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Measurement of prolidase activity in type 2 diabetes patients and associated biochemical variables.

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Abstract :

Background: The prolidase enzyme plays a vital role in collagen metabolism, a process that is disrupted in various pathological conditions, including type 2 diabetes mellitus (T2DM). T2DM is a widespread metabolic disorder, particularly after the age of 35, caused either by impaired insulin secretion from pancreatic beta cells or by insulin resistance.

Objective: Evaluates the activity of the prolidase enzyme in type 2 diabetes, which plays a role in collagen formation, and variables associated with its levels in T2D.

Method/Analysis This study included 90 individuals, comprising 60 individuals with type 2 diabetes and 30 healthy people, with a mean age range from 30 to 70 years for both patients and healthy controls. After collecting the data, prolidase activity was assessed, along with biochemical parameters of the type 2 diabetes patients.

Results: Activity of prolidase among type 2 diabetes subjects was measured. It was revealed that prolidase activity was decreased in type 2 diabetes patients compared to healthy individuals ($P = <0.001$). Increased inflammatory markers such as C-reactive protein (*CRP)* and tumor necrosis factor alpha (*TNF-α*) were observed ($P = <0.0001$). Increase in glucose and glycated hemoglobin levels ($P=<0.0001$), with a mild decrease in electrolytes (Mg+2, Ca+2) ($P=<0.0001$), kidney function tests (urea, creatinine, uric acid) ($P=<0.0001$), with an increase in lipid profile (*cholesterol, 2TRI, LDL, VLDL*) ($P=<0.0001$) except for a decrease in HDL ($P=<0.0001$), and body mass index (*BMI*) ($P=0.0006$). This suggests that both prolidase and inflammatory markers are linked to type 2 diabetes.

Keywords: diabetes mellitus, type 2 diabetes, clinical variables.

قياس نشاط البرولايديز لدى مرضى السكري من النوع الثاني والمتغيرات الكيموحية المرتبطة به

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مستخلص:

الخلفية: يعتبر انزيم البرولايديز امر حيوية في عملية إعادة تكوين الكولاجين لان هذه العملية مهمة في العديد من الحالات المرضية من ضمنها T2D الذي يعتبر هذه النوع من داء السكري من أكثر الأنواع شيوعًا بين البالغين خاصة بعد العمر 35 عاما، وينتج هذا النوع من السكري إما عدم قدرة خلايا بيتا في البنكرياس على إفراز كمية كافية من الأنسولين ، أو عن عدم استجابة الأنسجة للأنسولين بشكل جيد.

الهدف: يقوم بتقييم نشاط إنزيم البروليداز في مرض السكري من النوع 2، والذي يلعب دورًا في تكوين الكولاجين، والمتغيرات المرتبطة بمستوياته في مرض السكري من النوع 2.

الطريقة/ التحليل: شملت هذه الدراسة 90 فردًا، منهم 60 مريضًا بداء السكري من النوع الثاني و30 شخصًا من مجموعة الضبط، تتراوح أعمارهم بين 30 و70 عامًا في كل من المرضى والاصحاء. بعد جمع العينات، تم تقييم نشاط إنزيم البروليداز، بالإضافة إلى المتغيرات السريرية لمرضى السكري من النوع الثاني.

النتائج: تم قياس نشاط البروليداز في مرضى السكري من النوع 2. وقد وجد أنه انخفض لدى مرضى السكري من النوع 2 مقارنة بالمجموعة الضابط ($P = <0.001$) وقد لوحظت عوامل التهابية مرتفعة مثل بروتين سي التفاعلي (CRP) وعامل نخر الورم ألفا (TNF-α) ($P = <0.0001$) زيادة في مستويات الجلوكوز والهيموجلوبين السكري (HbA1c) ($P=<0.0001$)، بالإضافة إلى انخفاض طفيف في الشوارد (Mg^{+2}, Ca^{+2}) ($P=<0.0001$)، زيادة في وظائف الكلى (اليوريا، الكرياتينين، حمض البوليك) ($P=<0.0001$)، بالإضافة إلى زيادة في مستوى الدهون (الكوليسترول، 2TRI، LDL، VLDL) ($P=<0.0001$) باستثناء الانخفاض في HDL ($P=<0.0001$)، ومؤشر كتلة الجسم (BMI) ($P=0.0006$) . ويشير هذا إلى أن كل من إنزيم البروليديز والعلامات الالتهابية مرتبطة ارتباط بمرض السكري من النوع 2.

الكلمات المفتاحية: إنزيم البرولايديز ، داء السكري، داء السكري من النوع الثاني، المتغيرات السريرية.

Introduction :

The Prolidase enzyme is vital in the process of collagen reconfiguration. It is classified as cytoplasmic xopeptidase and has been isolated from multiple sources, including human erythrocytes. Its function is characterized by its primary role in the final phase of collagen degradation. Its activity was discovered about 75 years ago [1],[2]. Prolidase is characterized by a wide prevalence in human tissue, it may show particularly high activity in kidneys, red blood cells and intestinal mucosa, while its activity is relatively lower in plasma and liver [3]. The most important and active pillar with which proleydase enzyme reacts is Kleisin-Prolin. This enzyme belongs to the group of biphetydase-based methaloptydases such as Zn^{2+}, Mg^{2+}, Ca^{2+} and Co^{2+}. These cationics are essential auxiliaries for its catalytic activity [4],[5]. Human prolidase are a dual-symmetric enzyme consisting of two long chains, each containing 493 amino acids, where the total molecular weight of the enzyme is 54548 daltons, and the molecular weight of each series is estimated to be about 48 daltons. According to some studies, the enzyme is believed to have a role in regulating peptide hormones, and the decrease or absence of its activity has been associated with a rare disease known as proleydiz deficiency PD [6],[7].

Prolidase enzyme deficiency is a rare genetic disorder resulting from mutations in the PEPD gene, characterized by loss of prolidase enzyme activity. This condition leads to elevated levels of proline and hydroxyproline, resulting in impaired collagen production [8]. A deficiency in the activity of this enzyme causes impaired breakdown of collagen and proteins containing proline. These mutations lead to impaired wound healing and contribute to the appearance of characteristic skin symptoms. Mental retardation has also been observed in some people suffering from prolidase deficiency. This is due to high levels of proline, a compound that can negatively affect the functions of glutamatergic neurons in the central nervous system [9],[10].Diabetes is a highly common endocrine conditions , affecting more than 170 million people worldwide. This disease is characterized by inadequate insulin production , in some cases the hormone insulin

produced by the pancreas is ineffective. Diabetes is known as the "silent killer" because it is often discovered when health complications appear. Common problems faced by people with diabetes include failure to adhere to necessary care, such as a healthy diet and insulin, as well as a lack of knowledge about the disease and a lack of physical activity [11],[12]. In othe hand the Diabetes mellitus type 2 is the predominant form, representing 96% of diabetes among adults, and is commonly associated with genetic factors and lifestyle. The development of this disease is mainly a major factor: the first is the inability of beta cells in the pancreas to secrete insulin effectively, and the second is the lack of a good response of insulin-sensitive tissue to it [13],[14]. Chronic metabolic disorder is considered to cause high blood glucose levels. This condition is mainly due to insulin resistance and poor glucose tolerance, as well as the potential for insulin production shortages in some cases [15]. Type 2 diabetes begins with progressive development, with high blood sugar only observed after large meals. Unlike type 1 diabetes, where the function of pancreatic cells deteriorates further, the high level of blood sugar becomes more pronounced as the disease progresses in type 2. Studies suggest that about 40% of individuals with glucose intolerance can lose the mass of pancreatic cells, and this percentage increases to about 60% when type 2 diabetes is clinically diagnosed [16]. Drug selection is a critical element. Priority should be given to drugs that contribute to increased insulin sensitivity and support weight loss efforts. These drugs help improve the body's response to insulin, enhancing blood sugar level control. Conversely, the use of drugs that increase insulin levels in the body and promote weight gain should be reduced, as these factors can exacerbate the patient's state of health [17]. Therefore, this study aims to evaluate the activity of prolidase in T2DM patients and investigate its association with biochemical parameters .

Materials and Methods:

The study included (90) serum samples collected from external laboratories from Samarra and Tikrit districts, (60) samples of males and females with type 2 diabetes, aged between 30-70 years, and (30) samples of healthy

individuals as a control group, aged between 30-70 years. Blood was collected from the vein using a 5 ml capped syringe, and the blood was placed in tubes containing a gel tube, which facilitates the process of serum isolation. It was separated by a centrifuge at 3000 degrees Celsius for 10-15 minutes, then the serum was isolated and placed in tubes divided into two samples to ensure preservation, and the two samples were preserved at a temperature of (-20) until use.

Biochemical tests:Prolidase enzyme interacts with binary peptide (kleisin-proline) as an enzyme substrate core substance [18], amino acid-proline and claycine editors freely, measured by the amount of brulin emancipated by logne after its interaction with nanhydrin t 515nm wavelength based on proline as standard material.

Its level has been calculated according to the following formula[19] :

$$\text{ProlidasActivity(U/L)} = \textbf{Abs test-Ab}\boldsymbol{control}/\textbf{Abs }\boldsymbol{of standard} \times \mathbf{2.4} \times (\boldsymbol{S})$$

The study involved measuring several variable,including *TNF* (Tumor Necrosis Factor Alpha), *CRP* (C-Reactive Protein) from Elabscience in the ELISA System reader from Human Source Germany[20] while biochemical parameters including glucose, urea, creatinine, uric acid, calcium, magnesium, and lipid profile were assessed using commercial kits from Linear Chemicals (Spain) via ELISA System Reader.

Ethical Considerations: Ethical approval was obtained from the relevant institutional review board. Informed consent was obtained from all participants prior to sample collection.

Statistical analysis: SPSS was used to determine differences in biochemical parameters between T2D patients and the healthy contro group, using those F test at a statistically significant level ($P \leq 0.0001$) ROC curve analysis was employed to evaluate the diagnostic performance of these parameters in serum to differentiate between patients and the control group. Prolidase levels and the studied biochemical parameters were also determined, with the area under the curve (AUC) assessed.

Results and discussion :

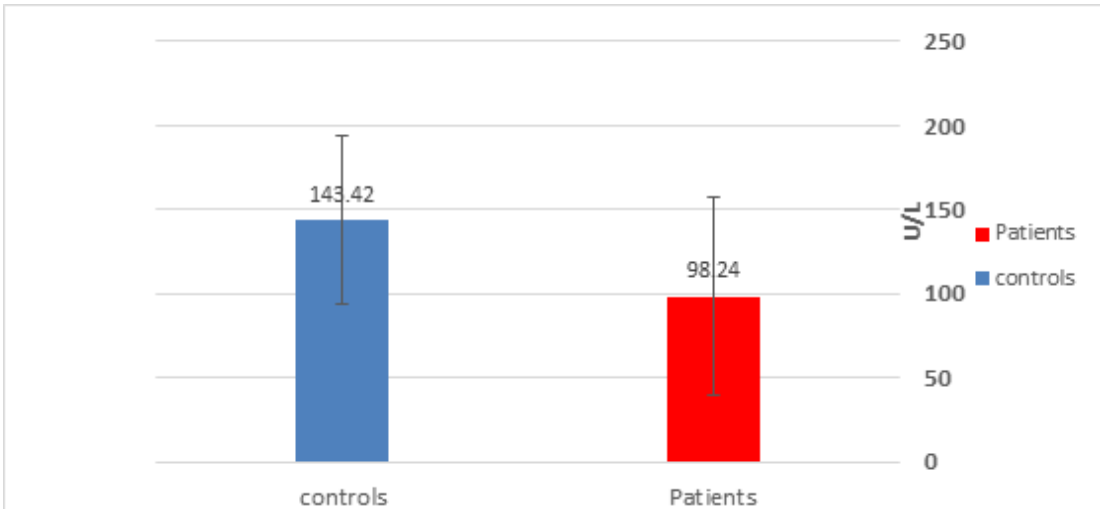
Table 1 shows a significant decrease in prolidase activity in T2DM patients compared to healthy controls *(P<0.001*). The findings of this research confirm the hypothesis that prolidase deficiency is associated with metabolic imbalance in T2DM. The reduced enzyme activity indicates possible defective collagen turnover in diabetic patients, which may be the cause of complications such as impaired wound healing.

Table 1:
levels of biochemical parameters in serum of patients with type 2 diabetes.

Variables	Control n=30 (Mean ± SD)	Patients n=60 (Mean ± SD)	*P-value*
Age (years)	40.2 ±10.03	55.63±12.8	0.0002
BMI (kg/m²)	25.7±1.35	27.83 ± 2.76	0.0006
prolidase(U/L)	143.4249.99±	98.2458.84±	<0.001
CRP (pg/mL)	8.21±0.68	13.83±3.36	<0.0001
TNF (pg/mL	80.04±20.86	121.03±28.98	<0.0001
Gluguse (mg/dl)	115.51±6.34	196.1±23.24	<0.0001
HbA1c	5.1±0.33	7.83±1.25	<0.0001
Urea(mg/dl)	22.93±1.59	38.42±8.63	<0.0001
Creatinin(mg/dl)	0.3±0.03	0.92±0.6	<0.0001
uric acid (mg/dl)	4.05±1.18	6.28±0.54	<0.0001
mg(mg/dl)	2.06±0.04	1.11±0.05	<0.0001
Ca(mg/dl)	10.67±0.34	8.21±0.33	<0.0001
CHOLE(mg/dl)	200.46±9.19	227.19±10.7	<0.0001
2 TRI(mg/dl)	193.24±6.77	223.41±9.4	<0.0001
3HDL(mg/dl)	47.27±12.96	21.71±1.75	<0.0001
VLDL(mg/dl)	38.64±1.35	44.68±1.88	<0.0001
LDL(mg/dl)	114.53±14.83	160.79±9.17	<0.0001

The ruslt shows that the BMI increased in body mass index of people with type 2 diabetes compared to healthy controls in (*27.83 ± 2.76vs 25.7±1.35,P = 0.0006*) as in Table (1) Obesity is a significant contributor to the rising incidence of type 2 diabetes, which is a chronic condition in which the body suffers from a deficiency in producing insulin sufficiently or the inability to use it effectively, causing high blood glucose levels [21]. Type 2 diabetes is characterized by a decrease in insulin secretion from the beta cells in the pancreas, along with resistance in the body's cells to insulin, which leads to an increase in fatty acid levels. These processes lead to a reduction in the ability to transport glucose to muscle cells, in addition to an increase in the breakdown of fats and the production of glucose in the liver [22]. According to studies that indicate that obesity occurs at a higher mass rate in the middle of life (50-40), and in all parts of the world, men are more susceptible to obesity than type 2 diabetics as a result of smoking rates, fat distribution, and food choices as a result of which men tend to store fat in the abdominal area. In contrast, according to studies, women usually store a larger amount of fat under the skin [23],[24] .

Prolidase enzyme : A significant decrease in prolidase levels was observed in type 2 diabetic patients compared to healthy individuals as in tabe (1) (*98.24 ± 58.84 vs. 143.42 ± 49.9, P < 0.001*). This enzyme deficiency is consistent with previous findings, indicating differential effects of diabetes on prolidase activity. Prolidase is an important enzyme in collagen metabolism and plays a crucial role in wound healing. Impaired prolidase activity can lead to wound healing problems, which impacts the patient's overall well-being. [10], [25]. Prolidase deficiency is a rare genetic disorder caused by mutations in the PEPD gene, resulting in a loss of function of the prolidase enzyme. This deficiency can lead to serious health complications, including skin and musculoskeletal problems. If left undiagnosed or untreated, it can increase the risk of chronic, difficult-to-treat skin ulcers [8].



Figer (1): : Mean ± SD for prolidase.

A decrease in the enzyme is associated with decreased collagen, and diabetes also affects collagen structure and metabolism in multiple ways, leading to various consequences. Among the mechanisms causing these changes are elevated blood sugar levels, rise in advanced glycation products, and increased oxidative stress [26]. Research indicates that type I collagen levels decline almost linearly with age, in both sexes. Studies also indicate a decrease in collagen production and synthesis in response to the aging process [27] .

Inflammatory factors: Both tumor necrosis factor alpha (*TNF-α*) and C-reactive protein (*CRP*) levels were higher in individuals with T2D compared to healthy controls ($p<0.0001$) as in tabe (1). This is consistent with this study [28],[29] . Tumor necrosis factor alpha (*α-TNF*) is key factor within stimulating tissue inflammation and thereby increasing the risk of type 2 diabetes. This factor plays an important role in the etiology of tissue insulin insensitivity, a condition considered a primary risk factor for disease developmen. In addition, impaired beta cell function in the pancreatic islets is correlated with elevated *TNF-α* production in fatty tissue. Studies indicate that elevated levels of this factor in fatty tissue is closely correlated with obesity-induced insulin resistance, leading to the development[30]. C-reactive protein (*CRP*) is an important biomarker stimulated by pro-inflammatory cytokines from fat cells, the liver produces. *CRP* levels are naturally low in healthy individu-

als, but chronically elevated in patients with type 2 diabetes, demonstrating a correlation between blood sugar and elevated inflammatory markers [31]. *CRP* plays a crucial role in various inflammatory processes. Furthermore, exacerbated inflammation, mediated by increased *CRP* levels, is associated with decreased beta cell function in patients with type 2 diabetes, negatively impacting blood sugar control [32].

Blood sugar tests : High levels of glucose and HbA1c were found in diabetic individuals with the condition versus healthy controls at a probability value ($p<0.0001$) as in tabe (1). High levels of glycated hemoglobin (*HbA1c*) indicate poor blood glucose control. It has been observed that a greater number of diabetic patients have high levels of *HbA1c*. This is partly due to inadequate dietary practices, lack of appropriate diets, and lack of exercise. Some studies have confirmed a positive association between better blood glucose management and improved HbA1c, reflecting the importance of lower *HbA1c* levels and better glycemic control[33]. Elevated plasma glucose levels stimulate insulin secretion. The combination of hyperinsulinemia and hyperglycemia contributes to (1) accelerated glucose uptake by visceral tissues, such as the intestine and liver, as well as peripheral tissues, especially muscle, and (2) inhibition of endogenous glucose production, particularly within the body [34]. Elevated blood glucose levels result in insulin resistance, a key feature of type 2 diabetes. This condition has two main aspects: increased insulin resistance in both skeletal muscle and liver, and impaired pancreatic beta cell function. According to this study, increased insulin resistance begins early in the development of type 2 diabetes and is temporarily compensated for by increased insulin secretion from beta cells [35].

Kidney function: we observed an increase in urea, creatinine, and uric acid in type 2 diabetes patients compared to the control group at a probability ($p<0.0001$) as in tabe (1). This is consistent with studies [36] suggesting that uncontrolled diabetes leads to elevated blood glucose levels, which in turn leads to elevated serum creatinine and urea levels. These biomarkers are important for diagnosing any kidney dysfunction, while urea production is affected by many factors such as liv-

er performance, protein intake, and protein degradation rate. Serum creatinine is a more reliable indicator for estimating glomerular filtration rate, which clearly reflects kidney function. Measuring serum creatinine and urea contributes to the early detection of diabetes-related kidney disease, helping prevent serious complications. Kidney disorders resulting from diabetes are known as diabetic nephropathy [37] , Urea levels increased with age, with the lowest levels in the 30-49 age group. Creatinine levels also recorded a noticeable increase, especially in the age group over 60 years [38]. Studies indicate that increased serum blood uric acid levels may increase probability of type 2 diabetes. Data show high uric acid levels account for about a quarter of diabetes cases levels. These findings corroborate earlier studies. Some research in the 1980s suggested a link relationship between circulating uric acid levels as well as an increased probability of developing diabetes mellitus. At the time, it was hypothesized that hyperuricemia might be a result of insulin resistance. However, recent evidence suggests implying that uric acid plays a part in the progression of the disease. “Elevated uric acid levels are associated with increased oxidative stress and tumor necrosis facto production, both of which are involved in the development of type 2 diabetes [39], Also, there is observed an increase in uric acid levels among men because the hormone estrogen enhances the secretion of uric acid, while its levels increase in women after menopause, which contributes leading to higher uric acid levels in diabetics with advancing asge [40].

Electrolytes (Mg^{+2}, Ca^{+2}): We observed lower magnesium and calcium levels between type 2 diabetes patients and controls at a probability value ($P<0.0001$) as in tabe (1). This is consistent with studies indicating that magnesium (Mg) is an essential element in the body, playing an important role in insulin regulation activity and glucose absorption by insulin, in addition to its effect on vascular tone [41]. Low intracellular magnesium concentrations can lead to a defect in tyrosine kinase activity.Magnesium is heavily involved in regulating insulin signaling resulting in an impaired insulin response after binding to its receptors, further impairing the insulin response

in diabetics [42],[43] .Evidence suggests that low magnesium intake and increased urinary loss are major factors that may be a factore to magnesium depletionin the context of type 2 diabetes, especially those who struggle to control blood glucose levels [44]. Diabetes-related hyperglycemia can lead to decreased calcium rates efflux from cells. Increased calcium entry and decreased calcium exit result in a persistently elevated cytoplasmic calcium level. Studies have shown that calcium concentrations in patients with type 2 diabetes, whether they have complications or not, are significantly lower than in healthy controls [45]. Some studies have also observed a significant decrease in serum prolidase levels in patients suffering from osteoporosis, a calcium deficiency disease, which can implies diminished collagen regeneration [1].

Lipid profile we observed an increase in lipid levels, except high-density lipoprotein (*HDL*), between type 2 diabetes patients and controls at a probability ($P<0.0001$)as in tabe (1). Diabetes"is associated with elevated blood cholesterol levels, which accelerate the development of atherosclerosis, a major long-term complication of diabetest this ruslt agrees with Murwan et.al [46] indicate that elevated low-density lipoprotein (*LDL*) in diabetic patients enhance *LDL* receptor expression, leading to elevated *LDL* cholesterol levels. Elevated triglyceride levels, resulting from increased production of very-low-density lipoprotein (*VLDL*), also increase plasma triglyceride levels. During the exchange process by cholesterol ester transfer protein (*CETP*), *HDL* cholesterol levels decrease, resulting in diminished glucose metabolism and, consequently, "elevated glycemia. In diabetes, tissues are unable to utilize blood glucose, increasing its plasma concentration. Additionally, fatty acids stored in adipose tissue are used as an energy source, leading to the accumulation of excess fatty acids in the liver and their conversion to triglycerides [47] . In other hand that lipid profile levels tend to rise with age in both sexes. the data from the current the study shows that women with diabetes aged 45 or older are at increased risk of lipid disorders versus men. The data show higher LDL cholesterol levels along with triglyceride levels, with a sig-

nificant decrease in HDL cholesterol concentrations, especially among older adults. Studies also found that high total cholesterol and high triglycerides co-occur almost equally, highlighting that women are more prone to developing high LDL cholesterol compared to men in in general , some studies indicate that all types of lipid disorders are significantly more common among women with diabetes [49] . [48]. The study also included Receiver Operating Characteristic (ROC) curve analysis for the evaluated parameters, as illustrated in Table 2 and Figures 1 to 10. These analyses were performed to assess the diagnostic performance and discriminative ability of each parameter in distinguishing between the studied groups.

Table: (2) AUC, cut off points , sensitivity analysis, and specificity for biochemical parameters via ROC analysis in patients versus controls.

parameter	Cut off	sensitivity analysis	Specificity analysis	+PV	-PV	AUC
CRP	>9.022	80	100	100	50	0.888
TNF	>86.002	80.00	75.00	95.2	37.5	0.870
Glucose	>125.156	100	100	100	100	1
Urea	>25.359	100	100	100	100	1
Creatinine	>0.344	96	100	100	80	0.99
uric acid	>5.482	88	100	100	57.1	0.97
mg	≤1.217	100	100	100	100	1
Ca	≤8.964	100	100	100	100	1
CHOLE	>209.152	96	100	100	80	0.98
2TRI	>200.793	100	100	100	100	1
3HDL	≤24.24	100	100	100	100	1
VLDL	>40.1586	100	100	100	100	1
LDL	>137.7638	100	100	100	100	1
HbA1c	>5.6	100	100	100	100	1
BMI	>26.5118	72.00	80.00	94.7	36.4	0.784
AGE	>45	76.00	80.00	95.0	40.0	0.824

As illustrated in Figures 1 to 10, Receiver Operating Characteristic (ROC) curve analysis indicated excellent diagnostic performance for most biochem-

ical and clinical parameters. Of significance, CRP (0.888), TNF-α (0.870), creatinine (0.990), uric acid (*0.970*), total cholesterol (0.980), BMI (0.784), age (0.824), and HbA1c (1.000) had high Area Under the Curve (AUC) values, indicating good discriminative ability. Other parameters like glucose, triglycerides (TRI), HDL, VLDL, and LDL presented high values of AUC, further supporting their potential utility as diagnostic markers for the disease in question.

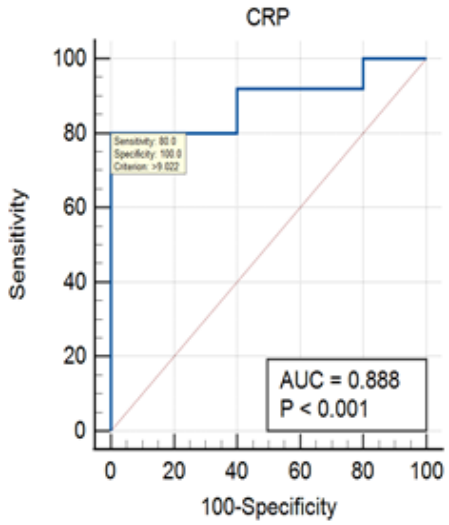


Fig.1. ROC analysis of CRP in disease and control groups

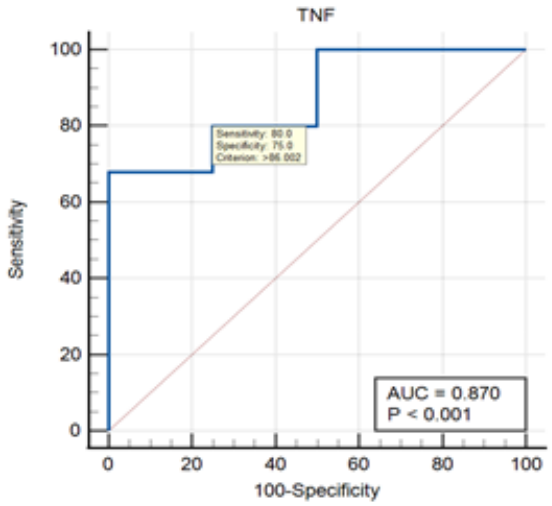


Fig.2. ROC analysis of TNF-α in disease and control.

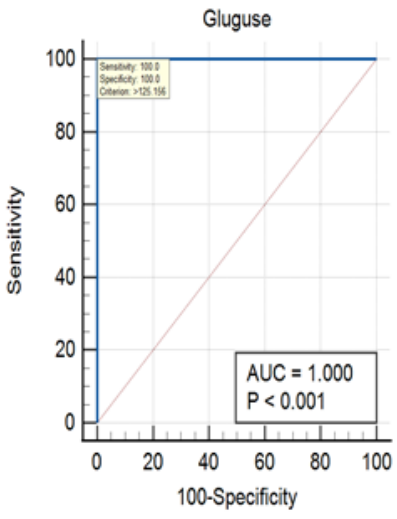


Fig. 3. ROC analysis of Glucose in disease and control.

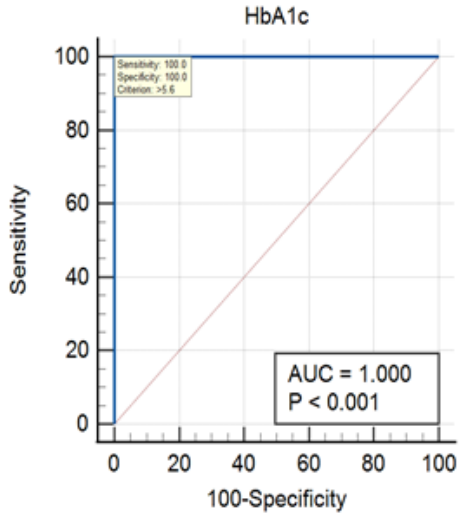


Fig.4. ROC analysis of Hba1c in disease and control.

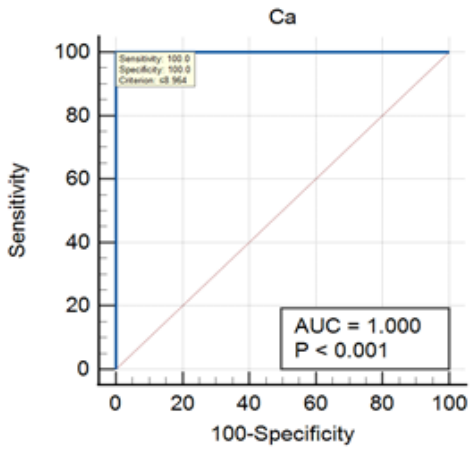


Fig.5. ROC analysis of Ca in disease and control.

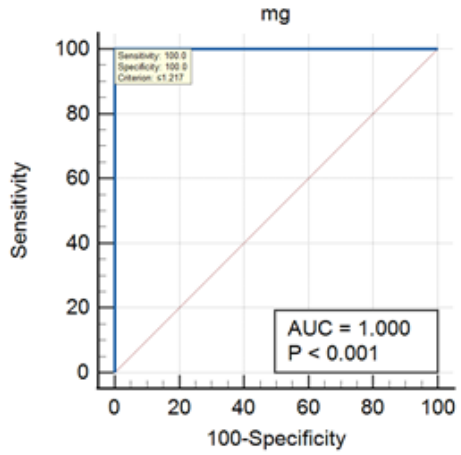


Fig.6. ROC analysis of Mg in patient and control

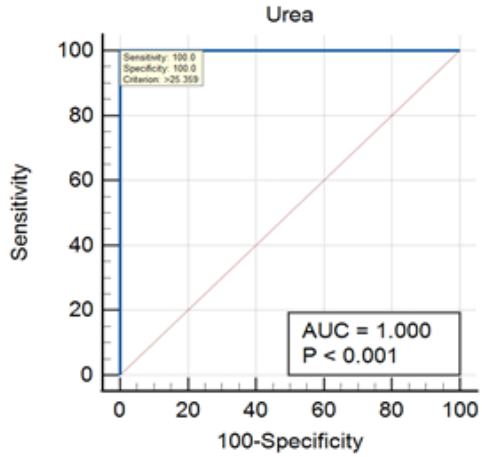


Fig.7. ROC analysis of urea in disease and control.

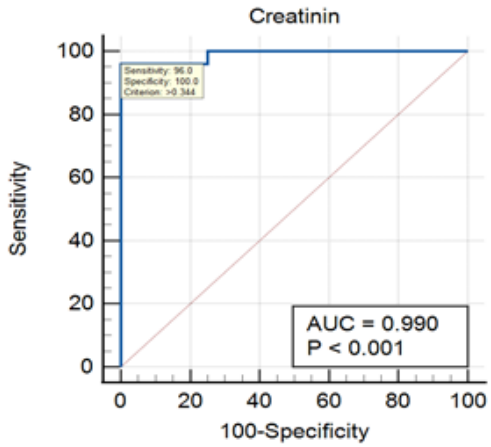


Fig.8. ROC analysis creatinine disease and control

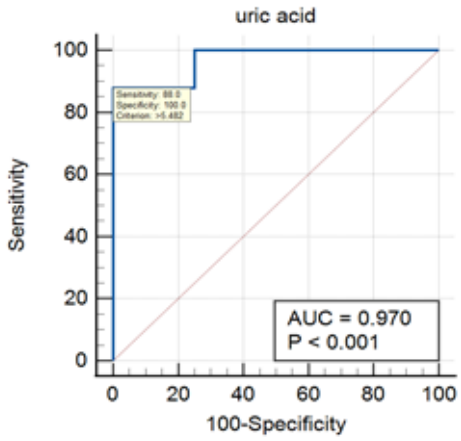


Fig.9. ROC analysis of uric acid in disease and control

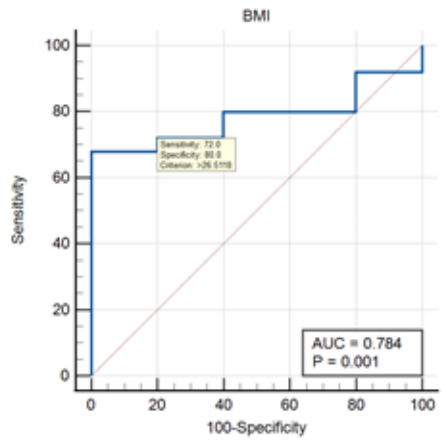


Fig.10. ROC analysis of uric BMI in patient and control

Conclusions

In conclusion, the study demonstrated significantly decreased prolidase activity in type 2 diabetes patients, together with increased inflammatory markers and impaired biochemical parameters. The findings suggest the potential role of prolidase and related biochemical markers in the early diagnosis and follow-up of T2DM complication.

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