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INDICADORES DE OSTEOPOROSE EM MULHERES DIABÉTICAS: A INTERAÇÃO DE CALCITONINA, CÁLCIO, VITAMINA D E FOSFATASE ALCALINA

OSTEOPOROSIS INDICATORS IN DIABETIC WOMEN: THE INTERPLAY OF CALCITONIN, CALCIUM, VITAMIN D, AND ALKALINE PHOSPHATASE

مؤشرات هشاشة العظام لدى مرضى داء السكري من النساء: تداخل هورمون الكالسيتونين، الكالسيوم، فيتامين د، وأنزيم الفوسفاتيز القلوي

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RESUMO

Introdução: O diabetes tipo 2 é caracterizado por hiperglicemia e hiperinsulinemia. O diabetes mellitus é comum em todo o mundo porque a corrente sanguínea de um adulto médio tem 100 miligramas por decilitro de glicose em cinco a dez gramas. Doenças renais e cardiovasculares relacionadas ao diabetes possuem maior custo financeiro e medicamente mais onerosas. Uma doença óssea comum é a osteoporose, caracterizada por ossos enfraquecidos que aumentam o risco de fratura, causando desconforto, função prejudicada e menor qualidade de vida. **Objetivo**: Este estudo visa avaliar algumas variáveis bioquímicas para pacientes diabéticas (Calcitonina, Ca, Vitamina D, ALP). **Métodos**: O estudo incluiu amostras de sangue de 120 mulheres. As amostras foram classificadas em dois grupos, o grupo controle com 40 amostras e um grupo de pacientes diabéticas com 80 amostras. O estudo foi realizado no Centro de Endocrinologia e Diabetes em Maysan Governorate, entre janeiro de 2023 e abril de 2023. As amostras de mulheres foram verificadas por um médico especialista em diabetes e foram diagnosticadas com diabetes, e algumas delas são obesas de acordo com os níveis de (IMC e HbA1c). Mulheres grávidas e fumantes foram excluídas. Foi utilizado o método ELISA usando um kit de calcitonina SUNLONG para determinar o nível sérico de calcitonina; os kits (Ca, ALP) fabricados pela A15 BioSystems foram utilizados para determinar cálcio e fosfatase alcalina, enquanto a vitamina D utilizou um kit VIDAS. **Resultados**: Os resultados revelaram um efeito significativo ($p<0.05$) em todas as variáveis em pacientes com D.M. que foram detectadas em comparação com o grupo de controle (O grupo de controle foi escolhido com base no fato de que os indivíduos estão livres de diabetes, obesidade e outras doenças crônicas e dentro do grupo etário de 20 a 65 anos). Os resultados mostram um efeito significativo na Calcitonina e Cálcio ($p<0.05$), e um aumento não significativo na Vitamina D e ALP ($p>0.05$) em pacientes com D.M., que foi detectado em comparação com as concentrações do grupo de controle. **Discussão**: Os resultados do estudo indicam que a calcitonina reduz o cálcio circulante, levando à teoria de que seu papel fisiológico na hipercalcemia pode estar envolvido no retorno das concentrações séricas médias de cálcio. Uma deficiência de vitamina D compromete a capacidade das células beta pancreáticas de secretar insulina em resposta à glicose, as células pancreáticas expressam receptores de vitamina D que se ligam à forma ativa circulante, 1,25 (O.H.)2D. **Conclusões**: O estudo destaca as mudanças significativas nos níveis de calcitonina e cálcio em pacientes diabéticas em comparação com controles saudáveis, sugerindo a necessidade de monitoramento cuidadoso dessas variáveis bioquímicas no manejo do diabetes. Os resultados oferecem novos insights sobre os aspectos metabólicos do diabetes, destacando áreas potenciais para intervenção terapêutica.

Palavras-chave: *Bioquímica do Diabético, Impacto da Calcitonina, Desequilíbrio de Cálcio, Deficiência de Vitamina D, Níveis de ALP.*

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ABSTRACT

Background: Type 2 diabetes is characterized by hyperglycemia and hyperinsulinemia. Diabetes mellitus is common worldwide because an average adult's bloodstream has 100 milligrams per deciliter of glucose at five to ten grams. Diabetes-related renal and cardiovascular diseases are more expensive and medically burdensome. One common bone disease is osteoporosis, characterized by weakening bones that increase fracture risk, causing discomfort, impaired function, and lower quality of life. **Aim:** This study aims to evaluate some biochemical variables for diabetic female patients (Calcitonin, Ca, Vitamin D, ALP). **Methods:** The study included blood samples from 120 women. The samples were classified into two groups, a control group with 40 samples and a group of diabetic patients with 80 samples. The study was conducted at the Endocrinology and Diabetes Center in Maysan Governorate from January 2023 to April 2023. The women's samples were medically checked by a diabetes specialist and were diagnosed with diabetes, and some of them are obese according to (BMI and HbA1c) levels. Pregnant women and smokers were excluded. The ELISA method using a SUNLONG calcitonin kit was used to determine the serum level of calcitonin; the (Ca, ALP) kits manufactured by A15 BioSystems were used to determine calcium and Alkaline Phosphatase, while Vitamin D used a VIDAS kit. **Results:** The current findings revealed a significant effect ($p<0.05$) in all variables in D.M. patients that were detected compared to the control group (The control group was chosen on the basis that individuals are free of diabetes, obesity, and other chronic diseases and within the age group 20-65 years). The results show a significant decrease in Calcitonin and Calcium ($p<0.05$), and non-significant differences in Vitamin D and ALP ($p>0.05$) in D.M. patients, which was detected in comparison with the control group concentrations. **Discussion**: The current study's findings indicate that calcitonin reduces circulating calcium, leading to the theory that its physiological role in hypercalcemia may be involved in returning average serum calcium concentrations. A vitamin D shortage compromises pancreatic beta cells' ability to secrete insulin in response to glucose, pancreatic cells express vitamin D receptors that bind to the active circulating form, 1,25 (O.H.)2D. **Conclusions:** The study underscores the significant changes in calcitonin and calcium levels in diabetic female patients compared to healthy controls, suggesting the need for careful monitoring of these biochemical variables in diabetes management. The findings offer new insights into the metabolic aspects of diabetes, highlighting potential areas for therapeutic intervention.

Keywords: *Diabetic Biochemistry, Calcitonin Impact, Calcium Imbalance, Vitamin D Deficiency, ALP Levels.*

الملخص

الخلفية: يتميز مرض السكري من النوع الثاني بأرتفاع السكر في الدم وفرط أنسولين الدم. يعد داء السكري شائعًا في جميع أنحاء العالم حيث يحتوي مجرى الدم لدى الشخص البالغ في المتوسط على 100 ملليجرام لكل ديسيلتر من الجلوكوز بمعدل خمسة إلى عشرة جرامات. تعتبر أمراض الكلى والقلب والأوعية الدموية المرتبطة بمرض السكري أكثر تكلفة ومرهقة طبيا. أحد أمراض العظام الشائعة هو هشاشة العظام. يتميز بضعف العظام مما يزيد من خطر الكسر، مما يسبب عدم الراحة، وضعف الوظيفة، وانخفاض نوعية الحياة. **الهدف**: تهدف هذه الدراسة إلى تقييم بعض المتغيرات الكيموحيوية لدى مريضات السكري (هرمون الكالسيتونين، الكالسيوم، فيتامين د، وأنزيم الفلوسفاتيز القلوي). **طرائق العمل**: شملت الدراسة عينات دم لمائة وعشرون امرأة. تم تصنيف العينات إلى مجموعتين، مجموعة السيطرة مكونة من اربعون عينة و مجموعة مرضى السكري مكونة من ثمانون عينة. اجريت الدراسة في مركز الغدد الصماء والسكري في محافظة ميسان للفترة من كانون الثاني 2023 الى نيسان 2023. تم فحص عينات النساء طبيا من قبل اخصائي السكري وتم تشخيص اصابتهن بمرض السكري وبعضهن يعانين من السمنة المفرطة حسب (مستويات مؤشر كتلة الجسم ونسبة السكر التراكمي). تم استبعاد النساء الحوامل والمدخنات. تم استخدام طريقة ELISA بأستخدام عدة SUNLONG الكالسيتونين لتحديد مستوى الكالسيتونين في مصل الدم؛ تم استخدام عدة (Ca, ALP) المصنعة من قبل شركة A15 BioSystems لتحديد الكالسيوم والفوسفاتيز القلوي، بينما فيتامين د بإستخدام عدة VIDAS. **النتائج**: أظهرت النتائج الحالية وجود تأثير معنوي ($P<0.05$)في جميع المتغيرات في مرضى داء السكري الذين تم اكتشافهم مقارنة بمجموعة السيطرة (تم اختيار مجموعة السيطرة على أساس خلو الأفراد من مرض السكري والسمنة والأمراض المزمنة الأخرى وضمن الفئة العمرية 20-65 سنة). أظهرت النتائج انخفاضاً معنوياً في هرمون الكالسيتونين والكالسيوم عند ($p<0.05$) ، ولا يوجد فرق معنوية في فيتامين د وانزيم الفوسفاتيز القلوي عند ($p>0.05$) في مرضى داء السكري، بالمقارنة مع تراكيز مجموعة السيطرة. **المناقشة**: تشير نتائج الدراسة الحالية إلى أن هرمون الكالسيتونين يقلل من الكالسيوم في الدورة الدموية، مما يؤدي إلى نظرية مفادها أن دوره الفسيولوجي في فرط كالسيوم الدم قد يكون له دور في إعادة متوسط تراكيز الكالسيوم في الدم. يؤدي نقص فيتامين د إلى إضعاف قدرة خلايا بيتا البنكرياسية على إفراز الأنسولين استجابة للجلوكوز، وتعبر خلايا البنكرياس عن مستقبلات فيتامين د التي ترتبط بالشكل النشط في الدورة الدموية، 1,25(O.H.)2D. **الاستنتاجات**: تؤكد الدراسة على التغيرات الكبيرة في مستويات هرمون الكالسيتونين والكالسيوم لدى مريضات السكري مقارنة بالأشخاص الأصحاء، مما يشير إلى الحاجة إلى مراقبة دقيقة لهذه المتغيرات البيوكيميائية في إدارة مرض السكري. تقدم النتائج رؤى جديدة حول الجوانب الأيضية لمرض السكري، وتسلط الضوء على المجالات المحتملة للتدخل العلاجي.

الكلمات المفتاحية: *الكيمياء الحيوية لمرضى السكري، تأثير الكالسيتونين، اختلال توازن الكالسيوم، نقص فيتامين د، مستويات انزيم الفوسفاتيز القلوي.*

1. INTRODUCTION:

The most frequent consequence is an increased risk of hip or spine fracture, which is between 2.4 and 7 times more than in T1DM and 2-3 times greater in T2DM than in the general healthy population (Dawood *et al.,* 2023). Bone health can be improved by understanding the factors that affect patients with D.M. (Abusaib *et al*., 2020).

A decrease in BMD, alterations to the microarchitecture, and an increased risk of fracture as a direct result of these changes are all signs of the dangerous health condition osteoporosis. Numerous people of various racial and gender identities are affected by osteoporosis. With age, the condition is anticipated to worsen. It is a condition that goes unnoticed until fractures happen, which can have significant secondary health effects and problems, including death. Patients with T2DM have repeatedly demonstrated the link between osteoporosis and diabetes mellitus. The causes of more significant bone mineral density loss include diabetes mellitus diagnosis at an earlier age, longer duration and higher insulin dosages, and extended periods of poor glycemic control (Dawood *et al.,* 2023). Low bone turnover and advanced glycation end products (AGEs) buildup can affect bone fragility. Reduced bone strength, which increases the risk of fracture and causes pain, diminished function, lower quality of life, and debilitation, is a prevalent skeletal illness called osteoporosis (Jiang and Xia, 2018).

Diabetes mellitus is a condition of macromolecule metabolism characterized by a decreased capacity of the body to produce or respond to endocrine, which helps maintain healthy blood sugar levels (glucose). Malady is a chronic condition that develops when the duct gland can no longer create endocrine or the body cannot utilize the endocrine it produces (Dubey and Sciences 2020). Despite having different underlying mechanisms, Type 1 Diabetes Mellitus (T1DM) and Type 2 Diabetes Mellitus (T2DM) both increase the risk of fracture, which is brought on by a variety of reasons and can be partially explained by Bone mineral density (BMD) loss.

In humans, the para-follicular or C cells of the thyroid gland release calcitonin when the amount of serum calcium rises. In the bone, where it acts primarily, calcitonin considerably lowers osteoclast activity and bone resorption (Marte *et al.*, 2021). Given that calcitonin lowers blood calcium levels, it has been hypothesized that one of its physiological functions in hypercalcemia is to bring serum calcium levels back to normal. When hypercalcemia was directly induced by calcium injection or infusion, the thyroid gland's existence was required to lower the skeleton, the stomach, and the kidney calcium homeostasis. These organs maintain blood calcium levels between a predetermined range of 8.5 and 10.5 mg/dL (Naot *et al.,* 2019).

Clinical research data suggested that calcium and vitamin D supplements may positively impact glucose metabolism (Cipriani *et al*., 2020). Calcium is absorbed more quickly in the intestine thanks to vitamin D, which also lowers the risk of fractures while increasing bone mineral density. (Tamura *et al*., 2020). By utilizing the Vitamin D Receptors (VDR), vitamin D exerts its insulinotropic effect, increasing calcium influx through the cell membrane. By encouraging insulin production in pancreatic beta cells, vitamin D impacts insulin secretion(Bhosle and Mubeen, 2018). The most recent meta-analysis showed calcium and vitamin D supplementation significantly lower fasting glucose, homeostasis model assessment of insulin resistance (HOMA-IR), and insulin levels (Cipriani *et al*., 2020). The tissue non-specific alkaline phosphatase (ALP) is a hydrolase enzyme that is widely expressed in human tissues, particularly the liver, bone, and kidney. (Zhang *et al*., 2020). The aim of this study: evaluate biochemical variables for diabetic patients including Calcitonin, Ca, Vitamin D and ALP.

2. MATERIALS AND METHODS:

2.1. Materials

Determination of calcium (Ca) and Alkaline Phosphatase (ALP) assayed by A15 BioSystems Kit components. The VIDAS kit uses Calcitonin components for Vitamin D, and the ELISA method uses the SUNLONG calcitonin kit to determine the serum level of calcitonin

2.2. Methods

2.2.1. Study population

The current study was conducted in the Endocrinology and Diabetes Center and some private laboratories in Maysan Governorate, Iraq from January 2023 to April 2023. The whole blood sample included 120 women aged 20-65years, divided into two groups:

- Control group 40 (healthy females).
- Diabetic group (T2DM) 80 females.

The women samples have been checked medically by a Diabetes specialist and have been diagnosed with diabetes, and some of them are obese according to (BMI and HbA1c) levels. Pregnant women and smokers have been excluded. A questionnaire has been designed to obtain the actual information about the sample individuals in Maysan Province, after obtaining the consent form and justification of the patients.

2.2.2. Statistical analysis

The results are expressed as mean ± Standard Deviation (SD), this analysis was completed using IBM SPSS statistics, version 26 (IBM Co., Armonk, NY, USA), by utilizing a t-test under the significant threshold ($p \leq 0.05$) (Steel *et al.*, 1997).

3. RESULTS AND DISCUSSION:

3.1. Results

3.1.1. Determination of Ca

The results in Table 1 show a significant decrease at ($p<0.05$) by comparing D.M. patients (8.956± 0.4272 mg/dL) and the control group (9.164± 0.2914 mg/dL).

3.1.2. Determination of calcitonin

The results in Table 1 show a significant decrease at ($p<0.05$) by comparing D.M. patients (3.574±1.0402 mg/dL) and the control group (5.604± 3.3009 mg/dL).

3.1.3. Determination of Vitamin D

The results in Table 1 show a non–significant differences at ($p>0.05$) by comparing D.M. patients (15.086±14.8622 mg/dL) and the control group (15.356±8.1023 mg/dL).

3.1.4. Determination of ALP

The results in Table 1 show a non–significant differences at ($p>0.05$) by comparing D.M. patients (106.98±39.175 mg/dL) and the control group (98.48±24.641 mg/dL).

3.2. Discussion

The thyroid gland was necessary to lower the calcium concentrations in the circulation when calcium injection or infusion caused hypercalcemia (Xie *et al*., 2020). This study does not agree with Cheng and colleagues (Cheng 2022). The finding that calcitonin reduces circulating calcium led to the theory that its physiological role in hypercalcemia may be involved in returning average serum calcium concentrations. This idea was investigated in several in vitro experiments using rats, and many had parathyroidectomies without cells that secrete PTH without cells that secrete both PTH and calcitonin (C-cells). The results of this study do not agree with Deepak (Bhosle and Mubeen, 2018). The health benefits of vitamin D are both preventative and reparative. Researchers in recent years have linked low vitamin D levels to diabetes and insulin resistance. Evidence shows that vitamin D allows the body to secrete more insulin and may also increase insulin sensitivity. A non-significant and even inverse connection between 25 (O.H.) D3 and HbA1c levels in diabetes individuals were found in the results of various earlier investigations. In cohort research, it was discovered that there was an inverse correlation between glycosylated hemoglobin levels and 25 (O.H.) D3 levels in patients with T2DM as compared to the control group. These findings suggest that 25 (O.H.) D3 levels may influence the regulation of glucose in T2DM (Kostoglou-Athanassiou *et al.,* 2013; Zoppini *et al.,* 2013).

Furthermore, there is still dispute regarding the involvement of vitamin D in the insulin pathway despite the known connections between T2DM and vitamin D insufficiency being previously noted. Preclinical research suggests vitamin D regulates calcium influx into cells, insulin production, and cell survival. A vitamin D shortage compromises pancreatic beta cells' ability to secrete insulin in response to glucose, whereas supplementing with vitamin D appears to improve this ability(Bourlon *et al.,* 1999) (CADE and NORMAN, 1986). Pancreatic cells exhibit vitamin D receptor binding of the circulating active form, 1,25(O.H.)2D (Bhatt *et al.,* 2018).

Moreover, the insulin gene was revealed to include a vitamin D response element (Cheng *et al.,* 2011) and the presence of vitamin D receptors in skeletal muscle (Dobnig, 2008). Given that 1,25(O.H.)2D increases the transcription of insulin receptor genes, the renin gene is also suppressed, which decreases the

hyperglycemia-induced rise in renin levels in pancreatic cells. A fresh target for treating diabetes has been suggested: blocking renin-angiotensin activity (Cheng *et al.,* 2011).

The results of this study do not agree with (Madhu *et al*., 2023), ALPI's bone-specific component most likely contributes to vascular calcification in T2DM. ALP elevations in T2DM have been reported in the past without explanation. Furthermore, it has been observed that T2DM is associated with higher levels of bone-specific alkaline phosphatase (BAP). It is intriguing how BAP causes an increase in arterial calcification. Vascular smooth muscle cells express BAP. The matrix vesicles released by vascular smooth muscle cells serve as a nidus for vascular calcification. While some matrix-bound vehicles include calcification inhibitors such as fetuin-A (non-calcifying vehicles), others have large amounts of BAP. BAP speeds up calcification in many ways. Bone Alkaline Base Phosphatase deactivates polyphosphates, particularly pyrophosphate, which prevent calcification. Dephosphorylation of osteopontin has also been hypothesized as a mechanism for osteopontin inhibition. Additionally, BAP can hydrolyze organic phosphate esters, releasing phosphate, a substrate for calcification. Even if serum phosphate levels are not increased, changes in the phosphate-to-pyrophosphate ratio in the vascular tissue can cause vascular calcification (Madhu *et al*., 2023)**.**

Finally, pregnant women and smokers in such a study are usually excluded to avoid getting wrong results because the effect of smoking on the bones also appears at the age of fifty in women, making the decrease in bone density occur faster than others (Nieves, 2021). Additionally, during pregnancy, the fetus gets the nutrients it needs, including calcium from the mother`s body. If the mother`s body does not contain enough calcium, the fetus will get what it needs to form the bones and form the basic material of the teeth, from the mother`s bones and teeth, which exposes them to osteoporosis and weak teeth (Farias *et al*., 2020). In summary, this study extends the current understanding of diabetes by highlighting the nuanced interactions between the disease and various biochemical markers. The findings suggest a complex web of biochemical pathways affected by diabetes, with implications for both the management of the disease and the potential for future therapeutic strategies. Further research inspired by these findings could lead to improved clinical practices and patient outcomes.

4. CONCLUSIONS:

The study meticulously gathered data, revealing variations in the biochemical variables that were presented as both significant and non-significant when juxtaposed with the control group's data. A thorough medical evaluation by a diabetes specialist was a cornerstone of the study, which involved not only the confirmation of diabetes in participating women but also the detection of obesity in some participants, as evidenced by Body Mass Index (BMI) and Hemoglobin A1c (HbA1c) levels. These two metrics are crucial for diagnosing and managing diabetes and its related metabolic complications.

The core analytical technique used in this study, the ELISA method, allowed for the precise quantification of the serum levels of the biochemical variables in question. Through this method, the study found a notable pattern regarding the role of calcitonin. It was observed that increased levels of calcitonin were associated with lower levels of circulating calcium, suggesting a potential regulatory role of this hormone in managing elevated calcium levels, a condition known as hypercalcemia. The research also delved into the relationship between Vitamin D levels and pancreatic function. It emerged that a deficiency in Vitamin D could potentially impair the pancreatic beta cells' ability to secrete insulin in response to blood glucose levels. This is particularly significant as it underscores the hormone's role in glucose metabolism. The detection of Vitamin D receptors on pancreatic cells, which bind to the biologically active form of Vitamin D, 1,25(O.H.)2D, points to a direct mechanism through which Vitamin D may influence pancreatic function and insulin secretion.

5. DECLARATIONS

5.1. Limitations

The study is limited to the sample size and the methods applied during the study.

5.2. Acknowledgements

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this study.

5.3. Funding source

The authors funded this research.

5.4. Competing Interests

No conflict of interest exists in this publication.

5.5. Open Access

6. HUMAN AND ANIMAL-RELATED STUDIES

6.1. Ethical Approval

The ethical approval authority was obtained from the Training and Human Development Center at the Misan Health Directorate, Ministry of Health (Approval No: 11, Date: 08-01-2023).

6.2. Informed Consent

The consent form sought participants' authorization to publish data from their involvement in our present research study. The objective of this publication is to disseminate our discoveries within the scientific community. We ensured participants that their data would be anonymized to safeguard their privacy. We emphasized that their involvement was entirely voluntary, with the freedom to withdraw consent at any stage. Participants were informed that their signature on the form would signify their agreement to the utilization of their data in our publication.

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Table 1. *and Inferential statistics of Calcitonin (C.T.), Ca, Vitamin D, and ALP levels in D.M. patients and control group*

Parameters	**Diabetes (80) Mean ± SD(mg/dL)**	**Control (40) Mean±SD(mg/dL)**	**P- value**
Calcitonin	3.574± 1.0402	5.604± 3.3009	0.000
Ca	8.956± 0.4272	9.164± 0.2914	0.032
Vitamin D	15.086±14.8622	15.356±8.1023	0.933
ALP	106.98±39.175	98.48±24.641	0.326