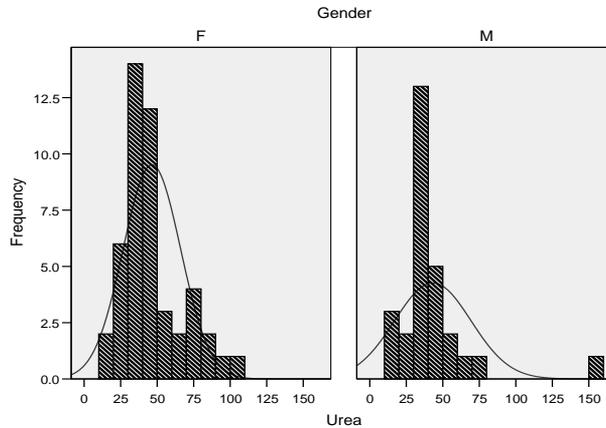


Figure 1. Distribution of “Urea” indicator according to gender, for the patients with high values of HbA1c.

Urea has a limited role in the evaluation of the renal function, as is influenced also by a number of extra renal factors (a richer protein diet, increased protein catabolism, etc.).

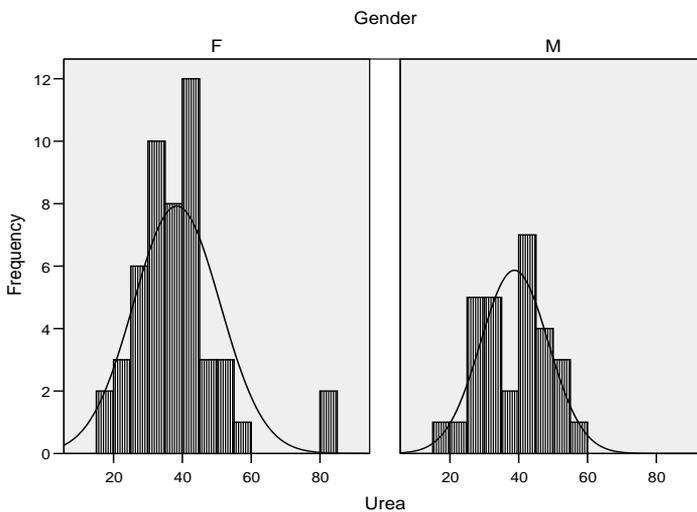


Figure 2. Distribution of “Urea” indicator according to gender, for the patients with normal values of HbA1c.

The reported percentages of urea that are slightly higher in the case of poorly controlled diabetes can be explained in the context of a further intensification of protein catabolism and a low use of glucose, without being able to be denied here and the role of a richer protein diet that can be found in the case of a properly unbalanced diabetes. The dehydration should also be considered, that especially in the case of patients with poorly controlled diabetes may cause elevated values of urea.

In order to compare the values of creatinine and urea indicators between the patients of the two groups a t-test was also applied.⁸ The results of this inferential test are reported in Table 2.

Table 2. The results of t-test for creatinine and urea indicators in patients with high (glic_cresc) and normal (glic_norm) HbA1c values (the group of patients poorly controlled vs the group of patients well controlled).

	Mean – Hb glic_cresc	Mean – Hb glic_norm	t- value	df	p	Valid N Hb_glic cresc	Valid N Hb_glic norm	Std.Dev. Hb_glic cresc	Std.Dev. Hb_glic norm
Creatinine	0.907	0.846	1.200	189	0.231	89	102	0.297	0.388
Urea	45.163	36.658	2.904	156	0.004	75	83	22.225	14.028

Mean-Hb_glic_cresc - average values for the group of poorly controlled patients; Mean-Hb_glic_norm - average values for the group of well controlled patients; t-value - the value of the t-statistic (Student); df - the number of degrees of freedom; p - the significance level of the test, that is to be compared to the coefficient of risk unanimously accepted, that is 0.05; Valid N-Hb_glic_cresc – the number of determinations for the group of poorly controlled patients; Valid N-Hb_glic_norm - the number of determinations for the group of well controlled patients; Std.Dev.-Hb_glic_cresc - standard deviation for the group of poorly controlled patients; Std.Dev.-Hb_glic_norm - standard deviation for the group of well controlled patients.

The t-test, applied for generating the results in Table 2, makes the supposition that, based on a relative small number of values of the two samples that are to be compared, there can be suggested suppositions at a inferential level (at a level of population statistics, from which these samples were extracted).

The statistics importance means that the difference in average for a certain variable, natural to any comparison between 2 samples, is significant (evidently, in a statistical sense) and is not due to the statistical chance, as it should be understood just by simply finding the difference between the 2 samples, if the t-test would not be applied. Taking into account the fact that any statistical determination of inferential-type is significant if only the level of significance, conventionally noted in literature with “p”, is inferior to the risk limit accepted by 0.05, it can be concluded, at the t-test, for the mentioned risk coefficient, that for urea there are significant statistical differences between the two groups ($t = 2.904$, $p < 0.05$), and for creatinine, there are no significant statistical differences between the two groups ($t = 1.200$, $p > 0.05$).

The comparative analysis in the case of creatinine will be made different according to gender (Figure 3 and Figure 4). In this way, in the case of women prevail patients with normal values of creatinine (95.9%) in the case of the well controlled group, 88.9% being registered in the case of the poorly controlled group. In terms of patients having a lower value than the reference one, there is no case for none of the two groups, and for those with higher values than normal, the percentage is 4% for the well controlled group and 11% for the poorly controlled group.

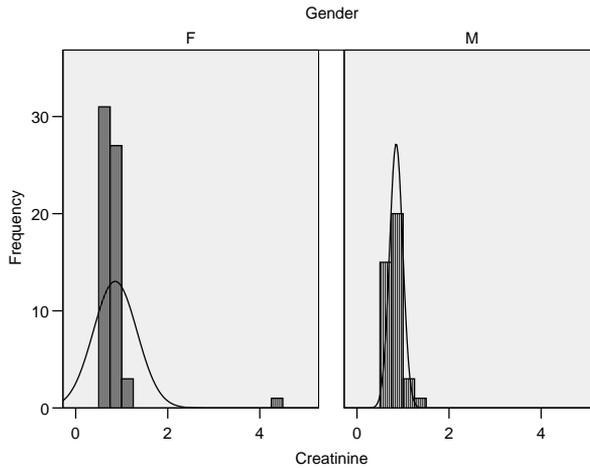


Figure 3. Distribution of “Creatinine” indicator values according to gender, for patients presenting high values of HbA1c.

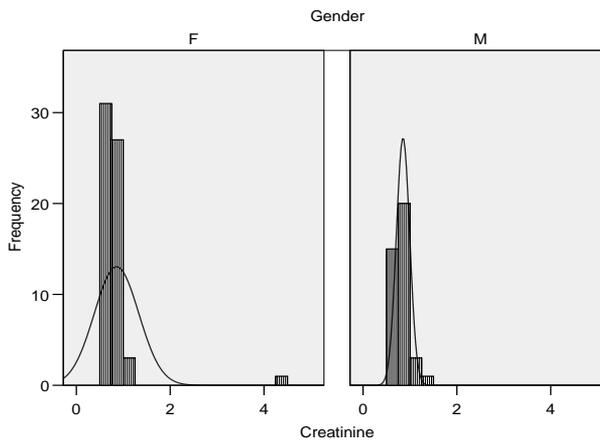


Figure 4. Distribution of “Creatinine” indicator values according to gender, for patients presenting normal values of HbA1c.

Creatinine values for men suggest that the largest percent is for the cases with normal values (84.5% for patients with unbalanced diabetes and 100% for those that are balanced in terms of control of diabetes). Values lower than normal do not appear at none of the studied groups and values higher than normal appear at the poorly controlled group (16% compared to 0% at the well controlled group).

Creatinine is influenced by the level of muscle mass, and is not a sensitive indicator in case of mild to moderate renal injuries. It can be said that in the case of these patients, presenting elevated values of creatinine, this parameter could indicate a certain level of renal insufficiency. Such disease is associated in a larger extent with poorly controlled diabetes in the context of dehydration, also not being excluded the possibility of association with a muscle or endocrine disorder, which could be accompanied by increased creatinine.

Also it is possible that due to interferences (related to methodology) in the context of formation of acetoacetate, the level of creatinine to have falsely elevated values.

In terms of comparative analysis of the two parameters linked to protein metabolism, urea and creatinine, we can classify this study in five possible variants.

Both increased creatinine and urea, situation in which fits 1.3% of people that are in both groups can be found in prerenal azotemia (congestive heart failure, shock, increased diuresis, sweat), or in post-renal azotemia with obstruction of the urinary tract or renal azotemia (of glomerular cause).

The absence of low values of creatinine and urea can be noted in both groups.

Normal creatinine and elevated urea, possibility that face 43.4% from the well controlled group, than 48.6% from the poorly controlled group, under the terms of intensifying protein catabolism, more accentuated in the second version, can be attributed to the excessive protein degradation in an attempt of the organism to compensate for the lack of energy.

For the version normal creatinine, normal urea can be found 53.2%

from the well controlled group, in the other group are registered 44.3% of the cases, with a higher percentage of normal values for the first group.

Another possibility would be the one that corresponds to a normal creatinine level associated to a low urea value in the case of the well controlled group being 0% cases, and for the unbalanced group in terms of control, it has been stated the presence of 1.4% cases that can appear in the context of a more intense protein glycosylation, what can cause a decrease of protein catabolism, thus, implicitly of the synthesis of urea.

Besides the presented situations, it is possible for other versions to appear and that have nothing to do with the basic condition.

Conclusions

In the case of comparative characterization of the two groups, we notice that for urea and creatinine (women) are found small increases of the values above the limit of reference for the poorly controlled group, than for the well controlled group. At creatinine (men), larger differences occur in favour of the group with a more poorly control.

The study by association of the two parameters of investigation of the protein metabolism leads to the conclusion that the most well represented is the group with normal creatinine and normal urea, as well as the group with normal creatinine and elevated urea.

In the case of the group with normal creatinine and normal urea, we meet a higher percentage of normal values in the case of the group with well controlled diabetes.

For the group normal creatinine and elevated urea, there is a greater proportion of cases for the poorly controlled group.

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