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Original Articles

Case Reports

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September / Eylül **2020** **Vol** / Cilt: **24** **No** / Sayı: **3**

CONTENTS

Original Articles

TÜRKİYE ENDOKRİNOLOJİ VE METABOLİZMA DERNEĞİ

Turkish Journal of Endocrinology and Metabolism

JOURNAL OF THE SOCIETY OF ENDOCRINOLOGY AND METABOLISM OF TURKEY

Case Report

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JOURNAL OF THE SOCIETY OF ENDOCRINOLOGY AND METABOLISM OF TURKEY

EDITORIAL

Dear esteemed readers of TurkJEM Family,

Obesity is one of the greatest public health challenges of the 21st century. Its prevalence has tripled in many countries of the WHO European Region since the 1980s. Efforts for obesity mainly focus on policy and environmental strategies to make healthy eating and active living accessible and affordable for everyone.

"These are unusual times, and unusual times call for unusual actions. A substantial fraction of people with active COVID disease who are hospitalized with an endocrine-related disease and their endocrine problems will impact their treatment." – Raghavendra G. Mirmira, MD.

Obesity is a chronic repeating condition affecting a rapidly increasing number of people globally. Obesity-related conditions seem to worsen the effect of COVID-19; indeed, the Centers for Disease Control and Prevention (CDC) in US reported that people with heart disease and diabetes are at higher risk of COVID-19 complications. Statistics show that high percentage of population who will contract COVID-19 will also have a BMI over 25. Unfortunately lockdown during the first months of COVID-19 led to;

- Limit their physical activity;
- Have lower access to healthy and fresh foods;
- Have less access to preventive and health promotion services;

Knowing that physical activity contributes to both our physical and mental health, apart from treatment difficulties COVID-19 contributed to obesity while reducing the chance for cure on obese CCOVID-19 patients. World Health Organization developed rigorous physical exercise manuals that can be used under quarantine. Obesity, an increasingly common chronic disease globally, is significantly associated with progression to severe COVID-19 in adults hospitalized with SARS-CoV-2 infection. As COVID-19 may continue to spread worldwide, clinicians should pay close attention to obese patients. Obese patients should be carefully monitored and managed with prompt and aggressive treatment.

We have an engaging and inspiring set of works this week from our researchers: "T helper 1 cytokines and their relationship with β-cell function in type 1 diabetes", "Effect of vitamin D deficiency on the frequency of lipohypertrophy occurrence in patients with type 2 Diabetes Mellitus under injectable treatment ","The screening of comorbid depressive disorders and associated risk factors in adult patients with type 2 diabetes ","Association of types of diabetic macular edema with different anti-diabetic therapies", "The effects of low-carbohydrate diet and protein-rich mixed diet on insulin sensitivity, basal metabolic rate and metabolic parameters in obese patients", "The effect of falsely highlighted intestinal intraluminal areas and the fat in paraspinal muscles on abdominal adipose tissue measurements using computed tomography", "Factors affecting survival in adrenocortical cancers: Single-center experience ", "The relationship between TSH level and stage of differentiated thyroid carcinoma ", "Effects of isotretinoin treatment on levels of hormones involved in the etiopathogenesis of acne ","Thrombotic microangiopathy after spontaneous pheochromocytoma rupture: a rare MEN 2A case".

Wish you all a very healthy, pleasant late summer time and a smooth 2nd expected COVID-19 wave during fall.

With my best regards,

Nilgün Başkal MD
Editor-in-Chief

Turk J Endocrinol Metab. 2020;24:177-183

T Helper 1 Cytokines and Their Relationship with Beta Cell Function in Type 1 Diabetes

T Helper 1 Sitokinleri ve Tip 1 Diyabette Beta Hücre Fonksiyonu ile İlişkileri

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Abstract

Objective: In type 1 diabetes (T1D), T helper (Th) 1 cells affect β cell functions significantly. This study aims to explore the association between serum levels of Th1 cytokines [interferon-gamma (IFN-γ), interleukin (IL)-2 and tumor necrosis factor-alpha (TNF-α)] and β cell function in T1D. **Material and Methods:** The study included 110 patients with T1D (TIDPs) and 31 healthy controls. The β cell functions in T1DPS were assessed by calculating mixed-meal stimulated C-peptide levels. T1DPs were categorized into three groups depending on results of this test (1a-lowest, 1b, 1c-highest). Cytokine levels, IFN-γ/IL-2, and TNF-α/IL-2 ratios in T1DPs were compared with that in controls. Correlation analysis between cytokine levels and diabetes-related parameters was then carried out. **Results:** IFN-γ, TNF-α, IL-2 levels, and TNF-α/IL-2 of T1DPs were higher (p=0.02, p=0.01, p=0.008, and p=0.01, respectively) than that of controls. The highest IFN-γ/IL-2 and TNF-α/IL-2 ratios were observed in group 1b (p=0.03 and p=0.04, respectively) while the lowest IFN-γ/IL-2 and TNF-α/IL-2 ratios were observed in group 1a (p=0.03 and p=0.04, respectively). The TNF-α levels were found to be negatively correlated with fasting glucose levels (r^2=-0.003, p=0.031). However, after adjustment for age and gender, this correlation diminished (r^2=-0.028, p=0.076). **Conclusion:** IFN-γ, IL-2, and TNF-α may exhibit a triggering role in the pathogenesis of T1D. IFN-γ/IL-2 and TNF-α/IL-2 ratios possibly have more significant roles in the progression of β cell dysfunction than other cytokines (ClinicalTrials.gov number: NCT02389335).

Keywords: Type 1 diabetes; IFN-γ; IL-2; TNF-α; T helper-1 cell

Özet

Amaç: Tip 1 diyabette (T1D) T helper (Th) 1 hücrelerinin β hücre fonksiyonuna belirgin etkisi vardır. Bu çalışmada, T1D tanısı olan hastalarda serum Th1 sitokin [interferon-gama (IFN-γ), interlökin (IL)-2 ve tümör nekrozis faktör-alfa (TNF-α)] seviyeleri ile β hücre disfonksiyonu arasında bir ilişki saptanması amaçlandı. **Gereç ve Yöntemler:** Toplam 110 tip 1 diyabetli hasta (T1DH) ve 31 sağlıklı kontrol çalışmaya alındı. T1DH'lerin β hücre fonksiyonu, karışık öğün testi ile stimüle edilmiş C-peptit düzeyi ile ölçüldü. Hastalar, bu testin sonuçlarına göre üç gruba ayrıldı (1a- en düşük, 1b,1c- en yüksek). Sitokin seviyeleri ve IFN-γ/IL-2 ile TNF-α/IL-2 oranları kontroller ile karşılaştırıldı. Sitokin seviyeleri ile diyabet ilişkili parametreler arasında korelasyon analizi yapıldı. **Bulgular:** T1DH'lerin IFN-γ, TNF-α, IL-2 seviyeleri ve TNF-α/IL-2 oranı kontrollerden istatistiksel olarak anlamlı şekilde yüksekti (sırasıyla, p=0,02, p=0,01, p=0,008, p=0,01). En yüksek IFN-γ/IL-2 ve TNF-α/IL-2 oranı grup 1b'de gözlendi (sırasıyla, p=0,03, p=0,04) ve en düşük IFN-γ/IL-2 ve TNF-α/IL-2 oranı grup 1a'da görüldü (sırasıyla, p=0,03, p=0,04). TNF-α seviyesi, açlık glukoz seviyesi ile negative korele olarak saptandı (r^2=-0,003, p=0,031). Ancak, yaş ve cinsiyete göre düzeltme yapıldığında bu korelasyon gözlenmedi (r^2=-0,028, p=0,076). **Sonuç:** IFN-γ, IL-2 ve TNF-α'nın T1D patogenezinde daha büyük rolü olabilir. IFN-γ/IL-2 ve TNF-α/IL-2 oranlarının β hücre disfonksiyonunun ilerlemesindeki rolü de diğer sitokinlerden fazla olabilir (Bu çalışmanın klinik araştırma kayıt numarası NCT02389335).

Anahtar kelimeler: Tip 1 diyabet; IFN-γ; IL-2; TNF-α; T helper 1 hücresi

This study was publicized during the poster presentation at Endo 2015 Congress, San Diego, California, USA, March 5-8, 2015.

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Introduction

In type 1 diabetes (T1D), pancreatitis destroys β cells. Cytokines of T helper (Th) 1 cell play a prominent role in the inflammation of the pancreas and impaired beta (β) cell function (1,2). The molecular alterations that occur in T cells before insulitis are poorly understood. Also, the mechanism of breakdown of T-cell tolerance in T1D has not been clarified. It may result from an increase in effector function and/or loss of regulatory function. A few studies point to the loss of regulatory T cell (Treg) number or function, which can trigger the breakdown in tolerance in T cells. Hughson A. et al. observed a stable increase in effector function and a transient decrease in Treg suppression in type 1 diabetes patients (T1DPs) (3). Other mechanisms associated with the progression of this disease include increased levels of inflammatory cytokines, increased insulin resistance, and glucotoxicity resulting in reduced β cell function (4,5).

Cytokines secreted from Th1 cells, exert pro-inflammatory or anti-inflammatory effects at the onset of T1D (6). This study aims to investigate the association between serum levels of Th1 cytokines [interferon-gamma (IFN-γ), interleukin (IL)-2 and tumor necrosis factor-alpha (TNF-α)] and β cell function in T1D.

Material and Methods

The study was conducted at Istanbul Medeniyet University Goztepe Training and Research Hospital between 2013 and 2014. Patients were recruited from an endocrinology clinic.

Subjects

The study involved 110 T1DPs and 31 healthy subjects (control group; CG). The ethical committee of Istanbul Medeniyet University approved the study protocol and was conducted following the Declaration of Helsinki (24.01.2013, 30/I). Informed consent was obtained from all the participants before their inclusion in the study, which was registered with an approved clinical trial registry. The clinical trial registration number of this study is NCT02389335.

The presence of inflammatory diseases, the disease likely to interfere with glucose metabolism, malignancies, hemoglobinopathies, recent trauma or antibiotic treatment, use of drugs affecting β cell function and insulin sensitivity, and the use of drugs with suppressive effects on inflammation formed the exclusion criteria for the study. Besides, fasting glucose intolerance and impaired glucose tolerance were the exclusion criteria for the CG.

T1D was diagnosed as per the criteria given by the American Diabetes Association (7). All patients were administered basal-bolus insulin treatment. The β cell function of the patients was evaluated by assessing mixed-meal stimulated C-peptide levels. T1DPs were categorized into three groups according to the C-peptide levels after Mixed-Meal Tolerance Test (MMTT)s: patients with undetectable ≤0.03 nmol/l (0.1 ng/mL) (group 1a, n=35) C-peptide levels; C-peptide levels between 0.03-0.26 nmol/l (0.1-0.8 ng/mL) i.e. minimal (group 1b, n=30); and sustained ≥0.26 nmol/L (0.8 ng/mL) (group 1c, n=45) (in normal range) C-peptide levels.

Laboratory Procedures

After at least 12 h of fasting and abstinence from smoking, and having used the long-acting insulin the previous day and but not prandial morning insulin, the levels of IFN-γ, IL-2, and TNF-α, venous glucose, C-peptide, HbA1c were assessed. After fasting samples were collected, a mixed meal comprising 33 g of carbohydrate, 15 g of protein, and 6 g of fat (240 kcal totals) was administered in less than 15 min for MMTT. As per the Diabetes Control and Complications Trial protocol (8), venous glucose and C-peptide levels were measured at the 90th minute.

Glucose was measured by the hexokinase method; high-performance liquid chromatography (Tosoh G7 and 2.2, Tokyo, Japan) was used to measure HbA1c level, and direct electrochemiluminescence immunoassay (Immulite 2000, Siemens, Germany) was employed to measure C-peptide levels. A C-peptide level ≤0.03 nmol/L (a common historical limit of detection) was considered as undetectable (9), that between 0.26-2.58 nmol/l as normal range and between 0.033-0.26 nmol/l as detectable levels (10,11). Limit of blank (LoB) and limit of detection (LoD) values for C-peptide 0.02 nmol/L and 0.03 nmol/L (0.08 ng/mL), respectively.

To measure serum levels of IFN-γ, Il-2, and TNF-α after overnight fasting, antecubital

venous blood samples were collected in non-anticoagulated tubes. They were then centrifuged at 2,000*g for 10 min at 4°C and serum was aliquoted and stored at -80°C for further use. Serum TNF-α and IFN-γ levels were calculated by the ELISA method using reagents produced by Assaypro LLC (MO, USA). The intra-assay and inter-assay coefficient of variability (CV) of TNF-α was 4.8% and 7.1%, respectively. The intra-assay and inter-assay CVs of IFN-γ was 4.8% and 7.0%, respectively. The minimum detectable doses of TNF-α and IFN-γ tests were nearly 0.016 and 0.01 ng/mL, respectively.

Serum IL-2 levels were measured by an ELISA reagent (eBioscience Inc., CA, USA). The intra-assay and inter-assay IL-2 were 7.0% and 5.0%, respectively. The minimum detectable dose of the test was 9.1 pg/mL.

Statistical Analysis

Statistical analysis was performed using the SPSS software version 16. The normality of variables was tested using visual (histogram) and analytic methods (Kolmogorov-Smirnov/Shapiro-Wilk's test). Kruskal-Wallis and Mann-Whitney U tests were used to compare groups. Pearson's and Spearman's correlations were used for testing the correlation between variables. Bonferroni correction was employed to analyze multiple comparisons. A *p*-value of less than 0.05 was identified as considered significant.

Table 1. Demographic and clinical characteristics between patients and controls.

	Patient Group (n=110)	Control Group (n=31)	p
Age (year)			0.13
Median	29	30	
Interquartile range	22-36	27-38	
Gender (Male/Female)	58/52	13/18	0.56
Duration of diabetes (month)		-	-
Median	44		
Interquartile range	18-108		
Fasting blood glucose (mg/dL)			<0.001
Median	175	86	
Interquartile range	122-251	84-92	
HbA1c (%)			<0.001
Median	8.4	5.2	
Interquartile range	7.1-10.1	5.1-5.4	
Fasting C peptide (nmol/L)			<0.001
Median	<0.001	1.35	
Interquartile range	0.01-0.78	1.05-1.66	
IFN-γ (pg/mL)			0.02
Median	31.7	21.7	
Interquartile range	10.3-66.9	10.1-30.8	
IL-2 (pg/mL)			0.008
Median	45.2	41.8	
Interquartile range	41.5-48.4	38.8-46.4	
TNF-α (pg/mL)			0.01
Median	90.5	30.0	
Interquartile range	29.8-101.3	26.7-90.3	
IFN-γ/IL-2 ratio			0.05
Median	0.66	0.51	
Interquartile range	0.26-1.40	0.26-0.64	
TNF-α/IL-2 ratio			0.01
Median	1.93	0.73	
Interquartile range	0.73-2.11	0.69-1.89	

HbA1c: Hemoglobin A1c; IFN: Interferon; IL: Interleukin; TNF: Tumor necrosis factor.

Results

Characteristics of T1DPs and controls have been shown in Table 1. Serum levels of IFN-γ, IL-2, and TNF-α were higher (p=0.02, p=0.008, and p=0.01, respectively) in T1DPs than in controls. No difference was observed between IFN-γ/IL-2 ratios of T1DPs and controls (p=0.05). However, TNF-α/IL-2 ratio of T1DPs was higher than that of controls (p=0.01). No correlation was found either between stimulated C-peptide levels and IFN-γ (r^2=0.022; p=0.349), IL-2 (r^2=0.005; p=0.667) and TNF-α (r^2=0.001; p=0.286) levels nor between stimulated C-peptide levels and IFN-γ/IL-2 (r^2=0.023; p=0.178) and TNF-α/IL-2 (r^2=0.001; p=0.217) ratios in T1DPs.

Characteristics of group 1a, group 1b, and group 1c have been depicted in Table 2. No difference was found between serum levels of IFN-γ, IL-2, and TNF-α in group 1a, group

Table 2. Comparison of groups 1a, 1b, 1c in terms of demographic and biochemical characteristics.

	Group 1a (n=35)	Group 1b (n=30)	Group 1c (n=45)	p
Age (year)				0.19
Median	31	28	28	
Interquartile range	24-38	22-33	21-38	
Gender (Male/Female)	15/20	17/13	26/19	0.53
Duration of diabetes (month)				<0.001
Median	96	35	24	
Interquartile range	65-159	12-92	7-60	
Fasting blood glucose (mg/dL)				0.07
Median	182	207	154	
Interquartile range	118-293	144-282	122-216	
HbA1c (%)				0.72
Median	8.0	8.6	8.3	
Interquartile range	7.2-9.1	7.5-10.1	6.6-10.7	
Fasting C peptide (nmol/L)				<0.001
Median	0.01	0.23	0.86	
Interquartile range	0.01-0.01	0.15-0.26	0.48-1.26	
IFN-γ (pg/mL)				0.06
Median	27.5	45.1	31.4	
Interquartile range	10.1-46.6	27.4-128.4	10.1-54.7	
IL-2 (pg/mL)				0.35
Median	44.0	46.1	44.6	
Interquartile range	40.9-47.4	42.3-49.0	41.2-48.7	
TNF-α (pg/mL)				0.05
Median	33.2	95.8	89.6	
Interquartile range	28.1-95.8	88.5-108.5	30.5-100.6	
Age at diagnosis				0.12
Median	19	23	25	
Interquartile range	16-29	15-29	19-31	
90th-minute C peptide (nmol/L)				<0.001
Median	0.01	0.64	2.26	
Interquartile range	0.01-0.01	0.27-0.71	1.69-4.14	
IFN-γ/IL-2 ratio				0.03
Median	0.57	0.93	0.63	
Interquartile range	0.25-0.99	0.57-2.65	0.25-1.12	
TNF-α/IL-2 ratio				0.04
Median	0.83	2.01	1.94	
Interquartile range	0.67-1.99	1.89-2.21	0.75-2.06	

HbA1c: Hemoglobin A1c; IFN: Interferon; IL: Interleukin; TNF: Tumor necrosis factor.

1b, and group 1c (p>0.05, p>0.05, p>0.05, respectively). However, group 1b expressed the highest IFN-γ/IL-2 and TNF-α/IL-2 ratio (p=0.03, p=0.04, respectively) while group 1a presented the lowest IFN-γ/IL-2 and TNF-α/IL-2 ratio (p=0.03, p=0.04, respectively) among T1DPs.

The TNF-α level was found to be negatively correlated with fasting glucose level (r^2=- 0.003, p=0.031). However, this correlation diminished (r^2=-0.028, p=0.076) after adjustment for age and gender.

IFN-γ level was negatively correlated with the duration of diabetes (r^2=-0.021, p=0.007) and age at diagnosis (r^2=-0.073, p=0.027). After adjustment for age and gender, the correlation between IFN-γ and age at diagnosis and that between IFN-γ and duration of diabetes disappeared (r^2=-0.003, p=0.453; r^2=-0.062, p=0.283, respectively).

Discussion

The present study proved that serum levels of IFN-γ, IL-2, TNF-α, and TNF-α/IL-2 ratio were significantly higher in T1DPs than in healthy controls. Although IFN-γ/IL-2 ratio was lower in CG than in T1DPs, this difference was insignificant. IFN-γ/IL-2 and TNF-α/IL-2 ratios were higher in T1DPs with detectable and normal C-peptide levels than in T1DPs with undetectable C-peptide levels. These findings suggest the presence of higher levels of autoimmune inflammation in T1DPs with better β cell function than in T1DPs with undetectable C-peptide levels. Inflammation of β cells is the major pathology in T1D (11). β cell apoptosis is stimulated by cytokines such as IL-1β, TNF-α, and IFN-γ (12). In contrast with the previous studies, the present study found higher IFN-γ and TNF-α levels, IFN-γ/IL-2, and TNF-α/IL-2 ratio in T1DPs with better β cell function than in T1DPs with undetectable β cell function. The process of dedifferentiation and/or differentiation of β cells may occur in T1D depending on the level of inflammation involved. It is, therefore, possible that IFN-γ and TNF-α may play protective roles for β cells against the phagocytosis and/or against the destruction by natural killers. However, it could also signify the ongoing inflammatory process in these patients, and high cytokine levels can be attributed to increased inflammation in group 1b. Since only scarce beta cells remained in group 1c, the inflammation is thought to have diminished over time.

The only Th 1 cytokine found in β cells is IFN-γ (13). Some studies reported that ablation or blockage of IFN-γ causes delayed or decreased incidence of T1D (14). IFN-γ has been found to increase the toxic effects of macrophages and T lymphocytes on β cell function (15-17).

Improvement in β cell function in T1D is considered to be a result of insulin sensitivity and reduced inflammatory/autoimmune process in islets (18). However, Kaas et al. established that stimulated C-peptide levels and IFN-γ levels did not have any link (19). The results of the present study showed no correlation between stimulated C-peptide and IFN-γ levels, which were consistent with those of Kaas's study.

Several studies have suggested the role of the upregulation of inflammatory factors and the downregulation of anti-inflammatory mechanisms (IL-10 and Tregs) in T1D pathogenesis (20). TNF-α has been confirmed to have an accelerator role in the development of T1D (21). Hotamisligil et al. identified that TNF-α downregulates the tyrosine kinase activity of the receptor (22). TNF-α could induce serine/threonine phosphorylation of the insulin receptor substrate, hinder normal phosphorylation of tyrosine, and diminish signal transduction of insulin, by increasing the activities of the NF-κB transcriptional factor, protein kinase C, amino-terminal kinase, and inhibitor kinase. This finally results in insulin resistance, or TNF-α may result in the destruction of pancreatic beta cells and lead to the development of T1DM (23,24).

TNF- α interacts with beta cells via different mechanisms. Li et al. elucidated that Th17 cells may promote the development of T1D, and TNF-α could mediate diabetes in response to either Th17 cells or Th1 cells (25). A comprehensive interpretation of the levels of cytokines that could affect beta cells is important because studies prove that there is no single pathway to this.

The proportion of effector T cells was stable in T1D during pancreatitis; however, IL-2 treatment was determined to reduce IFN-γ levels, particularly in the pancreas (26). While TNF-α and IFN-γ reflect autoimmune

activity, IL-2 indicates regulatory activity, which in turn suppresses autoimmunity. The present study investigated the effect of IFN-γ/IL-2 and TNF-α/IL-2 balance on β cell function and found that T1DPs with higher autoimmune response have better β cell function compared to other T1DPs.

The sources of IFN-γ include activated T lymphocytes, natural killer (NK) cells, and sometimes β cells (6,27), while the sources of TNF-α are monocytes, macrophages, $CD4^+$ and $CD8^+$ T cells, B cells, lymphokine-activated killer (LAK) cells, NK cells, endothelial cells, non-hematopoietic tumor cell lines, mast cells, and neutrophils. Nevertheless, the only source of IL-2 is Th1 cells (28). T1DPs with better β cell function had higher IFN-γ/IL-2 and TNF-α/IL-2 ratios in the present study; this may reflect the effect of these cytokine sources in the process of autoimmune T1D.

This study is the first to explore the effects of balanced IFN-γ/IL-2 and TNF-α/IL-2 on β cell function. One limitation of this study is that autoantibody levels of T1DPs with high, moderate, and low β cell function could not be compared as the autoantibody levels of the patients were measured in different laboratories. However, all T1DPs had high levels of autoantibody against 65-kDa glutamic acid decarboxylase. Also, this cohort comprised only adult patients. A larger cohort with the inclusion of children may have presented different results.

Conclusion

IFN-γ, IL-2, and TNF-α may act as a triggering factor in the pathogenesis of T1D. IFN-γ/IL-2 and TNF-α/IL-2 ratio may be more significant than IFN-γ, IL-2, and TNF-α levels themselves in the progress of β cell dysfunction. Yet, it is not clear whether decreased β cell mass and function in T1D is the cause or the result of decreased Th1 cytokine levels.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Gonca Tamer; Design: Gonca Tamer, Banu İşbilen Başok; Control/Supervision: Gonca Tamer, Burcu Doğan; Data Collection and/or Processing: Osman Köstek, Gonca Tamer; Analysis and/or Interpretation: Özge Telci Çaklılı, Osman Köstek; Literature Review: Özge Telci Çaklılı; Writing the Article: Özge Telci Çaklılı; Critical Review: Gonca Tamer; References and Fundings: Gonca Tamer; Materials: Gonca Tamer.

References

1. Nunemaker CS. Considerations for defining cytokine dose, duration, and milieu that are appropriate for modeling chronic low-grade inflammation in type 2 diabetes. J Diabetes Res. 2016;2016:2846570. [Crossref] [PubMed] [PMC]
2. Ramadan JW, Steiner SR, O'Neill CM, Nunemaker CS. The central role of calcium in the effects of cytokines on beta-cell function: implications for type 1 and type 2 diabetes. Cell Calcium. 2011;50:481-490. [Crossref] [PubMed] [PMC]
3. Hughson A, Bromberg I, Johnson B, Quataert S, Jospe N, Fowell DJ. Uncoupling of proliferation and cytokines from suppression within the CD4+ CD25+ Foxp3+ T-Cell compartment in the 1st year of human type 1 diabetes. Diabetes. 2011;60:2125-2133. [Crossref] [PubMed] [PMC]
4. Karlsson FA, Bjork E. Beta-cell rest: a strategy for the prevention of autoimmune diabetes. Autoimmunity. 1997;26:117-122. [Crossref] [PubMed]
5. Mortensen HB, Swift PG, Holl RW, Hougaard P, Hansen L, Bjoerndalen H, de Beaufort CE, Knip M; Hvidoere Study Group on Childhood Diabetes. Multinational study in children and adolescents with newly diagnosed type 1diabetes: association of age, ketoacidosis, HLA status, and autoantibodies on residual beta- cell function and glycemic control 12 months after diagnosis. Pediatr Diabetes. 2010;1:218-226. [Crossref] [PubMed]
6. Lu J, Liu J, Li L, Lan Y, Liang Y. Cytokines in type 1 diabetes: mechanisms of action and immunotherapeutic targets. Clin Transl Immunology. 2020;16;9:e1122. [Crossref] [PubMed] [PMC]
7. American Diabetes Association. Classification and diagnosis of diabetes: standards of medical care in diabetes-2019. Diabetes Care. 42(Supplement 1):S13-S28. [Crossref] [PubMed]

8. Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. New England Journal of Medicine. 1993;329: 977-986. [Crossref] [PubMed]
9. National Committee for Clinical Laboratory Standarts: Procedures for the collection of diagnostic blood specimens by venipuncture; approved standart. 4th ed. NCCLS document H3-A4. Wayne, PA: NCCLS, 1998.
10. CLSI. Protocols fort he Determination of Limits of Quantitation; Approved Guideline. CLSI document EP17-A. Vol 24. No:34. USA: CLSI; 2004.
11. Solidin OP, Dahlin JRB, Gresham EG, King J, Soldin SJ. Immulite 2000 age and sex-spesific reference intervals for alfa fetoprotein, homocysteine, insulin, insulin-like growth factor-1, insulin-like growth factor-1 binding protein-3, C-peptide, immunoglibulin E and intact parathyroid hormone. Clin Biochem. 2008;41:937-942. [Crossref] [PubMed] [PMC]
12. Steffes MW, Sibley S, Jackson M, Thomas W. Beta-Cell function and the development of diabetes –related complications in the diabetes control and complications trial. Diabetes Care. 2003;26:832-836. [Crossref] [PubMed]
13. Eizirik DL, Colli ML, Ortis, F. The role of inflammation in insulitis and beta-cell loss in type 1 diabetes. Nat Rev Endocrinol. 2009;5:219-226. [Crossref] [PubMed]
14. He JS, Xie PS, Luo Ds, Sun CJ, Zhang YG, Liu FX. Role of immune dysfunction in pathogenesis of type 1 diabetes mellitus in children. Asian Pac J Trop Med. 2014;7:823-826. [Crossref]
15. Foulis AK, McGill M, Farquharson MA. Insulitis in type 1 (insulin-dependent) diabetes mellitus in manmacrophages, lymphocytes, and interferon-γ containing cells. The Journal of Pathology. 1991;165: 97-103. [Crossref] [PubMed]
16. Bazzaz JT, Amoli MM, Taheri Z, Larijani B, Pravica V, Hutchinson IV. TNF-α and IFN-γ gene variation and genetic susceptibility to type 1 diabetes and its microangiopathic complications. J Diabetes Metab Disord. 2014;13:46. [Crossref] [PubMed] [PMC]
17. Stanley WJ, Trivedi PM, Sutherland AP, Thomas HE, Gurzov EN. Differential regulation of pro-inflammatory cytokine signalling by protein tyrosine phosphatases in pancreatic β-cells. J Mol Endocrinol. 2017;59:325-337. [Crossref] [PubMed]
18. Schloot NC, Hanifi-Moghaddam P, Aabenhus-Andersen N, Alizadeh BZ, Saha MT, Knip M, Devendra D, Wilkin T, Bonifacio E, Roep BO, Kolb H, Mandrup-Poulsen T. Association of immune mediators at diagnosis of Type 1 diabetes with later clinical remission. Diabetic Med. 2007;24:512-520. [Crossref] [PubMed]
19. Kaas A, Pfleger C, Kharagjitsingh AV, Schloot NC, Hansen L, Buschard K, Koeleman BPC, Roep BO, Mortensen HB, Alizadeh BZ: on behalf of the Hvidoere Study Group on Childhood Diabetes. Association between age, IL-10, IFN-γ, stimulated C-peptide and disease progression in children with newly diagnosed type 1 diabetes. Diabet Med. 2012;29:734-741. [Crossref] [PubMed]
20. El Samahy MH, Adly AA, Ismail EA, Salah NY. Regulatory T cells with CD62L or TNFR2 expression in young type 1diabetic patients: relation to inflammation, glycemic control and micro-vascular complications. J Diabetes Complications. 2015;29: 120-126. [Crossref] [PubMed]
21. Pickup JC. Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. Diabetes Care. 2004;27(3):813-823. [Crossref] [PubMed]
22. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1-mediated inhibitionof insulin receptor thyrosine kinase activity in TNF alpha- and obesity- induced insulin resistance. Science. 1996;2;271:665-668. [Crossref] [PubMed]
23. Qiao YC, Chen YL, Pan YH, Tian F, Xu Y, Zhang XX, Zhao HL. The change of serum tumor necrosis factor alpha in patients with type 1 diabetes mellitus: a systematic review and meta-analysis. PloS one. 2017;20;12:e0176157. [Crossref] [PubMed] [PMC]
24. Buzzetti R, Zampetti S, Pozzilli P. Impact of obesity on the increasing incidence of type 1 diabetes. Diabetes, Obesity & Metabolism. 2020;10. [Crossref] [PubMed]
25. Li CR, Mueller EE, Bradley LM. Islet antigen-spesific Th17 cells can induce TNF-α-dependent autoimmune diabetes. J Immunol. 2014;15;192:1425-1432. [Crossref] [PubMed] [PMC]
26. Hartemann A, Bourron O. Interleukin-2 and type 1 diabetes: New therapeutic perspectives. Diabetes & Metabolism. 2012;38:387-391. [Crossref] [PubMed]
27. Chan SH, Perussia B, Gupta JW, Kobayashi M, Pospísil M, Young HA, Wolf SF, Young D, Clark SC, Trinchieri G. Induction of interferon gamma production by natural killer cell stimulatory factor: characterization of the responder cells and synergy with other inducers. J Exp Med. 1991;1;173:869-879. [Crossref] [PubMed] [PMC]
28. Balkwill F. Tumor Necrosis factor or tumor promoting factor? Cytokine Growth Factor Rev. 2002;13: 135-141. [Crossref]

Original Article
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Effect of Vitamin D Deficiency on the Frequency of Lipohypertrophy Occurrence in Patients with Type 2 Diabetes Mellitus Under Injectable Treatment

D Vitamini Eksikliğinin Enjeksiyon Tedavisi Alan Tip 2 Diabetes Mellitus Hastalarında Lipohipertrofi Sıklığına Etkisi

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Abstract

Objective: Lipohypertrophy (LH) is one of the most common treatment-related cutaneous complications of injectable therapies. Although the etiology of LH cannot be clarified, it may be due to the lipogenic effect of insulin or recurrent tissue trauma caused by injections. In this respect, we aimed to evaluate the relationship between vitamin D level and LH. **Material and Methods:** Patients with Type 2 diabetes mellitus aged 18 years or older, who were under insulin and/or exenatide treatment for at least one year were included in this study. The injection sites of the patients were examined by inspection and palpation method. Patients were categorized into two groups according to vitamin D levels as below and above 20 ng/mL. **Results:** A total of 140 patients, including 91 women and 49 men, aged between 20-78 years with a mean age of 54.53±13.89 were included in the study. LH was detected in 91 (65%) of 140 patients. This study demonstrated that there was a significant relationship between gender and LH. Statistically, the frequency of LH was higher in female patients (p=0.001). Further, a relationship between vitamin D levels and LH was also observed (p=0.006). **Conclusion:** Besides calcium metabolism, the effects of vitamin D on lipogenesis are also known. Vitamin D inhibits the differentiation of pre-adipocytes to mature adipocytes. This is the first study showing the relationship between vitamin D and LH in our knowledge.

Keywords: Diabetes mellitus; exenatide; insulin treatment; lipohypertrophy; vitamin D

Özet

Amaç: Lipohipertrofi (LH), enjekte edilebilir tedavilerin, tedaviyle ilişkili en yaygın kutanöz komplikasyonlarından biridir. LH'nin etiyolojisi tam olarak açıklanamasa da, insülinin lipojenik etkisinden veya enjeksiyonun neden olduğu tekrarlayan doku travmasından kaynaklandığı düşünülmektedir. Bu bağlamda D vitamini ve LH arasındaki ilişkiyi değerlendirmeyi amaçladık. **Gereç ve Yöntemler:** En az 1 yıl boyunca insülin ve/veya eksenatid tedavisi kullanan 18 yaş ve üzeri Tip 2 diabetes mellitus tanılı hastalar çalışmaya dâhil edildi. Hastaların enjeksiyon bölgeleri inspeksiyon ve palpasyon yöntemi ile incelendi. Hastalar, D vitamini seviyelerine göre 20 ng/mL'nin altında ve üstünde olmak üzere iki gruba ayrıldı. **Bulgular:** Yaş ortalaması 54.53±13.89 yıl olan 20-78 yaşları arasında 91'i kadın, 49'u erkek toplam 140 hasta çalışmaya dâhil edildi. 140 hastanın 91 (%65)'inde LH saptandı. Bu çalışma, cinsiyet ile LH arasında anlamlı bir ilişki olduğunu göstermekteydi. İstatistiksel olarak kadınlarda LH sıklığının daha fazla olduğu görüldü (p=0.001). D vitamini düzeyleri ile LH arasında da anlamlı bir ilişki vardı (p=0.006). **Sonuç:** Kalsiyum metabolizması üzerindeki etkilerine ek olarak, D vitamininin lipogenez üzerindeki etkileri de bilinmektedir. D vitamini, preadipositlerin olgun adipositlere farklılaşmasını inhibe eder. Bu makale, bilgimiz dâhilinde D vitamini ile LH arasındaki ilişkiyi gösteren ilk çalışmadır.

Anahtar kelimeler: Diabetes mellitus; eksenatid; insülin tedavisi; lipohipertrofi; D vitamini

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Introduction

The occurrence of diabetes mellitus (DM) is increasing worldwide, and the number of patients diagnosed with DM is expected to reach 642 million in 2040 (1). Thirty-five percent of these patients use insulin therapy (2), causing an increase in the cost of both treatment and treatment-related complications. One of the most common cutaneous complications due to incorrect insulin use is lipohypertrophy (LH), and daily insulin requirement is higher in patients with LH than in patients without LH. According to a study by Blanco et al. the increased requirement of insulin due to LH in the Spanish healthcare system was estimated to cost € 122 million per year (3). Besides the treatment cost, the frequency of hypoglycemia is six-times higher, and the glycemic fluctuations are seven-times higher in patients with LH (3). Although the etiology of LH has not yet been clarified, it is thought to be due to the lipogenic effect of insulin or recurrent tissue trauma caused by injections (4). According to the examination method and the examiner, in various studies conducted globally, the frequency of LH was found to be quite variable, between 1.9% and 77% (5). The most significant cause of LH development was found to be insufficient rotation between injection sites (3). In addition, the frequency of injections, the total daily dose of insulin, and the needle length were also found to be associated with LH (6). Further, the fact that there is a large difference in the frequency of LH between these studies and the presence of LH in other subcutaneous treatments other than insulin such as pegvisomant, long-acting exenatide and anti-tumor necrosis factor, suggest that this complication is not related to recurrent trauma only (7). In this respect, we aimed to evaluate the relationship between vitamin D, which is known to play a role in lipogenic effect, and LH.

Material and Methods

Patients who applied at our outpatient clinic between May-August 2019 for routine tests, who had been using insulin and/or exenatide treatment for at least one year, were included in the study. All patients were older than 18 years. The exclusion criteria were evidence of dermatitis and cutaneous disease. The patients were classified according to the body mass index (BMI) classification of the World Health Organization, as, 18.5-24.9 kg/m^2, normal; 25-29.9 kg/m^2, overweight; 30-39.9 kg/m^2, obese; and ≥40 kg/m^2, morbidly obese. The injection sites of the patients were examined by inspection and palpation method, and the LH regions were recorded by the education nurse working in our clinic. Patient data of HbA1c and vitamin D levels determined for routine tests were acquired from the hospital system. Vitamin D analyses were made with liquid chromatography-mass spectrometry API 3200 (ABSCIEX, USA), while HbA1c was measured by glycohemoglobin analyzer (G7 HPLC Analyzer, Tosoh Bioscience, USA). Patients were categorized into two groups according to vitamin D levels as below and above 20 ng/mL. Our study was carried out after the approval of the Ethics Committee of Selçuk University Faculty of Medicine dated 17.04.2019 and numbered 2019/04. Written informed consent was obtained from each patient.

All statistical analyses of the study were performed in SPSS (version 21.0 for Windows) program. The variables were investigated using visual (histograms and probability plots) and Kolmogorov-Smirnov test to determine whether or not they are normally distributed. The categorical variables were expressed as numbers and percentages, whereas continuous variables were summarized as mean with standard deviation, median, and minimum-maximum, wherever appropriate. While investigating, the associations between non-normally distributed variables and their significance were calculated using Spearman's test or Kruskal-Wallis test. A p-value of <0.05 was considered statistically significant.

Results

A total of 140 patients, including 91 women (65%) and 49 men (35%), aged between 20 and 78 years (mean age of 54.53±13.89), were included in the study. Thirteen (9.3%) patients had normal BMI [mean BMI=21.94 (interquartile range; IQR=19.65-24.25)] while 40 (28.6%) patients were overweight [mean BMI=27.6 (IQR=26.4-28.84)], 65 (46.4%) patients were obese [mean value of BMI: 33.97

(IQR 31.6-36.13)] and 22 (15.7%) patients were morbidly obese [mean value of BMI=41.68 (IQR=40.02-44.08)]. When the treatments of the patients were evaluated, 93 (66.4%) patients were receiving insulin, 28 (20%) patients were receiving exenatide twice daily, and 19 (13.6%) patients were receiving insulin+exenatide twice daily. LH was detected in 91 of 140 patients (65%).

While mean HbA1c levels of the patients with LH were 9.43±2.97, the mean HbA1c levels of the patients without LH were 8.42±1.85. Vitamin D levels of 96 (68.5%) patients were less than 20 ng/mL and 44 (31.4%) patients were 20 ng/mL and above. Spearmen's correlation analysis demonstrated a significant negative correlation between levels of vitamin D and the presence of LH (Spearmen's ρ=|-0.2|, p=0.006) (Table 1). Kruskal-Wallis test showed no significant relationship between LH and BMI (p=0.057) (Table 1). This study indicated the absence of significant correlations between types of injectable treatments and the presence of LH (Spearmen's ρ=0.17, p=0.296) (Table 1). There was no correlation between LH and HbA1c levels, and the age of the patients (Spearmen's ρ=|-0.11|, p=0.18, and ρ=0.13, p=0.11, respectively). We also observed a significant relationship between gender and LH. Statistically, the frequency of LH was higher in female patients (Spearmen's ρ=|-0.34|, p=0.001) (Table 1).

Discussion

Vitamin D is one of the primary hormones that play a role in calcium, phosphorus hemostasis, and bone metabolism. Due to the low vitamin D content of foods, the main source of vitamin D endogenous synthesis is through the ultraviolet B rays from cholesterol in the skin (8). Although different vitamin D ranges are used in different studies, generally accepted normal vitamin D levels have been determined as 20-50 ng/mL in a healthy population (9).

In addition to affecting the bone metabolism, vitamin D plays a role in the etiology and progression of many different diseases such as cardiovascular diseases, autoimmune diseases, and malignancies (10-12). In addition to these effects, the effects of vitamin D on lipogenesis are known. In a study performed by Borges et al. on obese mice, vitamin D deficiency was found to increase lipogenesis in the liver, reduce beta-oxidation, and increase the risk of fatty liver disease (13). Vitamin D inhibits differentiation of pre-adipocytes to mature adipocytes by inhibiting characteristic mature adipocyte genes such as peroxisome proliferator-activated receptor-γ, and

Table 1. Relationship between LH and gender, BMI, vitamin D, and the patient's treatment.

	Lipohypertrophy		
	Present	**Absent**	**p**
Gender			
Male	21	28	0.001
Female	70	21	
Body mass index (kg/m²)			
Normal	12	1	0.057
Over-weight	21	19	
Obese	42	23	
Morbid obese	16	6	
Vitamin D (ng/mL)			
<20	55	41	0.006
≥20	36	8	
Patient's treatment			
Insulin	57	36	0.296
Exenatide	22	6	
Insulin+exenatide	12	7	

sterol regulatory element-binding protein-1c (14). Vitamin D has also been shown to be protective against obesity due to its negative effect on adipocytes by increasing dinucleotide concentration and Sirtulin 1 activity to nicotinamide adenine (15). LH is the adipose tissue that appears as a tumor-like swelling at subcutaneous injection sites. Histopathologically, it is seen that the dermal reticular layer consists of mature adipocytes of volume larger than that of normal adipocytes. Some lipid droplets may occur in the periphery of some of the adipocytes in LH tissue, possibly due to the stimulation of lipogenesis (16). This study was planned based on the above-mentioned effects of vitamin D on lipogenesis, and on assessing the relationship between LH and vitamin D, the frequency of LH was found to increase significantly in patients with low vitamin D levels (p=0.006). There are conflicting results on the effects of the replacement of vitamin D on levels of HbA1c and fasting blood glucose in DM patients (17). However, studies showing the positive effects of vitamin D replacement on glycemic control in Type 1 DM patients who were treated with insulin alone (18,19) suggest that this effect of vitamin D may improve LH and exert positive effects on glucose metabolism.

Although the incidence of LH in our country varied in previous studies, the LH rate was 27.4% in a study published in 2018 with 29 different centers, whereas this rate was 48.8% in another study with 215 patients (20,21). In our study, the incidence of LH was found to be 65%. This difference may be due to the low number of patients included in our study; it also indicates that we need more efforts on insulin treatment education in our center.

While no relationship was observed between gender and LH in previous studies, LH was more common in women in our study (3). Contrary to the studies reporting that increased BMI increases the risk of LH, we observed no statistically significant relationship between BMI and LH in our study (6). These conflicting results maybe because of the sample size of our study.

There are some limitations to our study. We focused only on the relationship between vitamin D and LH. The other factors which could affect LH, for example, rotation of the injection site, injection regimen, needle syringe length, etc., needs to be evaluated in this study. Another limitation was, as indicated earlier, the sample size. As the third limitation of the study, we evaluated LH by the inspection and palpation methods. Evaluating the presence of LH by radiological methods would provide more sensitive and specific results.

Conclusion

To our knowledge, this is the first study showing the relationship between vitamin D and LH. Further randomized controlled trials are needed to clarify this relationship. LH is a significant issue that cannot be underestimated considering its negative effects on the frequency of hypoglycemia, quality of life of the patients, glycemic control, and cost of treatment.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Cem Onur Kıraç, Cuma Gönüllü; Design: Cem Onur Kıraç, Cuma Gönüllü, Süleyman Baldane; Control/Supervision: Süleyman Baldane, Levent Kebapçılar; Data Collection and/or Processing: Cem Onur Kıraç, Cuma Gönüllü; Analysis and/or Interpretation: Levent Kebapçılar; Literature Review: Cem Onur Kıraç; Writing the Article: Cem Onur Kıraç, Süleyman Baldane; Critical Review: Süleyman Baldane, Levent Kebapçılar; References and Fundings: Cem Onur Kıraç, Cuma Gönüllü, Süleyman Baldane.

References

1. International Diabetes Federation. IDF Diabetes Atlas. 7th ed. (Last update December 13, 2017.) [Link]
2. Mohan V, Shah SN, Joshi SR, Seshiah V, Sahay BK, Banerjee S, Wangnoo SK, Kumar A, Kalra S, Unnikrishnan AG, Sharma SK, Rao PV, Akhtar S, Shetty RV, Das AK, DiabCare India 2011 Study Group et al. Current status of management, control, complications and psychosocial aspects of patients with diabetes in India: Results from the DiabCare India 2011 Study. Indian J Endocrinol Metab. 2014;18:370-378. [Crossref] [PubMed] [PMC]
3. Blanco M, Hernández MT, Strauss KW, Amaya M. Prevalence and risk factors of lipohypertrophy in insulin-injecting patients with diabetes. Diabetes Metab. 2013;9:445-453. [Crossref] [PubMed]
4. Hanuer H, Stockamp B, Haastert B. Prevalence of lipohyperthyropy in insulin-treated diabetic patients and predisposing factors. Exp Clin Endocrinol Diabetes. 1996;104:106-110. [Crossref] [PubMed]
5. Gentile S, Strollo F, Guarino G, Diabetologists; Nurses. Why are so huge differences reported in the occurrence rate of skin lipohypertrophy? Does it depend on method defects or on lack of interest? Diabetes Metab Syndr. 2019;13:682-686. [Crossref] [PubMed]
6. Barola A, Tiwari P, Bhansali A, Grover S, Dayal D. Insulin-related lipohypertrophy: lipogenic action or tissue trauma? Front Endocrinol (Lausanne). 2018;9:638. [Crossref] [PubMed] [PMC]
7. Gentile S, Strollo F, Ceriello A, AMD-OSDI Injection Technique Study Group. Lipodystrophy in insulin-treated subjects and other injection-site skin reactions: are we sure everything is clear? Diabetes Ther. 2016;7:401-409. [Crossref] [PubMed] [PMC]
8. Nair R, Maseeh A. Vitamin D: the 'sunshine' vitamin. J Pharmacol Pharmacother. 2012;3:118-126. [PubMed]
9. Munns CF, Shaw N, Kiely M, Specker BL, Thacher TD, Ozono K, Michigami T, Tiosano D, Mughal OM, Ramos-Abad L, Ward L, DiMeglio LA, Atapattu N, Cassinelli H, Braegger C, Pettifor JM, Seth A, Idris HW, Bhatia V, Fu J, Goldberg G, Sävendahl L, Khadgawat R, Pludowski P, Maddock J, Hyppönen E, Oduwole A, Frew E, Aguiar M, Tulchinsky T, Butler G, Högler W. Global Consensus Recommendations on Prevention and Management of Nutritional Rickets. J Clin Endocrinol Metab. 2016;101:394-415. [Crossref] [PubMed] [PMC]
10. Norman PE, Powell JT. Vitamin D and cardiovascular disease. Circ Res. 2014;114:379-393. [Crossref] [PubMed]
11. Murdaca G, Tonacci A, Negrini S, Greco M, Borro M, Puppo F, Gangemi S. Emerging role of vitamin D in autoimmune diseases: An update on evidence and therapeutic implications. Autoimmun Rev. 2019;18:102350. [Crossref] [PubMed]
12. Kulling PM, Olson KC, Olson TL, Feith DJ, Loughran Jr TP. Vitamin D in hematological disorders and malignancies. Eur J Haematol. 2017;98:187-197. [Crossref] [PubMed] [PMC]
13. Borges CC, Salles AF, Bringhenti I, Mandarim-DE-Lacerda CA, Aguila MB. Vitamin D deficiency increases lipogenesis and reduces beta-oxidation in the liver of diet-induced obese mice. J Nutr Sci Vitaminol (Tokyo). 2018;64:106-115. [Crossref] [PubMed]
14. Kang EJ, Lee JE, An SM, Lee JH, Kwon HS, Kim BC, Kim Sj, Kim JM, Hwang DY, Jung YJ, Yang SY, Kim SC, An BS. The effects of vitamin D3 on lipogenesis in the liver and adipose tissue of pregnant rats. Int J Mol Med. 2015;36:1151-1158. [Crossref] [PubMed]
15. Chang E, Kim Y. Vitamin D decreases adipocyte lipid storage and increases NAD-SIRT1 pathway in 3T3-L1 adipocytes. Nutrition. 2016;32:702-708. [Crossref] [PubMed]
16. Fujikura J, Fujimoto M, Yasue S, Noguchi M, Masuzaki H, Hosoda K, Tachibana T, Sugihara H, Nakao K. Insulin-induced lipohypertrophy: report of a case with histopathology. Endocr J. 2005;52:623-628. [Crossref] [PubMed]
17. Lee CJ, Iyer G, Liu Y, Kalyani RR, Bamba N, Ligon CB, Varma S, Mathioudakis N. The effect of vitamin D supplementation on glucose metabolism in type 2 diabetes mellitus: a systematic review and meta-analysis of intervention studies. J Diabetes Complications. 2017;31:1115-1126. [Crossref] [PubMed] [PMC]
18. Ordooei M, Shojaoddiny-Ardekani A, Hoseinipoor SH, Miroliai M, Zare-Zerdini H. Effect of vitamin D on HbA1c levels of children and adolescents with diabetes mellitus type 1. Minerva Pediatr. 2017;69:391-395. [PubMed]
19. Aljabri KS, Bokhari SA, Khan MJ. Glycemic changes after vitamin D supplementation in patients with type 1 diabetes mellitus and vitamin D deficiency. Ann Saudi Med. 2010;30:454-458. [Crossref] [PubMed] [PMC]
20. Dagdelen S, Deyneli O, Olgun N, Siva ZO, Sargin M, Hatun S, Kulaksızoğlu M, Kaya A, Gürlek CA, Hirsch LJ, Strauss KW, ITQ Turkish Study Group. Turkish insulin injection techniques study: complications of injecting insulin among turkish patients with diabetes, education they received, and the role of health care professional as assessed by survey questionnaire. Diabetes Ther. 2018;9:1615-1628. [Crossref] [PubMed]
21. Vardar B, Kizilci S. Incidence of lipohypertrophy in diabetic patients anda study of influencing factors. Diabetes Res Clin Pract. 2007;77:231-236. [Crossref] [PubMed]

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The Screening of Comorbid Depressive Disorders and Associated Risk Factors in Adult Patients with Type 2 Diabetes

Erişkin Tip 2 Diyabet Hastalarında Depresif Bozuklukların ve İlişkili Risk Faktörlerinin Taranması

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Abstract

Objective: Elevated depressive symptoms and disorders affect one in five patients with diabetes. Current guidelines recommend screening depression in the diabetic population. Turkey has the highest (13.7%) prevalence of diabetes in Europe. However, there are limited data about the prevalence of depressive disorders among diabetic patients in Turkey. We aim to investigate the prevalence of a comorbid depressive disorder in Type 2 diabetic patients who were referred to the Endocrinology outpatient unit of a tertiary hospital. **Material and Methods:** All the Type 2 diabetic patients admitted to our endocrinology department were consecutively included in the study. Their sociodemographics, concomitant diseases and medications, macro and microvascular complications, lifestyle and personal habits, and treatment regimens were obtained by a specifically designed questionnaire. Laboratory data were obtained from the hospital records. The Patient Health Questionnaire-9 (PHQ-9), a depression screening tool, was used as a screening method for depression. Patients with a score of 10 or above determined high risk for depressive disorder according to PHQ-9. The scores were re-evaluated by a psychiatrist to minimize the false negative and positive results. **Result:** A total of 460 patients with Type 2 diabetic were enrolled in this cross-sectional study. 18.9% (n=87) of the participants were found to have depressive disorders according to the psychiatric evaluation done after the PHQ-9 questionnaire. Patients with depressive disorders were predominantly female (69.0% vs. 55.5%; p=0.022), younger (57.2±10.5 vs. 60.0±9.5; p=0.014), had higher HbA1c (8.51±2.51 vs. 7.98±2.05; p=0.042), total cholesterol (205.6±44.2 vs. 194.2±46.0; p=0.045), LDL-cholesterol (123.1±37.8 vs. 113.1±35.4; p=0.026) and non-HDL-cholesterol (158.5±41.61 vs. 146.6±42.7; p=0.024). These patients had frequent neuropathy (37.3% vs. 19.0%, p=0.001), they were less likely to perform exercise (31.8% vs. 53.1%; p<0.001) while smoke in excess (31.4% vs. 14.3%; p<0.001). The analysis showed that female gender (OR=4.4; 95% CI=1.6-12.8; p=0.005) and smoking (OR=7.6; 95% CI=2.8-20.5, p<0.001) are independent determinants of a depressive disorder. **Conclusion:** Approximately one-fifth of diabetic patients had a depressive disorder, and their metabolic parameters were worse than those without a depressive disorder. Therefore, to assess a diabetic patient from all aspects, screening for depressive disorder should be made an indispensable part of the evaluation process.

Keywords: Diabetes; depressive disorders; depression; PHQ-9; Patient Health Questionnaire

Özet

Amaç: Depresif bozukluk ve artmış depresif durumlar her beş diyabet hastasından birini etkilemektedir. Güncel rehberler, diyabetik popülasyonda depresif semptom ve bozuklukların taranmasını önermektedir. Türkiye, Avrupa'da en yüksek (%13,7) diyabet prevalansına sahip ülke konumundadır. Bununla birlikte, Türkiye'de diyabet hastaları arasında depresif bozukluğun sıklığı hakkında kısıtlı veri bulunmaktadır. Biz bu çalışma ile bir üçüncü basamak Endokrinoloji polikliniğine başvuran Tip 2 diyabetik hasta popülasyonunda eşlik eden depresif bozukluk prevalansını saptamayı amaçladık. **Gereç ve Yöntemler:** Endokrinoloji polikliniğine başvuran tüm tip 2 diyabet hastaları ardışık olarak çalışmaya dâhil edildi. Katılımcıların sosyodemografik özellikleri, eşlik eden hastalıkları ve uygulanan tedaviler, makro ve mikro komplikasyonlar, yaşam tarzı ve kişisel alışkanlıkları hazırlanan sorgu formu ile kayıt altına alındı. Laboratuvar verileri hastane bilgi sisteminden alındı. Depresif bozukluk taraması için "Patient Health Questionnaire-9 (PHQ-9)" depresyon tarama aracı kullanıldı. PHQ-9 puanı 10 ve üzeri olan hastalar, depresif bozukluk açısından yüksek riskli olarak değerlendirildi ve olası yanlış negatif-pozitif sonuçları en aza indirgemek amacıyla bir psikiyatri uzmanı tarafından yeniden değerlendirildi. **Bulgular:** Çalışmaya toplam 460 Tip 2 diyabet hastası dâhil edildi. PHQ-9 skorları ve akabinde yapılan psikiyatri değerlendirmesi sonrası hastaların %18,9 (n=87)'unda depresif bozukluk saptandı. Depresif bozukluk saptanan hastalar ağırlıklı olarak kadın cinsiyette (%69,0'a karşı %55,5; p=0,022), daha genç yaşta (57,2±10,5'e karşı 60,0±9,5; p=0,014), daha yüksek HbA1c (8,51±2,51'e karşı 7,98±2,05; p=0,042), total kolesterol (205,6±44,2'ye karşı 194,2±46,0; p=0,045), LDL kolesterol (123,1±37,8'e karşı 113,1±35,4; p=0,026) ve non-HDL-kolesterol (158,5±41,61'e karşı 146,6±42,7; p=0,024) seviyelerine sahip idi. Ek olarak, bu hastalarda nöropati daha sık (%37,3'e karşı %19,0, p=0,001), egzersiz yapma oranları daha düşük (%31,8'e karşı %53,1; p<0,001) ve sigara içme sıklığı daha yüksek (%31,4'e karşı %14,3; p<0,001) idi. Kadın cinsiyette olmak (OR=4,4; %95 GA=1,6-12,8; p=0,005) ve sigara içmek (OR=7,6; %95 GA=2,8-20,5; p<0,001) depresif bozukluğa sahip olmanın bağımsız öngördürücüleri idi. **Sonuç:** Diyabetik hastaların yaklaşık beşte birinde depresif bozukluk mevcuttu ve metabolik parametreleri depresif bozukluk olmayanlara göre daha kötü idi. Bu nedenle, bir diyabet hastasını kapsamlı bir şekilde değerlendirebilmek için depresif bozukluk taraması hasta değerlendirmesinin ayrılmaz bir parçası olarak uygulanmalıdır.

Anahtar kelimeler: Diyabet; depresif bozukluklar; depresyon; PHQ-9; Halk Sağlığı Anketi

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Introduction

Diabetes is a chronic disease and complicated to manage due to the associated mood and emotional problems. Depression is a frequent comorbidity in Type 1 and Type 2 diabetic patients. Depression affects one in four patients with diabetes (1). In other words, diabetic patients are at 1.4-3 times higher at risk of comorbid depression (1-3). Depressive disorders may complicate the management of diabetes and negatively affect the achieving of glycemic and metabolic targets (4-6). Thus, American Diabetes Association (ADA), US Preventive Services Task Force (USPSTF), and National Institute for Health and Care Excellence (NICE) diabetes guidelines recommend routine screening of depressive symptoms in a high-risk population (2,3,7).
The prevalence of depression is reported as 20.3-29.7% in European countries, and 8.3% in North America (8-10). But there are only a few studies available about the prevalance of depressive disorders for among diabetic patients in Turkey (11-13).
We hypothesize that frequency of depressive symptoms was similar throughout European countries and was associated with metabolic disturbances.
In this cross-sectional study, we aim to determine the frequency of depressive disorders in Type 2 diabetic patients referred to the Endocrinology Outpatient Unit of a tertiary hospital. Our secondary aim was to assess the relationship between comorbid depressive disorder and the metabolic consequences in Type 2 diabetic patients.

Material and Methods

Study Design and Population

This cross-sectional study was carried out from January 2017 to May 2018 in a tertiary endocrine unit. The study was approved by the local ethics committee (08.02.2017 Keçiören Training and Research Hospital Ethical Committee-Ankara/Turkey/2012-KAEK-15/1338), and the study protocol was designed as per the international agreements (Helsinki Declaration revised 2013). All patients signed informed consent before data collection. Type 2 diabetic patients over the age of 18 were enrolled consecutively in the study. Patients were excluded if pregnant, younger than 18 years, had Type I diabetes, decompensated liver disease, malignancy, chronic inflammatory disorders, or were undergoing renal replacement therapy.

Data Collection

The sociodemographics (age, gender, marital status, education, occupation, and income), concomitant diseases and medications, macro and microvascular complications, lifestyle and personal habits [exercise, smoking, alcohol use], and treatment regimens were obtained by a specifically designed questionnaire given to all the participants by their physicians. Laboratory data were obtained from hospital records.
The following are the evaluations done cross-sectionally,

Anthropometrics and Blood Pressure Measurement

Height, weight, and waist circumferences (WC) of the patients in their underclothes were recorded according to the standard protocol. Body mass index (BMI) was computed as the ratio of weight to the square of height (kg/m^2). WC was measured on the line between the iliac crest and the lower costal margin parallel to the ground, once the patients exhaled. Arterial blood pressure (ABP) was recorded using automatic BP monitors (Omron M2, HEM-7121-E) after at least 5 min of rest in a seated position. Three consecutive measurements were taken from the same arm, and the mean was recorded.

Laboratory Data

For biochemical analyses, all the blood samples were collected from the antecubital vein between 08:00-10:00 AM after overnight fasting. All laboratory parameters were measured using standard procedures. The levels of fasting blood glucose concentration, total, and high-density lipoprotein-cholesterol (HDL-C), and triglycerides (TG) were measured enzymatically. low-density lipoprotein (LDL-C) was calculated using Friedewald's equation [LDL-C=total cholesterol-(HDL-C+TG/5)] if TG was found less than 400 mg/dL (14). Glycohemoglobin (HbA1c) was measured using high-performance liquid chromatography (HPLC).

Patient Health Questionnaire-9 a Depression Screening Tool

To screen the depressive disorders, Patient Health Questionnaire-9 (PHQ-9), a depres-

sion screening tool was used. PHQ-9 is a valid and reliable tool for screening depressive disorders in diabetic individuals (15,16). All the diabetic patients who were at the risk of a depressive disorder according to PHQ-9 score were re-evaluated by a psychiatrist to minimize the false negative and positive results according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) in terms with the major depressive disorder. PHQ-9 is an instrument to perform criteria-based diagnoses of depressive disorders commonly encountered in primary care. A PHQ-9 score of ≥10 has a sensitivity and specificity of 88% in diagnosing depressive disorders (17). Depressive symptoms were screened by the PHQ-9 questionnaire. The PHQ-9 query form consists of 9 questions. The scores obtained by each answer are collected and evaluated on a scale. If the patient has not marked one of the 3 to 4 options given for the first two questions, the questionnaire cannot be evaluated as a depressive disorder regardless of the total score. Patients with a score of 10 or higher as per the rules were referred to a psychiatrist for a re-evaluation of comorbid depressive disorder. The diagnosis of comorbid depressive disorder is made by a psychiatrist according to the DSM-5 criteria. Also, regardless of the PHQ-9 score, all the patients who used prescribed antidepressants were referred to a psychiatrist and re-evaluated for depressive disorder to avoid overdiagnosis.

Definitions

An internationally accepted definition for Type 2 diabetes was used by the physicians (18). Hypertension was defined as an average office BP>140/90 mmHg on two different visits or an individual undergoing antihypertensive treatment. Dyslipidemia was defined as TG>150 and/or LDL-C>100, and/or low HDL-C (men <40, women <50 mg/dL), or receiving medications for dyslipidemia. Obesity was defined as BMI>30 kg/m^2 (19). Treatment targets were defined as HbA1c<7%, office ABP<140/90 mmHg, and LDL-C<100 mg/dL according to the national (20) and international (2) diabetes guidelines. Achieving all the goals, such as glycemia, BP, and lipid levels by an individual patient, indicate triple metabolic control being established. The exercise was defined as meeting both these criteria, performing exercise more than two days per week, and more than thirty minutes per day. Marital status was dichotomized as married and unmarried. Self-reported income status was categorized according to their ability to meet up basic needs and save. A low education level was defined as less than eight years of formal education. Macrovascular complications were either self-reported: having a history of coronary artery disease, angina, heart attack, cerebrovascular event or peripheral artery disease; or recorded by the physicians according to their findings such as non-palpable extremity pulses, low ankle-brachial index values (≤0.9), positive findings on coronary or peripheral arteriography, and carotid or peripheral arterial duplex ultrasound examination. Retinopathy was self-reported by the patients based on being identified with an eye problem related to diabetes mellitus. Nephropathy was recorded by the physicians if the patients had albuminuria and/or decreased estimated glomerular filtration rate. Neuropathy was also self-reported or recorded by the physicians if the patients had symptoms related to bilateral symmetric distal neuropathy or other autonomous neuropathies attributed to diabetes mellitus.

Statistical Analyses

Statistical analyses were performed in SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Data were expressed as mean±SD and median (minimum-maximum value) for continuous variables or as a percentage for categorical variables. To identify the variables associated with depression state (depressive/not depressive), the univariate analyses were performed using Chi-square, Fisher exact, Student's t, and Mann-Whitney U tests, where ever appropriate. For the multivariate analysis, binomial logistic regression was performed to ascertain the association of different variables. The criteria for inclusion in the model were having statistical significance ($p<0.05$) in the univariate analysis and a clinical rationale to have a potential association with glycemic control. The variables were gender, BMI (<25 vs. 25-29.9 vs. ≥30 kg/m^2), BP (<140/90 mmHg vs. higher), having microvascular and

macrovascular complications, smoking, exercise (≤2/week vs. higher), alcohol consumption, statin treatment, insulin usage, education level, and monthly income. The odds ratios with 95% confidence intervals (CI) are given in Figure 1. The p-value is two-tailed with a significance level of 0.05.

Results

A total of 460 patients with Type 2 diabetes were enrolled in the study. Based on the predefined criteria, 18.9% (n=87) of the participants had comorbid depressive disorders. The clinical and demographical characteristics of the patients are given in Table 1.

The patients with comorbid depressive disorders were predominantly female (69.0% vs. 55.5%; p=0.022), younger (57.2±10.5 vs. 60.0±9.5; p=0.014), had higher HbA1c (8.51±2.51 vs. 7.98±2.05; p=0.042), total cholesterol (205.6±44.2 vs. 194.2±46.0; p=0.045), LDL-C (123.1±37.8 vs. 113.1±35.4; p=0.026) and non-HDL-C (158.5±41.61 vs. 146.6±42.7; p=0.024) (Table 1, Table 2). Additionally, these patients had frequent neuropathy (37.3% vs. 19.0%, p=0.001) and were less likely to perform exercise (31.8% vs. 53.1%; p<0.001) while smoke in excess (31.4% vs. 14.3%; p<0.001) (Table 1).

According to the multivariate analyses, being female [odds ratio (OR)=4.4; 95% CI=1.6-12.8; p=0.005] and smoking (OR=7.6; 95% CI=2.8-20.5; p<0.001) were independent determinants of comorbid depressive disorders in type 2 diabetic patients (Figure 1).

Discussion

The result shows that approximately one-fifth of Type 2 diabetic patients have comorbid depressive disorders. Patients with depressive disorders were predominantly fe-

Table 1. The demographic parameters of patients with and without depressive disorders.

Variables	Total patients (n=460)	Without depressive disorder (n=373; 81.1%)	With depressive disorder (n=87; 18.9%)	p
Gender (female) (n, %)	267 (58.0%)	207 (55.5%)	60 (69.0%)	0.022
Age (years)	59.48±9.72	60.02±9.46	57.17±10.48	0.014
Higher education (n, %)	217 (48.3%)	171 (47.3%)	46 (52.9%)	0.345
Patients with lower income (n, %)	324 (75.7%)	262 (75.3%)	62 (77.5%)	0.677
Marital status (married) (n, %)	366 (82.2%)	303 (83.9%)	63 (75.0%)	0.054
Smoking (n, %)	79 (17.6%)	52 (14.3%)	27 (31.4%)	<0.001
Alcohol intake (n, %)	7 (1.6%)	6 (1.7%)	1 (1.2%)	0.766
Regular exercise (n, %)	217 (49.0%)	190 (53.1%)	27 (31.8%)	<0.001
Diabetes duration (year)	10.94±8.14	10.69±8.16	12.15±8.02	0.186
Macrovascular complications (n, %)	85 (22.4%)	68 (21.7%)	17 (25.8%)	0.516
Coronary artery disease	100 (23.8%)	79 (23.0%)	21 (27.3%)	0.422
Peripheral artery disease	8 (1.9%)	6 (1.8%)	2 (2.7%)	0.578
Cerebrovascular disease	10 (2.3%)	9 (2.6%)	1 (1.3%)	0.497
Microvascular complications (n, %)	107 (36.8%)	83 (34.6%)	24 (47.1%)	0.093
Retinopathy	61 (17.3%)	50 (17.1%)	11 (18.3%)	0.813
Nephropathy	45 (13.6%)	36 (13.3%)	9 (15.0%)	0.734
Neuropathy	95 (22.2%)	67 (19.0%)	28 (37.3%)	0.001
Obesity (n, %)	256 (57.7%)	207 (57.3%)	49 (59.0%)	0.778
Hypertension (n, %)	338 (73.5%)	280 (75.1%)	58 (66.7%)	0.110
Dyslipidemia (n, %)	303 (78.0%)	264 (76.5%)	69 (84.1%)	0.134
Insulin treatment (n, %)	191 (41.5%)	148 (39.7%)	43 (49.4%)	0.097
Statin treatment (n, %)	110 (23.9%)	89 (24.3%)	21 (22.3%)	0.689
Treatment with anti-depressants (n, %)	61 (13.3%)	0 (0.0%)	61 (70.1%)	< 0.001

Table 2. The PHQ-9 scores, laboratory parameters, and rates of achieving metabolic targets of patients with and without depressive disorders.

Variable total (n=460)	Total patients (n=460)	Without depressive disorder (n=373; 81.1%)	With depressive disorder (n=87; 18.9%)	p
PHQ-9 score	7.04±5.14	5.88±3.96	11.97±6.51	<0.001
BMI (kg/m^2)	31.71±5.97	31.50±6.06	32.62±5.55	0.126
SBP office (mmHg)	130.84±18.96	131.41±18.95	128.37±18.91	0.191
DBP office (mmHg)	79.43±10.19	79.55±10.42	78.89±9.11	0.599
HbA1c (%)	8.08±2.15	7.98±2.05	8.51±2.51	0.042
Total-C (mg/dL)	196.46±45.84	194.23±46.01	205.61±44.25	0.045
HDL-C (mg/dL)	47.56±13.23	47.69±13.53	47.05±11.95	0.697
LDL-C (mg/dL)	115.10±36.07	113.17±35.42	123.11±37.82	0.026
TG (mg/dL)	184.51±105.45	180.61±102.87	200.83±114.87	0.121
Non-HDL-C (mg/dL)	148.99±42.76	146.62±42.77	158.56±41.61	0.024
Achieving metabolic targets				
ABP (<140/90 mmHg)	266 (60.6%)	211 (59.1%)	55 (67.1%)	0.183
LDL-C (<100 mg/dL)	151 (36.2%)	130 (38.7%)	21 (25.9%)	0.032
HbA1c (<7%)	162 (36.3%)	136 (37.4%)	26 (31.7%)	0.336
Triple target	34 (7.7%)	32 (8.9%)	2 (2.4%)	0.042

PHQ-9: Patients Health Questionnaire-9; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HbA1c: Glycated hemoglobin; Total-C: Total cholesterol; HDL-C: High-density lipoprotein-cholesterol; LDL-C: Low-density lipoprotein-cholesterol; TG: Triglycerides; ABP: Arterial blood pressure.

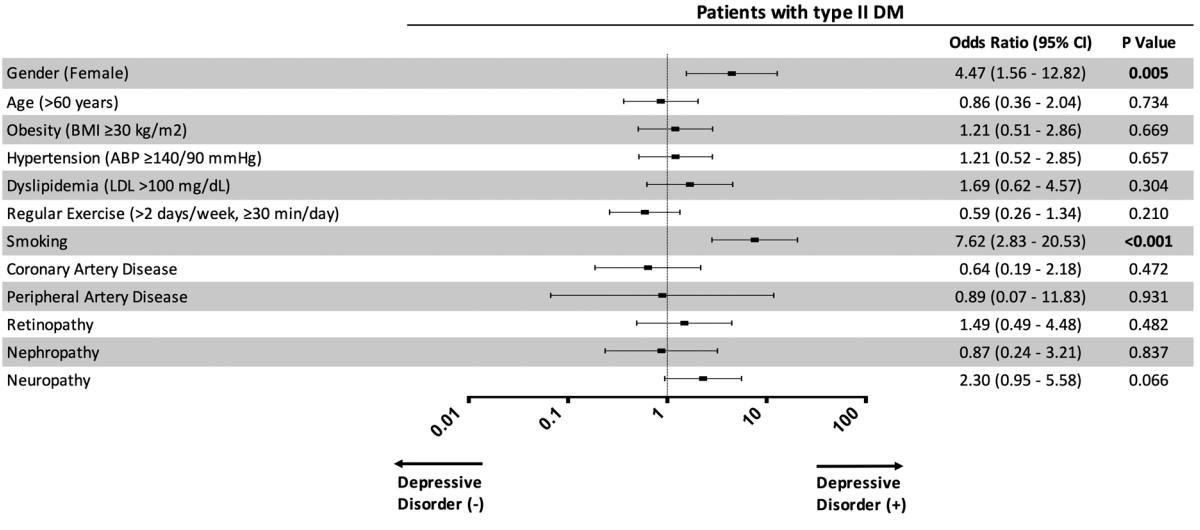

Figure 1: The factors associated with depressive disorder in patients with Type 2 diabetes.
BMI: Body mass index; ABP: Arterial blood pressure; LDL: Low-density lipoprotein.

male, younger, and had poor lipid levels. Being a female or a smoker were predictors of a depressive disorder.

A depressive disorder has a broad and heterogeneous diagnosis, where depressed mood and/or loss of pleasure are the most characteristic features. Many factors, including chronic diseases, can cause or exacerbate depressive disorders. Incidentally, the concomitant depressive disorder may also adversely affect the course of chronic diseases. The frequency of depressive dis-

orders is increasing all over the world (8,21-24). It is reported that the prevalence of depressive disorders in people with chronic diseases is twice higher than in the healthy population (25). Various studies have reported the prevalence of depressive disorders from 8% to 30% in diabetic patients (25-27). However, studies also show that only half of the patients with depressive disorders are diagnosed (25). The data on the prevalence of depressive disorders among diabetic patients in Turkey is limited. An international study that used the PHQ-9 questionnaire reported the prevalence of depressive disorder as 21% for both Type 1 and Type 2 diabetes, in Turkey (11). Another study found a prevalence of 26.4% by using Beck's Depression Inventory in 440 adult patients with Type 2 diabetes (13). The prevalence of depressive disorders in our study (18.9%) was similar to that of the reports of other national (11,13) and international studies (4,27,28). Depressive disorders may affect the course of diabetes, while it may also be affected by diabetes as well. It may also increase the risk of macro and microvascular complications in diabetic patients (29,30). Therefore, it is very important to determine the risk factors that develop depressive disorders in diabetic patients. Evidence shows that the prevalence of depression is moderately increased in prediabetic patients and significantly increased in diabetic patients (31). There may be a few reasons for an increased risk of depression in diabetic patients, such as diabetes, causing structural changes in the brain leading to atrophy (32). Studies show that atrophic changes may involve the hippocampus and that HbA1c may be an important determinant of hippocampal volume (33). Our study supports these findings by showing a higher HbA1c level in patients with depressive disorders. The patients with depressive disorder in our study had higher total cholesterol, LDL-C, and non-HDL-C levels. These findings are also consistent with the previous studies that reported higher cholesterol levels in Type 2 diabetic patients with depressive disorders (27,34). Lack of diet, medication adherence, and inadequate self-care in depressive patients may be the most important reasons for the poor metabolic features. Patients with depressive disorders in our study were younger than patients without depressive disorders. However, several studies show conflicting results of the effect of aging on depressive disorders, where many show a linear rise in the frequency of depressive disorders with increasing age (35-37), while others show a negative correlation (38). Also, different studies suggest a U-shaped relationship between age and depressive disorders (39).

Further, we showed that female gender and smoking were the independent determinants of depressive disorders in Type 2 diabetic patients. Patients with depressive disorders have poor self-care behaviors, such as overeating, drinking alcohol, smoking, limited physical activity, and poor medication adherence. For these reasons, it is not surprising that smoking is a determinant of a depressive disorder in this study. Studies also show that there may be a dose-dependent relationship between depressive disorder and smoking (40,41). In a study of Type 2 diabetic patients, heavy smokers were twice as likely as to experience major depression compared to nonsmokers (42). Diabetic patients may have more problems in quitting smoking because of the physical and emotional burdens associated with diabetes, where smoking may act as a stress-coping behavior (42). Many studies have also reported that depressive disorders are more common in women (43-45). The findings of a similar global female predominance suggest that the differential risk may primarily stem from the biological sex difference and is less dependent on race, culture, diet, education, and many other potentially confounding social and economic factors. Therefore, it was expected from our study to identify that the female gender is a risk factor in the development of depressive disorders.

This study may have several limitations. Firstly, as described in the definitions, PHQ is an instrument in performing criteria-based diagnoses of depression and other mental disorders. However, it is not a gold standard method for diagnosing depressive disorders. Also, in order to prevent an overdiagnosis, all the patients with high PHQ scores are further referred to a psychiatrist, with their progress being fully

monitored. Secondly, the study does not represent the whole country as it is performed in a local health center. Thirdly, we did not question erectile dysfunction in male patients, which is an important factor that causes depression in male diabetics. Additionally, the cross-sectional design of the study may preclude a causal relationship between predictive risk factors and depressive disorder in diabetic patients. There may also be a selection bias in our study since all the enrolled patients were followed-up in a tertiary endocrine unit, and also the enrollment of patients with multiple comorbidities and complications may have affected the results. Nevertheless, the result of our study is remarkable because it is one of the rare studies in our country that reports the prevalence and characteristics of depressive disorders in diabetic patients.

In conclusion, the prevalence of depressive disorders is considerably high in Type 2 diabetic patients, although they are being followed up in a tertiary outpatient endocrinology unit. To assess a diabetic patient from all aspects, screening for depressive disorders should be made an indispensable part of the evaluation process. The risk is higher if the patient is a female or a smoker. Further, prospective studies with a larger sample size may be required to reveal the relationship between depressive disorders and diabetes.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: İbrahim Demirci, Alper Sönmez; Design: Cem Haymana; Control/Supervision: Ömer Azal; Data Collection and/or Processing: Nazlı Kırnap, Orhan Demir, Aydoğan Aydoğdu; Analysis and/or Interpretation: Coşkun Meriç, Güven Oysul; Literature Review: Abdullah Bolu; Writing the Article: İbrahim Demirci, Cem Haymana; Critical Review: Alper Sönmez; References and Fundings: Neşe Ersöz Gülçelik.

References

1. Anderson RJ, Freedland KE, Clouse RE, Lustman PJ. The Prevalence of comorbid depression in adults with diabetes: a meta-analysis. Diabetes Care. 2001;24:1069-1078. [Crossref] [PubMed]
2. American Diabetes Association. Standards of Medical Care in Diabetes-2018. Diabetes Care. 2018;41(Suppl 1):S1-S153. [Crossref] [PubMed]
3. Siu AL, US Preventive Services Task Force (USPSTF); Bibbins-Domingo K, Grossman DC, Baumann LC, Davidson KW, Ebell M, García AR, Gillman M, Herzstein J, Kemper AR, Krist AH, Kurth AE, Owens DK, Phillips WR, Pignone MP. Screening for depression in adults: US Preventive Services Task Force Recommendation Statement. JAMA. 2016;26;315:380-387. [Crossref] [PubMed]
4. Van Buren DJ, Wilfley DE, Marcus MD, Anderson B, Abramson NW, Berkowitz R, Landis CL, Trief P, Yasuda P, Hirst K, TODAY Study Group. Depressive symptoms and glycemic control in youth with type 2 diabetes participating in the TODAY clinical trial. Diabetes Res Clin Pract. 2018;135:85-87. [Crossref] [PubMed] [PMC]
5. Schmitt A, Reimer A, Ehrmann D, Kulzer B, Haak T, Hermanns N. Reduction of depressive symptoms predicts improved glycaemic control: secondary results from the DIAMOS study. J Diabetes Complications. 2017;31:1608-1613. [Crossref] [PubMed]
6. Bădescu SV, Tătaru C, Kobylinska L, Georgescu EL, Zahiu DM, Zăgrean AM, Zăgrean L. The association between diabetes mellitus and depression. J Med Life. 2016;9:120-125. [PubMed]
7. National Institute of Clinical Excellence (NICE). Depression in adults with a chronic physical health problem: recognition and management. NICE. 2016;31:1-57.
8. Salinero-Fort MA, Gómez-Campelo P, San Andrés-Rebollo FJ, Cárdenas-Valladolid J, Abánades-Herranz JC, Carrillo de Santa Pau E, Chico-Moraleja RM, Bemaud-Victoria D, de Miguel-Yanes JM, Jimenez-Garcia R, Lópezde-de-Andres A, Ramallo-Fariña Y, de Burgos-Lunar C. Prevalence of depression in patients with type 2 diabetes mellitus in Spain (the DIADEMA Study): results from the MADIABETES cohort. BMJ Open. 2018;24;8:e020768. [Crossref] [PubMed] [PMC]

9. Knol MJ, Heerdink ER, Egberts ACG, Geerlings MI, Gorter KJ, Numans ME, Grobbee DE, Klungel OH, Burger H. Depressive symptoms in subjects with diagnosed and undiagnosed type 2 diabetes. Psychosom Med. 2007;69:300-305. [Crossref] [PubMed]
10. Li C, Ford ES, Strine TW, Mokdad AH. Prevalence of depression among U.S. adults with diabetes: findings from the 2006 behavioral risk factor surveillance system. Diabetes Care. 2008;31:105-107. [Crossref] [PubMed]
11. İlkova H, Damcı T, Karşıdağ K, Çömlekçi A, Ayvaz G. The International Diabetes Management Practices Study (IDMPS) - Turkey's 5th Wave Results. Turk J Endocrinol Metab. 2016;20:88-96. [Crossref]
12. Eker S. Prevalence of depression symptoms in diabetes mellitus. Open Access Maced J Med Sci. 2018;11;6:340-343. [Crossref] [PubMed] [PMC]
13. Altinok A, Marakoğlu K, Kargın NÇ. Evaluation of quality of life and depression levels in individuals with Type 2 diabetes. J Family Med Prim Care. 2016;5:302-308. [Crossref] [PubMed] [PMC]
14. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18:499-502. [Crossref] [PubMed]
15. van Steenbergen-Weijenburg KM, de Vroege L, Ploeger RR, Brals JW, Vloedbeld MG, Veneman TF, Hakkaart-van Roijen L, Rutten FFJ, Beekman ATF, van der Feltz-Cornelis CM. Validation of the PHQ-9 as a screening instrument for depression in diabetes patients in specialized outpatient clinics. BMC Health Serv Res. 2010;12;10:235. [Crossref] [PubMed]
16. Janssen EPCJ, Köhler S, Stehouwer CDA, Schaper NC, Dagnelie PC, Sep SJS, Dagnelie PC, Henry RMA, van der Kallen CJH, Verhey FR, Schram MT. The patient health questionnaire-9 as a screening tool for depression in individuals with type 2 diabetes mellitus: the maastricht study. J Am Geriatr Soc. 2016;64:e201-e206. [Crossref] [PubMed]
17. Kroenke K, Spitzer RL, Williams JBW. The PHQ-9: validity of a brief depression severity measure. J Gen Intern Med. 2001;16:606-613. [Crossref] [PubMed] [PMC]
18. Definition, diagnosis and classification of diabetes mellitus and intermediate hyperglycemia: report of a WHO/IDF consultation. World Health Organization Report, 2006.
19. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organization technical report series 894, 2000.
20. Satman İ, İmamoğlu Ş, Yılmaz C, Akalın S, Salman S, Dinççağ N. TEMD Diabetes Mellitus Çalışma ve Eğitim Grubu (Ed). Diabetes Mellitus ve Komplikasyonlarının Tanı, Tedavi ve İzlem Kılavuzu-2019. Türkiye Endokrinoloji ve Metabolizma Derneği (TEMD) Yayınları, Bayt Matbaacılık, Ankara, 2019. ISBN: 978-605-4011-38-4.
21. McManus P, Mant A, Mitchell PB, Montgomery WS, Marley J, Auland ME. Recent trends in the use of antidepressant drugs in Australia, 1990-1998. Med J Aust. 2000;6;173:458-461. [Crossref] [PubMed]
22. Hemels MEH, Koren G, Einarson TR. Increased use of antidepressants in Canada: 1981-2000. Ann Pharmacother. 2002;36:1375-379. [Crossref] [PubMed]
23. Lépine JP, Gastpar M, Mendlewicz J, Tylee A. Depression in the community: the first pan-European study DEPRES (Depression Research in European Society). Int Clin Psychopharmacol. 1997;12:19-29. [Crossref] [PubMed]
24. Carta MG, Carpiniello B, Kovess V, Porcedda R, Zedda A, Rudas N. Lifetime prevalence of major depression and dysthymia: results of a community survey in Sardinia. Eur Neuropsychopharmacol. 1995;5:103-107. [Crossref] [PubMed]
25. Egede LE, Simpson K. Epidemiology, treatment and costs of depression in adults with type 2 diabetes. Expert Rev Pharmacoecon Outcomes Res. 2003;3:251-262. [Crossref] [PubMed]
26. Andreulakis E, Hyphantis T, Kandylis D, Lacovides A. Depression in diabetes mellitus: a comprehensive review. Hippokratia. 2012;16:205-214. [PubMed]
27. Egede LE, Ellis C. The effects of depression on metabolic control and quality of life in indigent patients with type 2 diabetes. Diabetes Technol Ther. 2010;12:257-262. [Crossref] [PubMed] [PMC]
28. Andrade L, Caraveo-Anduaga JJ, Berglund P, Bijl RV, Graaf RD, Vollebergh W, Dragomirecka E, Kohn R, Keller M, Kessler RC, Kawakami N, Kiliç C, Offord D, Ustun TB, Ulrich Wittchen H. The epidemiology of major depressive episodes: results from the International Consortium of Psychiatric Epidemiology (ICPE) Surveys. Int J Methods Psychiatr Res. 2003;12:3-21. [Crossref] [PubMed] [PMC]
29. Clouse RE, Lustman PJ, Freedland KE, Griffith LS, McGill JB, Carney RM. Depression and coronary heart disease in women with diabetes. Psychosom Med. 2003;65:376-383. [Crossref] [PubMed]
30. M de Groot, Anderson R, Freedland KE, Clouse RE, Lustman PJ. Association of depression and diabetes complications: a meta-analysis. Psychosom Med. 2001;63:619-630. [Crossref] [PubMed]
31. Chen S, Zhang Q, Dai G, Hu J, Zhu C, Su L, Wu X. Association of depression with pre-diabetes, undiagnosed diabetes, and previously diagnosed diabetes: a meta-analysis. Endocrine. 2016;53:35-46. [Crossref] [PubMed]
32. Harten van B, de Leeuw FE, Weinstein HC, Scheltens P, Biessels GJ. Brain imaging in patients with diabetes: a systematic review. Diabetes Care. 2006;29:2539-2548. [Crossref] [PubMed]
33. Gold SM, Dziobek I, Sweat V, Tirsi A, Rogers K, Bruehl H, Tsui W, Richardson S, Javier E, Convit A. Hippocampal damage and memory impairments as possible early brain complications of type 2 diabetes. Diabetologia. 2007;50:711-719. [Crossref] [PubMed]
34. Gary TL, Crum RM, Cooper-Patrick L, Ford D, Brancati FL. Depressive symptoms and metabolic control in African-Americans with type 2 diabetes. Diabetes Care. 2000;23:23-29. [Crossref] [PubMed]
35. Stordal E, Mykletun A, Dahl AA. The association between age and depression in the general population: a multivariate examination. Acta Psychiatrica Scandinavica. 107:132-141. [Crossref] [PubMed]

36. Weiss Wiesel TR, Nelson CJ, Tew WP, Hardt M, Mohile SG, Owusu C, Klepin HD, Gross CP, Gajra A, Lichtman SM, Ramani R, Katheria V, Zavala L, Hurria A, Cancer Aging Research Group (CARG). The relationship between age, anxiety, and depression in older adults with cancer. Psychooncology. 2015;24:712-717. [Crossref] [PubMed] [PMC]
37. Rothermund K, Brandtstädter J. Depression in later life: cross-sequential patterns and possible determinants. Psychol Aging. 2003;18:80-90. [Crossref] [PubMed]
38. Lawton MP, Kleban MH, Dean J. Affect and age: cross-sectional comparisons of structure and prevalence. Psychol Aging. 1993;8:165-175. [Crossref] [PubMed]
39. Kessler RC, Foster C, Webster PS, House JS. The relationship between age and depressive symptoms in two national surveys. Psychol Aging. 1992;7:119-126. [Crossref] [PubMed]
40. Chaiton M, Cohen J, O'Loughlin J, Rehm J. Use of cigarettes to improve affect and depressive symptoms in a longitudinal study of adolescents. Addict Behav. 2010;35:1054-1060. [Crossref] [PubMed]
41. Boden JM, Fergusson DM, Horwood LJ. Cigarette smoking and depression: tests of causal linkages using a longitudinal birth cohort. Br J Psychiatry. 2010;196:440-446. [Crossref] [PubMed]
42. Clyde M, Smith KJ, Gariépy G, Schmitz N. The association between smoking and depression in a Canadian community-based sample with type 2 diabetes. Can J Diabetes. 2013;37:150-155. [Crossref] [PubMed]
43. Rai D, Zitko P, Jones K, Lynch J, Araya R. Country- and individual-level socioeconomic determinants of depression: multilevel cross-national comparison. Br J Psychiatry. 2018;202:195-203. [Crossref] [PubMed]
44. Cyranowski JM, Frank E, Young E, Shear MK. Adolescent onset of the gender difference in lifetime rates of major depression: a theoretical model. Arch Gen Psychiatry. 2000;57:21-27. [Crossref] [PubMed]
45. Baxter AJ, Scott KM, Ferrari AJ, Norman RE, Vos T, Whiteford HA. Challenging the myth of an "epidemic" of common mental disorders: trends in the global prevalence of anxiety and depression between 1990 and 2010. Depress Anxiety. 2014;31:506-516. [Crossref] [PubMed]

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Association of Types of Diabetic Macular Edema with Different Anti-Diabetic Therapies

Farklı Antidiyabetik Tedavilerle Diyabetik Makuler Ödem Tiplerinin İlişkisi

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Abstract

Objective: To evaluate and assess the association of diabetic macular edema with different anti-diabetic therapy regimens. **Material and Methods:** We recruited 340 patients with prediagnosed Type 2 diabetes mellitus attending the ophthalmology and medicine outpatient department. Patients were older than 30 years with Type 2 diabetes mellitus and on a specific anti-diabetic regimen (monotherapy/combination therapy) for ≥6 months, and who underwent macular edema assessment by using spectral domain optical coherence tomography. The patterns of macular edema per retinal morphology were grouped as diffuse retinal thickening, cystoid macular edema, and serous retinal detachment. **Results:** No significant association was found between edema pattern and dual therapy regimen (metformin+1 other oral hypoglycemic agent) (p=0.685) in the 680 eyes of the 340 patients. In patients on all the other triple therapy regimens (metformin+2 other oral hypoglycemic agents), diffuse retinal thickening was the most common type, except in patients on thiazolidinediones and insulin in conjunction with metformin in which cystoid macular edema was the most common. However, the difference between different triple therapy regimens was statistically significant (p=0.053). **Conclusion:** The most common form of macular edema was diffuse retinal thickening irrespective of the type and regimen of anti-diabetic therapy. Increased incidence of cystoid macular edema was observed in patients on triple therapy, including insulin. Because of the difference in the patterns, it is imperative to evaluate patients for different types of edema due to ongoing anti-diabetic treatment.

Keywords: Diabetes mellitus; diabetic retinopathy; macular edema; anti-diabetic therapy

Özet

Amaç: Diyabetik makuler ödemin farklı antidiyabetik tedavi rejimleriyle ilişkisinin değerlendirilmesi. **Gereç ve Yöntemler:** Oftalmoloji ve medikal oftalmoloji polikliniğine başvuran, önceden tanı almış Tip 2 diabetes mellituslu 340 hasta çalışmaya alındı. Otuz yaşından büyük, Tip 2 diabetes mellituslu ve ≥6 aydır spesifik bir antidiyabetik rejim (monoterapi/kombinasyon terapisi) alan hastalar, spektral alan optik koherens tomografisi kullanılarak makuler ödem değerlendirmesine tabi tutulmuşlardır. Her bir retina morfolojisi için makuler ödem paternleri; diffüz retina kalınlaşması, sistoid makuler ödem ve seröz retina dekolmanı olarak gruplandırıldı. **Bulgular:** Toplam 340 hastadaki 680 gözde ödem paterni ve ikili tedavi rejimleri (metformin+diğer 1 oral hipoglisemik ajan) (p=0,685) arasında anlamlı bir ilişki bulunmadı. Diğer tüm üçlü tedavi rejimlerindeki (metformin+diğer 2 oral hipoglisemik ajan) hastalarda, kistoid makuler ödemin en yaygın olduğu tiazolidindionlar ve metformin ile birlikte insülin kullanan hastalar hariç, diffüz retinal kalınlaşma en yaygın tipti. Bununla birlikte, farklı üçlü tedavi rejimleri arasındaki fark istatistiksel olarak anlamlıydı (p=0,053). **Sonuç:** Makuler ödemin en yaygın formu, antidiyabetik tedavinin tipi ve rejimine bakılmaksızın diffüz retinal kalınlaşma idi. İnsülin de dâhil olmak üzere üçlü tedavi gören hastalarda artmış kistoid makuler ödem insidansı gözlenmiştir. Paternlerdeki farklılık nedeni ile devam eden antidiyabetik tedaviye bağlı olarak hastaları farklı ödem tipleri açısından değerlendirmek zorunludur.

Anahtar kelimeler: Diabetes mellitus; diyabetik retinopati; makuler ödem; antidiyabetik tedavi

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Introduction

Diabetes mellitus is a group of disorders characterized by hyperglycemia and glucose intolerance due to insulin deficiency, impaired effectiveness of insulin action, or both (1). Retinopathy is probably the most common microvascular complication of diabetes. Diabetic macular edema (DME) results in severe vision loss in Type 2 diabetes because of fluid leakage due to increased vascular permeability, owing to anatomical and biochemical changes. Increased vascular permeability of retinal blood vessels and subsequent edema and hard exudates are the key features of DME. The Wisconsin Epidemiological Study of Diabetic Retinopathy (2) reported the prevalence to be 29% in younger-onset diabetic patients and 28% in older-onset diabetic patients, after 20 years. As there is no definitive cure for diabetes, glycemic control using oral hypoglycemic agents (OHAs) and insulin remains the current mainstay of treatment, which has side effects. DME is the warning sign associated with the development and progression of diabetic retinopathy (DR). However, some specific classes of OHAs alone or in combination with insulin are associated with fluid retention and peripheral edema (3-5). The treatment of diabetes with insulin may result in increased retinal vascular permeability, and then induce DR progression and visual impairment (4,6,7). Therefore, there is a need to review the treatment strategies for diabetes mellitus in this perspective. Various studies have recently assessed the association of DME with anti-diabetic treatments; however, data regarding the severity and morphology of DME are scarce.

Hence, we conducted this study to find an association between the different types of macular edema and different anti-diabetic treatment regimens.

Material and Methods

This is a hospital-based cross-sectional study conducted at a tertiary care center in North India. We recruited 340 patients with prediagnosed Type 2 diabetes mellitus attending the ophthalmology and medicine outpatient department of our facility. We obtained their informed and written consent after adhering to the tenets of the Declaration of Helsinki after approval of our institutional ethics committee (Institutional Ethics Committee, Era's Lucknow Medical College and Hospital; Approval No. ELMC/R_Cell/EC/2017/71; Dated: 14/02/2017). Patients older than 30 years with Type 2 diabetes mellitus and who were on a specific anti-diabetic regimen (monotherapy/combination therapy) for ≥6 months were included.

Patients with any other ocular disease altering macular thickness, hazy ocular media obscuring fundus examination and imaging, patients on other medications alongside anti-diabetic treatment, those with a history of recent (<3 months) intraocular surgeries or laser treatment, chronic renal disease, uncontrolled use of hypertensives, dyslipidemia, chronic smokers, and patients not willing to participate in the study were excluded.

Detailed history regarding the demographic profile of the patients and that of diabetes and anti-diabetic medications and symptoms indicative of macular pathology were obtained. Afterward, patients underwent a best-corrected visual acuity assessment by Snellen's Chart, anterior segment assessment using a slit lamp, and a fundus examination using indirect ophthalmoscopy and +90D lens. Fundus photography and fluorescein angiography were performed using Carl Zeiss Visucam724 (Carl Zeiss Meditech Inc.). Patients then underwent optical coherence tomography (macula) using Carl Zeiss cirrus HD-Optical Coherence Tomography (OCT), and 512x128 macular cube axial scans covering an area of 6 mmx6 mm were obtained. Center-involving DME was considered to be present based on the incidence of foveal intraretinal fluid SD-OCT in association with the clinical diagnosis of DR and concurrent appropriate fluorescein angiographic findings. The patterns of macular edema per retinal morphology were grouped as diffuse retinal thickening, cystoid macular edema, and serous retinal detachment.

Statistical analysis was performed using SPSS Version 21.0 0 statistical Analysis Software. The values are represented in number (%) and mean±SD. $p<0.05$ was considered significant, and $p<0.001$ as very highly significant.

Results

We aimed to find the correlation of different patterns of DME with the different types of anti-diabetic regimens for which we included 680 eyes of 340 subjects.

The mean age of patients was 52.0±9.67 years. Most of the patients (55.3%) were women. The duration of diabetes ranged from 1 to 15 years. HbA_{1C} values ranged from 6% to 14% (8.3%±1.2%). Mean fasting and postprandial blood glucose levels were 109.9±36.24 mg/dL and 174.1±45.6 mg/dL, respectively. Out of the 680 eyes of 340 patients, there were 598 (87.9%) with normal vision, 71 (10.4%) with low vision, and 11 (1.6%) with social blindness (vision 3/60 or diminution of the field of vision to 10°). No patients had economic blindness (inability of a person to count fingers from a distance of 6 m or 20 feet). Fifty-eight eyes (8.5%) showed DR. OCT measurements could not be performed in 6 eyes because of poor scan. The central subfield thickness of macula on OCT ranged from 106 µm to 468 µm (mean=238.81±39.94 µm). The mean cube average thickness was 263.41±27.34 µm. Seventy-two eyes (10.7%) showed macular thickening followed by cystoid macular edema (n=20; 3%) and serous retinal detachment (n=6; 0.9%). Twelve eyes (1.8%) had clinically significant macular edema (Table 1).

The majority of patients were on dual therapy taking metformin in combination with one other anti-diabetic medication (58.25%), followed by those on metformin alone (monotherapy) (26.2%) and triple therapy (metformin+2 other anti-diabetic medications) (17.1%).

Among patients receiving dual therapy, most (n=59) were receiving the combination Metformin+Sulfonylureas (M+S), followed by Metformin+DPP4 inhibitors (M+D) (n=53), Metformin+Thiazolidinediones (M+T) (n=38), Metformin+Insulin (M+I) (n=28), and Metformin+Alpha glucosidase inhibitor (M+A) (n=20). Thus, overall, five combinations were used.

Among patients receiving triple therapy, most (n=21) were receiving Metformin+Thiazolidinediones+Insulin (M+T+I), followed by Metformin+Suplhonylurea+Alpha glu-

Table 1. Demographic profile and characteristics of the study population (n=340).

SN	Characteristic	Statistic
1.	Mean age±SD (range) in years	52.00±9.67 (35-76)
2.	Gender Gender (n=340)	
	Male	154 (44.9%)
	Female	186 (55.3%)
3.	Diabetic retinopathy (n=680)	
	Present	58 (8.5%)
	NPDR	54
	PDR	04
	Absent	622
4.	Visual acuity (n=680)	
	≥6/6-6/18	598 (87.9%)
	<6/18-6/60	71 (10.4%)
	<6/60-NPL	0
	<3/60-Pl denied	11 (1.6%)
5	Macular edema (n=674)	72 (10.7%)
	Serous retinal detachment	6 (0.9%)
	Diffuse macular thickening	46 (6.8%)
	Cystoid macular edema	20 (3%)
6.	Clinically significant macular edema (n=674)	12 (1.8%)

SN: Serial number; SD: Standard deviation; NPDR: Non proliferative diabetic retinopathy; PDR: Proliferative diabetic retinopathy.

cosidase inhibitor (M+S+A) (n=11), Metformin+Sulfonylurea+Insulin (M+S+I) (n=9), Metformin+Alpha glucosidase inhibitor+DPP4 inhibitor (M+A+D) (n=7), Metformin+Sulfonylurea+Dipeptidyl Peptidase 4 (DPP4) inhibitor (M+S+D) (n=5), Metformin+Sulfonylurea+Thiazolidinediones (M+S+T) (n=4), and Metformin+Thiazolidinediones+Alpha glycosidase inhibitor (M+T+A) (n=1). Thus, overall, 7 combinations were used (Table 2).

Irrespective of therapy, diffuse retinal thickening was the dominant type. Although the proportion of cystoid macular edema was higher in mono and triple therapy groups than in the dual therapy group, this difference was not statistically significant (p=0.110) (Table 3).

Irrespective of dual therapy regimen, diffuse retinal thickening was most common. Statistically, there was no significant association between the pattern of edema and dual therapy regimen (p=0.685). Except for M+T+I and M+T+A, diffuse retinal thickening was the most common type for all the other triple therapy regimens. For M+T+I, cystoid macular edema was most common, whereas the lone edematous case of M+T+A was of serous type; however, the difference among different triple therapy regimens was not statistically significant (p=0.053) (Table 4).

Discussion

Diabetes mellitus is a metabolic disorder of multiple etiologies. The long term effects of diabetes mellitus, besides systemic effects, include the progressive development of

Table 2. Distribution of anti-diabetic treatment regimens (n=340).

SN	Treatment modality	Number	%
1.	Monotherapy (metformin)	84	24.7
2.	Dual therapy	198	58.2
	M+T (Metformin+Thiazolidinediones)	38	11.2
	M+A (Metformin+Alpha glucosidase inhibitor)	20	5.9
	M+D (Metformin+DPP4-inhibitors)	53	15.6
	M+I (Metformin+Insulin)	28	8.2
	M+S (Metformin+Suliphonylureas)	59	17.4
3.	Triple therapy	58	17.1
	M+S+T (Metformin+Sulfonylurea+Thiazolidinediones)	4	1.2
	M+A+D (Metformin+Alpha glucosidase inhibitor+DPP4 inhibitor)	7	2.1
	M+S+A (Metformin+Suplhonylurea+Alpha glucosidase inhibitor)	11	3.2
	M+S+I (Metformin+Sulfonylurea+Insulin)	9	2.6
	M+T+I (Metformin+Thiazolidinediones+Insulin)	21	6.2
	M+S+D (Metformin+Sulponylurea+DPP4 inhibitor)	5	1.5
	M+T+A (Metformin+Thiazolidinediones+Alpha glycosidase inhibitor)	1	0. 3

Table 3. Association between the pattern of macular edema and therapy (n=72).

	Monotherapy (n=19)		Dual therapy (n=29)		Triple therapy (n=24)		Total (n=72)	
Pattern	No.	%	No.	%	No.	%	No.	%
Serous retinal detachment	0	0	4	13.8	2	8.3	6	8.3
Diffuse retinal thickening	13	68.4	21	72.4	12	50.0	46	63.9
Cystoid macular edema	6	31.6	4	13.8	10	41.7	20	27.8

x^2=7.54 (df=4); p=0.110 (percentages have been calculated column wise).

Table 4. Pattern of edema by different multi therapies.

	Pattern							
Therapy	**Serous (n=4)**		**Diffuse (n=21)**		**Cystoid (n=4)**		**Total (n=29)**	
Dual therapy	**No.**	**%**	**No.**	**%**	**No.**	**%**		**Statistics**
M+T	3	20.0	11	73.3	1	6.7	15	x^2=5.66 (df=8); p=0.685
M+A	0	0.0	1	50.0	1	50.0	2	
M+D	0	0.0	1	100	0	0.0	1	
M+I	1	12.5	5	62.5	2	25.0	8	
M+S	0	0.0	3	100	0	0.0	3	
	Serous (n=2)		**Diffuse (n=12)**		**Cystoid (n=10)**			
Triple therapy	**No.**	**%**	**No.**	**%**	**No.**	**%**	**Total (n=24)**	
M+S+T	0	0.0	3	100	0	0.0	3	x^2=18.1 (df=10); p=0.053
M+A+D	0	0.0	2	66.7	1	33.3	3	
M+S+A	0	0.0	2	66.7	1	33.3	3	
M+S+I	0	0.0	1	100	0	0.0	1	
M+T+I	1	7.7	4	30.8	8	61.5	13	
M+T+A	1	100	0	0.0	0	0.0	1	

M: Metformin; T: Thiazolidinediones; A: Alpha glucosidase inhibitor; D: DPP4; I: Insulin; S: Sulfonylurea.

retinopathy with potential blindness (8). The total number of people with diabetes is expected to rise to an estimated 300 million by 2025, with the most significant increases occurring in developing countries, probably attributed to population growth, ageing, obesity, and sedentary lifestyles. DME is a vision-threatening complication of diabetic microangiopathy, which demands urgent treatment. The SN-DREAMS (9) study found that the chances of DME increase at the fifth and sixth decades of life and taper thereafter. OCT is a noninvasive, reproducible, and reliable method to evaluate macular thickness (10,11). Several authors have studied the possibility of OCT for the early diagnosis of ME and have suggested criteria to detect so-called subclinical macular edema. Among anti-diabetic medications, thiazolidineiones (12-14) and insulin (3,4,5,15) have been implicated in the rise of macular thickness, which may be due to the side effect of drugs or due to its role progression of retinopathy. However, the evidence obtained is not conclusive. Other anti-diabetic medications like DPP4 inhibitors (vildagliptin) (16-18) and glibenclamide and glicazide (19) have shown to possess a potential role against DR in obese diabetic rats; however, human studies using these medications have not been conducted as yet.

Various studies have speculated the role of anti-diabetic medications in the development of macular edema, but, to the best of our knowledge, there is no available literature regarding the association of type of macular edema with the use of anti-diabetic medications.

In our study, most of the patients were women (55.3%). The diabetic profile of patients revealed that the duration of diabetes was <1 year (59.10%), showing that a fairly large amount of patients had been recently diagnosed, irrespective of their awareness of disease status. The mean HbA_{1c} was 8.3%±1.2% (6%-14%), representing a fair glycemic control in the study population.

Intensive glycemic control was associated with 46% reduction in the incidence of DME at the end of trial, and a 58% reduction in four years later compared with the conventional group. Diabetes Control and Complications Trial (DCCT) reported incentive glycemic control progression of existing DR (65^{th} year treatment). Aggressive glycemic control (<6.5%) did not reduce DR risk and ME. Intensive glycemic control has a meta-

bolic memory, whereby there is low risk of DR/DME progression after 10 years of anti-diabetic therapy (DCCT), but our study did not have many patients with a duration of diabetes mellitus>10 years, but they had fairly controlled HbAlc levels. However, the duration of treatment was not known.

In our study, most of the patients were on dual therapy: metformin+OHA (58.2%), followed by monotherapy (metformin) and triple therapy (metformin+OHA1+OHA2) (6.2%), depicting a study population containing many patients who were not achieving glycemic target even after lifestyle modification and metformin monotherapy for a duration of 3 months (American Diabetes Association), or had a fairly disturbed blood sugar profile on the first diagnosis. Next in the majority were those patients who were fairly controlled on metformin alone and had been taking the drug for ≥6 months, irrespective of previous treatment. In addition, these patients were free of contraindications for metformin therapy. The number of patients on a triple drug regimen to achieve glycemic targets was very small. In our study, out of the 680 eyes of 340 patients, 594 eyes (92.5%) had no DR. The low prevalence of DR could be attributed to the fact that the study population was largely metabolically stable with a duration of diabetes <5 years in many patients with a fair glycemic control. Out of 340 patients, 72 patients had macular edema and 46 patients (63.9%) of them showed diffuse retinal thickening regardless of their anti-diabetic regimen. Jingi et al. evaluated the presence of macular edema in patients on various anti-diabetic medications and found that irrespective of staging, the frequency of DME was significantly higher in the insulin group than in the OHA group (43.5% vs. 19.8%; OR=3.1; 95% CI=1.2 to 8; p=0.019).

We evaluated the association between the pattern of macular edema and the type of anti-diabetic therapy taken by the patients having DME. Irrespective of the type of therapy, diffuse retinal thickening was most common in all anti-diabetic therapy groups. Overall, diffuse retinal thickening was observed in 19 patients on monotherapy (68.4%), 21 (72.4%) on dual therapy, and 12 (50%) on triple therapy. However, cystoid macular edema was common in monotherapy and triple therapy groups than in the dual therapy group, but the difference was not statistically significant. Among dual therapy subgroups, out of 29 eyes, diffuse retinal thickening was most common and most eyes were of patients on M+S (100%) and M+D (100%), followed by M+T (73.33%) and M+I (62.5%), but the difference was not statistically significant. Among triple therapy subgroups, the M+T+I subgroup had cystoid macular edema and the M+T+A subgroup had serous retinal detachment as the most common patterns of macular edema, but the difference was not statistically significant. Previous studies have shown that insulin treatment is a risk factor for DME. Aroca et al. (20) observed that insulin use was a risk factor for focal and diffuse DME in a 4-year prospective study, including 93 Type 2 diabetes mellitus patients.

Within the subgroups of 3 anti-diabetic therapy groups, no statistically significant pattern was associated with the type of anti-diabetic therapy. However, those on the triple combination showed more cystoid and serous retinal detachment (SRD) patterns, hinting at the presence of moderate to severe macular edema; however, the exact duration of treatment was not known. Jingi et al. (21) reported that out of the patients who had developed macular edema, majority of them were on insulin therapy (43.5%) compared with those on OHAs (19.8%). On comparing the severity of macular edema with the type of anti-diabetic regimen, the authors found that in all the subgroups, the number of patients on insulin therapy outnumbered the patients on OHA, where 8.7% of the patients on insulin developed severe macular edema compared with only 2.7% of the patients on OHAs.

Various studies have assessed the effect of anti-diabetic medications on the development of macular edema. Ryan et al. (14) reported the incidence of worsening of macular edema to be 1.5%-2.6%, detected using FFA alone. About 63% of their patients had DME in at least one eye. Among OHAs, Liazos et al., Oshitari et al., Fong et al., Tatti et al., and Agarwal et al. reported a positive association of macular edema with glitazones, but none of these authors studied the type of macular edema in relation to the anti-diabetic regimen. However, regardless

of the anti-diabetic regimen, diffuse retinal thickening was the most common pattern of DME in the previous studies (22-24).

The diffuse thickening pattern of macular edema represents the initial stage of macular edema formation, which may slowly progress to severe visual impairment eventually giving rise to other patterns of macular edema. Thus, this pattern of macular edema, which was present in the majority of our study population, represented the early stage of macular edema formation.

Although various studies have examined the role of oral hypoglycemics and insulin in the development of macular edema, to the best of our knowledge, no study has examined the correlation of various anti-diabetic regimens and different types of DME.

Conclusion

We conclude that the most common form of macular edema is diffuse retinal thickening, irrespective of the type and regimen of anti-diabetic therapy. An increase in the cystoid macular edema was observed among patients on triple therapy, including insulin. With a difference in the pattern of macular edema, the treatment modalities for the pattern might vary; hence, we must evaluate patients for the different types of edema due to ongoing anti-diabetic treatments. Changes are required in the currently available treatment options for diabetes to avoid the vicious cycle set up by certain anti-diabetic medications. Further studies regarding this concern involving a larger population size need to be conducted for in-depth understanding of this association.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

All authors contributed equally while this study preparing.

References

1. Toldo S, Boccellino M, Rinaldi B, Seropian IM, Mezzaroma E, Severino A, Quagliuolo L, Van Tassell BW, Marfella R, Paolisso G, Rossi F, Natarajan R, Voelkel N, Abbate A, Crea F, Baldi A. Altered oxido-reductive state in the diabetic heart: loss of cardioprotection due to protein disulfide isomerase. Mol Med. 2011;17:1012-1021. [Crossref] [PubMed] [PMC]
2. Klein R, Klein BE, Moss SE, Cruickshanks KJ. The wisconsin epidemiology study of diabetic retinopathy: XVII. The 14 years incidence and progression of diabetic retinopathy and associated risk factors in type l diabetes. Ophthalmology. 1998;105:1801-1815. [Crossref] [PubMed]
3. Niemeyer NV, Janney LM. Thiazolidinedione-induced edema. Pharmacotherapy. 2002;22:924-929. [Crossref] [PubMed]
4. Mudaliar S, Chang AR, Henry RR. Thiazolidinediones, peripheral edema, and type 2 diabetes: incidence, pathophysiology, and clinical implications. Endocr Pract. 2003;9:406-416. [Crossref] [PubMed]
5. Patel C, Wyne KL, McGuire DK. Thiazolidinediones, peripheral oedema and congestive heart failure: what is the evidence? Diabet Vasc Dis Res. 2005;2:61-66. [Crossref] [PubMed]
6. Henricsson M, Bemtorp K, Femlund P, Sundkvist G. Progression of retinopathy in insulin-treated type 2 diabetic patients. Diabetes Care. 2002;25:381-385. [Crossref] [PubMed]
7. Poulaki V, Qin W, Joussen AM, Hurlbut P, Wiegand SJ, Rudge J, Yancopoulos GD, Adamis P. Acute intensive insulin therapy exacerbates diabetic blood-retinal barrier breakdown via hypoxia-inducible factor-1alpha and VEGF. J Clin Invest. 2002;109:805-815. [Crossref] [PubMed]
8. Huang J, Liu X, Wu Z, Xiao H, Dustin L, Sadda S. Macular thickness measurements in normal eyes with time-domain and Fourier-domain optical coherence tomography. Retina. 2009;29:980-987. [Crossref] [PubMed] [PMC]
9. Munjal YP. Epidemiology and basic considerations of diabetes. In: Ramachandran A, Snehalatha C, eds. API Textbook of Medicine (9th ed). New Delhi: Jaypee; 2012;331-324.
10. Podoleanu AGh. Optical coherence tomography. Br J Radiology. 2005;78:976-988. [Crossref] [PubMed]
11. Paunescu LA, Schuman JS, Price LL, Stark PC, Beaton S, Ishikawa H, Wollstein G, Fujimoto JG. Reproducibility of nerve fiber thickness, macular thickness, and optic nerve head measurements using stratusOCT. Invest Opthalmol Vis Sci. 2004;45:1716-1724. [Crossref] [PubMed] [PMC]

12. Idris I, Warren G, Donnelly R. Association between thiazolidinedione treatment and risk of macular edema among patients with type 2 diabetes. Arch Intern Med. 2012;172:1005-1011. [Crossref] [PubMed]
13. Tarbett AK VanRoekel RC, Howard RS, Vigersky RA. The use of optical coherence tomography to determine the effectt of thiazolidinediones on retinal thickness in patients with type 2 diabetes. J Diabetes Sci Technol. 2011;5:945-951. [Crossref] [PubMed] [PMC]
14. Ryan Jr EH, Han DP, Ramsay RC, Cantrill HL, Bennett SR, Dev S, Williams DF. Diabetic Macular Edema Associated With Glitazone Use. Retina. 2006;26:562-570. [Crossref] [PubMed]
15. Hee MR, Puliafito CA, Wong C, Duker JS, Reichel E, Rutledge B, Schuman JS, Swanson EA, Fujimoto JG. Quantitative assessment of macular edema with optical coherence tomography. Arch Ophthalmol. 1995;113:1019-1029. [PubMed]
16. Antcliff R, Marshall J. The pathogenesis of edema in diabetic maculopathy. Semin Ophthalmol. 1999;14:223-232. [Crossref] [PubMed]
17. Raman R, Bhende M. Diabetic macular edema. Sci J Med & Vis Res Foun. 2015;XXXIII:50-56.
18. Photocoagulation for diabetic macular edema. Early Treatment Diabetic Retinopathy Study report number 1. Early Treatment Diabetic Retinopathy Study research group. Arch Ophthalmol. 1985;103:1796. [Crossref] [PubMed]
19. Zander E, Herfurth S, Bohl B, Heinke P, Herrmann U, Kohnert KD, Kerner W. Maculopathy in patients with diabetes mellitus type 1 and type 2: associations with risk factors. Br J Ophthalmol. 2000;84:871-876. [Crossref] [PubMed] [PMC]
20. Aroca PR, Salvat M, Fernández J, Méndez I. Risk factors for diffuse and focal macular edema. J Diabetes Complications. 2004;18:211-215. [Crossref] [PubMed]
21. Jingi AM, Noubiap JJN, Essouma M, Bigna JJR, Nansseu JRN, Ellong A, Mvogo CE. Association of insulin treatment versus oral hypoglycaemic agents with diabetic retinopathy and its severity in type 2 diabetes patients in Cameroon, sub-Saharan Africa. Ann Transl Med. 2016;4:395. [Crossref] [PubMed] [PMC]
22. Otani T, Kishi S, Maruyama Y. Patterns of diabetic macular edema with optical coherence tomography. Am J Ophthalmol. 1999;127:688-693. [Crossref] [PubMed]
23. Catier A, Tadayoni R, Paques M, Erginay A, Haouchine B, Gaudric A, Massin P. Characterization of macular edema from various etiologies by optical coherence tomography. Am J Ophthalmol. 2005;140:200-206. [Crossref] [PubMed]
24. Kim BY, Smith SD, Kaiser PK. Optical coherence tomographic patterns of diabetic macular edema. Am J Ophthalmol. 2006;142:405-412. [Crossref] [PubMed]

Original Article
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The Effects of Low-Carbohydrate Diet and Protein-rich Mixed Diet on Insulin Sensitivity, Basal Metabolic Rate and Metabolic Parameters in Obese Patients

Obez Hastalarda Düşük Karbonhidrat Diyeti ve Proteinden Zengin Karma Diyetin İnsülin Sensitivitesi, Bazal Metabolik Hız ve Metabolik Parametreler Üzerine Etkisi

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Abstract

Objective: Various diet plans with varying ratios of carbohydrates, proteins, and fat ensure weight loss in obesity. The primary aim of our study was to evaluate the effects of weight loss on metabolic parameters, and the secondary aim was to compare the successes of various weight loss regimens in maintaining weight loss. **Material and Methods:** A team of doctors comprising a dietary consultant and a psychologist developed a program that was followed throughout our study. Twenty-two patients were included in our study. Based on their preference, they were classified into two groups: low carbohydrate diet (Atkins) group and protein-rich mixed diet group. **Results:** The mean age of the patients was 52.4±3 years, and the mean body mass index (BMI) was 36.1±1.2 kg/m^2. Five patients followed the Atkins diet, whereas 17 followed the protein-rich mixed diet. Compared with the baseline values, in the 3rd, 6th, and 12th months, body weight (BW), BMI, and waist circumference decreased significantly (p<0.001) in all the patients. Basal metabolic rate decreased in the third and sixth months but increased in the 12th. Fasting blood glucose, fasting insulin, HbA1c, 120-minute blood glucose level in oral glucose tolerance test, total cholesterol, low-density lipoprotein, free fatty acids, and uric acid did not change significantly (p>0.05). In the Atkins group, BMI decreased significantly in the 6th month (p=0.03) but increased in the 12th month (p=0.29). In the protein-rich mixed diet group, BMI (basal 35.1±1.5 kg/m^2) decreased significantly (32.8±1.5, p<0.001) in the 6th month, and continued to decrease in the twelfth (31.5±1.2, p=0.007). **Conclusion:** In obesity, approximately 10% weight loss can change metabolic parameters moderately. The Atkins and protein-rich mixed diets caused similar weight loss ratios in the first six months, but a protein-rich mixed diet was more successful in terms of long-term sustainability and maintenance of weight loss.

Keywords: Atkins diet; diet plans; weight loss

Özet

Amaç: Değişen karbonhidrat, protein ve yağ oranlarına sahip diyet rejimleri obezitede kilo kaybını sağlar. Çalışmamızın birinci amacı, kilo kaybının metabolik parametreler üzerindeki etkilerini değerlendirmek, ikinci amacı ise çeşitli kilo kaybı rejimlerinin kilo kaybını sürdürmedeki başarılarını karşılaştırmaktır. **Gereç ve Yöntemler:** Çalışma boyunca doktor, diyet danışmanı ve psikoloğu içeren bir ekip tarafından program takip edildi. Yirmi iki hasta çalışmaya dâhil edildi. Tercihlerine göre hastalar, düşük karbonhidrat diyeti (Atkins) grubu ve proteinden zengin karma diyet grubu olmak üzere ikiye ayrıldı. **Bulgular:** Hastaların ortalama yaşı 52,4±3 yıl, ortalama beden kitle indeksi (BKİ) 36,1±1,2 idi. Hastaların 5'i Atkins diyetini, 17'si proteinden zengin karma diyeti takip etti. Başlangıç değerleri ile karşılaştırıldığında 3, 6 ve 12. aylarda, tüm hastaların vücut ağırlığı (VA), BKİ ve bel çevresi önemli ölçüde azaldı (p<0,001). Bazal metabolizma hızı 3 ve 6. aylarda azaldı, ancak12. ayda arttı. Açlık kan şekeri, açlık insülini, HbA1c, oral glukoz tolerans testinde 120. dk'da glukoz, total kolesterol, düşük yoğunluklu lipoprotein, serbest yağ asitleri ve ürik asit düzeylerinde anlamlı bir değişiklik olmadı (p>0,05). Atkins grubunda BKİ, 6. ayda anlamlı olarak azaldı (p=0,03), ancak 12. ayda arttı (p=0,29). Proteinden zengin karma diyet grubunda, 6. ayda BKİ (bazal 35,1±1,5 kg/m^2) anlamlı olarak azaldı (32,8±1,5; p<0,001) ve 12. ayda azalmaya devam etti (31,5±1,2; p=0,007). **Sonuç:** Obezitede yaklaşık %10 kilo kaybı metabolik parametrelerde orta düzeyde değişime neden olabilir. Atkins ve proteinden zengin karma diyetler, ilk 6 ayda benzer oranda kilo kaybına yol açar, ancak proteinden zengin karma diyet, kilo kaybının sürdürülmesi ve uzun dönem devam edilmesinde daha başarılı olmuştur.

Anahtar kelimeler: Atkins diyeti; diyet rejimleri; kilo verme

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Introduction

Obesity is a chronic disease considered to be a global epidemic with increasing incidence worldwide (1). It is associated with a significant increase in morbidity (including diabetes mellitus, hypertension, dyslipidemia, heart disease, stroke, sleep apnea, and cancer) and mortality (2). In weight loss, the aim is to prevent or revert the complications of obesity and increase the quality of life (3). The first step in weight loss management is the intervention of an extensive lifestyle that includes changes in diet, exercise, and behavior (4). Obesity has a multifactorial characteristic that originates from genetic, epigenetic, physiological, behavioral, sociocultural, and environmental factors, and leads to long-term imbalance between energy intake and expenditure. However, in most cases, obesity is caused by behaviors such as a sedentary lifestyle and increased calorie intake (5). To ensure weight loss in obesity treatment, all individuals need to receive consultation on diet, physical activity, behavioral changes, and weight loss goals (6). Data on the success of diet plans, which include varying ratios of dietary fat, protein, and carbohydrate, are controversial (7-10). The primary aim of this study was to evaluate the effects of weight loss on metabolic parameters, and the secondary aim was to compare the successes of various weight loss regimens in maintaining weight loss.

Material and Methods

Patients who volunteered to participate in the weight loss program were randomly selected and included. A total of twenty-two volunteers (nineteen females and three males) were included in the study. Before the weight loss program began, the patients were asked to record their diet for three days and were provided consultation on their habits. In the weight loss program, two different dietary strategies were implemented: a protein-rich mixed diet and a low-carbohydrate Atkins diet. The patients made the choice of diet for themselves. Calorie intake was set between 1,409 kcal and 2,090 kcal, depending on the patient.

The protein-rich mixed diet comprised of 33% protein, 33% fat, and 34% carbohydrate. Atkins diet is usually followed in three stages (11): Stage 1 diet includes 35% protein, 60% fat and 5% carbohydrate for one week; Stage 2 diet includes 35% protein, 35% fat and 30% carbohydrate for eight weeks; and Stage 3 diet includes 30% protein, 30% fat and 40% carbohydrate for a duration that is of the patient's preference. The patients in the Atkins diet group did not proceed to the third stage after the second but continued with a carbohydrate percentage of 30%.

During the first six months, twenty meetings were conducted for the patients, with each meeting lasting for 2.5 h. During the first 1.5 h of the first nine meetings, group training, which included practical cooking methods, were provided to the patients by the dietary consultants. In the final hour of every meeting, a mild sports activity, which included either gymnastics or water sports, was performed. A doctor was presenting every meeting, and a psychologist was presenting at least ten meetings to provide training. In the last six months, one meeting was conducted every month in the form of 1.5 h of group training, in which dietary consultation was provided (total of six meetings).

Physical examination, basal metabolic rate (BMR) measurement (MVmax29, Sensor Medics, USA), bioimpedance analysis (AKER SRL, 50136 Flana-Italy), and blood gas analysis (ABL 505, Radiometer Kopenhag, DK-2700 Bronshoj/Denmark) were performed in the beginning and in the third, sixth and twelfth months of the study. In addition, real-time serum total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride, free fatty acids, fasting blood sugar, HbA1c, creatinine, urea, uric acid, complete blood count and C-reactive protein levels were measured. A 75 g oral glucose tolerance test (OGTT), test was performed on each patient in the beginning and in the sixth month of the study. A euglycemic clamp test was performed on thirteen patients. Biochemical analyses were performed in the central laboratories of Benjamin Franklin University Hospital in Berlin, Germany.

Euglycemic Clamp Test

It was performed on patients after ten hours of fasting while the patients were lying in a supine position. Single-arm infusions of 40

mU/m^2/min human insulin (Actrapid, Novo Nordisk) and 10% dextrose were given to patients. When blood glucose levels were stable for at least two hours, blood samples were collected from the other arm. Capillary blood samples were collected at 5-minute intervals and analyzed using the glucose oxidase method. Insulin resistance was calculated according to the glucose infusion rate. The glucose level was calculated when glucose levels were stable for at least 2 h (80±10% mg/dL was considered stable). Two cannulas were inserted: one in an antecubital vein for the infusion of glucose and insulin, and the other in the opposite upper extremity radial artery or antecubital vein, which was warmed with a heating pillow to arterialize venous blood. When the glucose levels were stable, the blood glucose level was divided by the patient's weight to calculate the M-value. Homeostatic model assessment for insulin resistance (HOMA-IR) was calculated by using the formula: fasting insulin (mIU/L)×fasting glucose (mmol/L)/22.5 (12).

Statistical Analysis

Statistical analysis was performed by using SPSS Version 11.0 statistic software package (Chicago, USA). Normality distribution analysis of the data was performed by using the Kolmogorov-Smirnov test and the Shapiro-Wilk test. Normally distributed parametric data were presented as mean±SD, and the significance of intergroup variance was analyzed by using the Student t-test. Repeated measurements of the non-normally distributed data in the same individual were analyzed using the Wilcoxon test. Pearson's correlation coefficient was used for correlation analysis, and a p-value of<0.05 was considered significant.

Results

The mean age of patients was 52.4±3 years and the mean body mass index (BMI) was 36.1±1.2 kg/m^2. Five patients chose to follow the Atkins diet, whereas seventeen patients chose to follow the protein-rich mixed diet. Demographic data and the laboratory values measured in the patients at the beginning of the study are shown in Table 1. Of the twenty-two patients, four left the study during the first three months. Eighteen patients remained in the study for six months, and later, seven left, and eleven patients remained in the study for twelve months.

During the follow-up sessions, when all the patients were evaluated, it was found that in the third, sixth and twelfth months, the patients' body weight (BW), BMI and waist circumference values decreased significantly compared with their baseline values (p<0.001) (Table 2, Figure 1). BMR decreased in the third and sixth months but increased in the twelfth month (1,544-1,524-1,547 kcal). From the bioimpedance

Table 1. Demographic data and laboratory values of the patients at the beginning of the study.

Patients	Total (n=22)	Atkins diet (n=5)	Protein-rich mixed diet (n=17)	p
Age	52.4±3	51.6±7.1	52.6±3.4	0.26
Sex				0.63
Female, n (%)	19 (86.4)	4 (80)	15 (88.2)	
Male, n (%)	3 (13.6)	1 (20)	2 (11.8)	
BMI	36.12±1.3	39.7±2.2	35.1±1.50	<0.001
Waist circumference (cm)	115.4±2.64	121±2.65	114.1±3.12	0.01
Fat-free mass (kg)	60.45±1.55	63.03±2.04	59.7±1.90	<0.001
BIA-Fat mass (kg)	39.2±2.04	43.2±3.61	38.11±2.4	<0.001
BIA-Fat percentage (%)	39.33±0.9	40.53±2.5	39.0±1.0	0.01
BMR (kcal)	1544.1±53.8	1713.8±105.6	1495.64±57.53	<0.001
SBP (mmHg)	128.5±4.6	139.7±10.5	125.5±4.91	<0.001
DBP (mmHg)	80.6±3.0	86.33±11.9	79.00±2.5	<0.001

BMI: Body mass index; BIA: Bioimpedance analysis; BMR: Basal metabolic rate; SBP: Systolic blood pressure; DBP: Diastolic blood pressure.

Table 2. Demographic and metabolic follow-up parameters of all patients.

	Baseline n=22	3rd month n=18	p	6th month n=18	p	12th month n=11	p
BW (kg)	99.9±2.82	91.14±3.63	**<0.001**	92.54±2.70	**<0.001**	90.6±3.1	**0.001**
BMI (kg/m²)	36.12±1.3	32.6±1.55	**<0.001**	33.4±1.2	**<0.001**	32.82±1.33	**0.002**
BMR (kcal)	1,544.11±53.8	1,471.47±35.54	0.125	1,420.72±43.2	**0.002**	1,547.73±87.5	0.566
Waist circumference (cm)	115.4±2.6	105.83±3.2	**0.001**	106.53±2.6	**<0.001**	106.2±2.7	**0.009**
Fat-free mass (kg)	60.45±1.5	56.44±2.14	**<0.001**	56.9±1.72	**<0.001**	57.8±1.9	**0.022**
BIA-Fat mass (kg)	39.2±2.04	34.7±2.03	**0.007**	34.2±1.9	**<0.001**	32.9±1.73	**0.001**
BIA-Fat percentage (%)	39.33±0.9	36.9±1.0	**0.002**	37.32±1.0	**0.004**	36.2±1.11	**0.012**
SBP (mmHg)	128.5±4.6	119.8±7.42	0.193	121.6±3.81	0.138	132.5±5.6	0.748
DBP (mmHg)	80.6±3.0	73.6±2.7	**0.043**	75.0±2.9	0.133	82.3±2.0	0.419

BW: Body weight; BMI: Body mass index; BMR: Basal metabolic rate; BIA: Bioimpedance analysis; SBP: Systolic blood pressure; DBP: Diastolic blood pressure.

analysis, the initial fat mass was 39.2 kg and decreased to 34.7 kg in three months (p=0.007). It was 34.2 kg in the sixth month and remained at 34.9 kg in the twelfth month (p=0.001 compared with the baseline value). Fat-free mass, which represents the muscle mass, decreased from 60.4 kg to 56.4 kg in three months (p<0.001). It was 56.9 kg in the sixth month (p<0.001) and 57.8 kg in the twelfth month (p=0.22 compared with the baseline value). An insignificant decrease was detected in the systolic and diastolic blood pressure in the sixth month compared with the initial values, and an insignificant increase was detected in the twelfth month (Table 2). Fasting blood glucose decreased from 99.83 mg/dL to 93.96 mg/dL in six months (p=0.027). At the end of the twelfth month, fasting blood glucose, fasting insulin, HbA1c, and 120-minute blood glucose level in OGTT did not change significantly compared with the baseline values. Total cholesterol, LDL, TG, free fatty acids, and uric acid also did not change significantly compared with the baseline values. HDL cholesterol increased from an initial level of 1.22 mmol/L to 1.5 mmol/L in twelve months (p=0.008). C-reactive protein and adiponectin levels did not change significantly at the end of the study compared with the beginning of the study (Table 3).

In the Atkins diet group, BW, BMI, and BMR decreased significantly in the sixth month compared with the baseline value (baseline/follow-up values: 104.83/94.6 kg, p=0.03; 39.7/35.8 kg/m², p=0.03; 1,713/1,587.5 kcal, p=0.04, respectively). In the twelfth month, BW increased to 99.4 kg (p=0.3 compared with the baseline value),and BMI increased to 38.9 kg/m² (p=0.29 compared with the baseline value) (Figure 1, Table 4). BMR increased to 1,690.5 kcal (p=0.33 compared with the baseline value). No significant changes were detected in the blood pH value throughout the diet (Table 4).

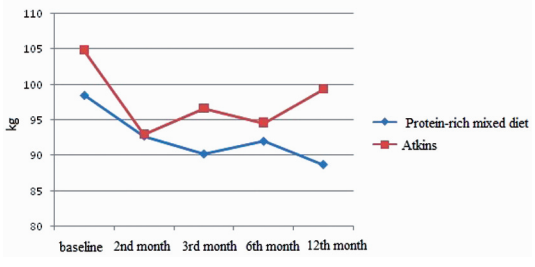

Figure 1: Weight change in Atkins and protein-rich mixed diet groups.

In the protein-rich mixed diet group, BW, BMI, and waist circumference values decreased significantly in the sixth month compared with the baseline values (baseline/follow-up values= 98.5/92 kg, p<0.001; 35.1/32.8 kg/m², p<0.001; 114.1/105.9 cm, p<0.001,respectively). The decrease in BW, BMI and waist circumference values continued in the twelfth month (88.7 kg; 31.5 kg/m²; 105.3 cm, p<0.005, p=0.007, p=0.036, respectively). The basal metabolic rate decreased from the baseline value of 1,695 kcal to 1,373 kcal in the sixth month (p=0.055) and increased back to 1516 kcal in the twelfth month (p=0.3 compared with the baseline value) (Table 5, Figure 1).

Table 3. Laboratory follow-up parameters of all patients.

	Baseline n=22	3rd month n=18	p	6th month n=18	p	12th month n=11	p
FBG (mg/dL)	99.83±3.03	92.5±6.1	**<0.001**	93.96±3.76	**0.027**	92.5±4.1	0.091
OGTT 120. min BS	132.2±11.3	126.2 ±9	0.39	125.14±13.7	0.413	130.2±10.2	0.09
HbA1c (%)	5.34±0.17	5.31±0.2	0.757	5.31±0.14	0.903	5.22±0.16	0.391
Insulin (mU/L)	15.2±2.6	15.7±2.9	0.8	14.7±2.6	0.510	15±2.9	0.9
HOMA-IR (kg/m^2)	3.9±0.76	3.7±0.66	0.09	3.5±0.7	0.098	3.8±0.56	0.7
Adiponectin (µg/mL)	5.1±0.6	5.9±0.8	0.06	6.1±0.73	0.057	6±0.8	0.06
M value (mg/kg/min)	2.9±0.4	3.2±0.4	0.09	3.35±0.32	0.210	3.2±0.22	0.15
Hemoglobin (g/dL)	13.54±0.34	14.6±0.42	**0.039**	13.44±0.31	0.759	13.31±0.4	0.744
Hematocrit (%)	40.2±0.96	44.13±1.2	0.14	40.4±0.90	0.476	38.4±1.03	0.359
Blood gas pH	7.44±0.006	7.43±0.07	0.323	7.42±0.01	**0.016**	7.42±0.01	0.163
TCHOL (mmol/L)	5.8±0.3	5.34±0.23	0.733	5.84±0.3	0.701	5.92±0.22	0.45
HDL (mmol/L)	1.22±0.07	1.5±0.11	**0.008**	1.3±0.07	0.114	1.7±0.11	**<0.001**
LDL(mmol/L)	3.5±0.2	3.3±0.2	0.187	3.51±0.24	0.669	3.54±0.2	0.604
TG (mmol/L)	1.8±0.18	1.3±0.95	**0.002**	1.74±0.25	0.878	1.62±0.21	0.974
FFA(mmol/L)	0.75±0.06	0.72±0.1	0.23	0.64±0.07	0.129	0.52±0.06	0.097
Urea (µmol/L)	270.23±16.7	287.72±13.2	0.202	287.94±15.8	0.319	263±17.9	0.080
Creatin (µmol/L)	81.41±2.75	87.3±4.03	0.086	76.2±3.5	0.070	84.44±4.21	**0.042**
Protein (g/L)	64.95±1.1	67.97±1.0	0.07	68.5±1.1	0.052	67.97±1.5	0.078
CRP (mg/L)	4.4±0.86	3.1±0.51	0.541	4.6±0.99	0.837	3.5±0.8	0.148
Urine albumin (mg/dL)	18.25±6.6	18.9±6.63	0.901	14.61±5.85	0.777	5.6±1.6	0.200

BMI: Body mass index; BIA: Bioimpedance analysis; BMR: Basal metabolic rate; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; FBG: Fasting blood glucose; HOMA IR: Homeostasis model assessmentinsulin resistance; OGTT: Oral glucose tolerance test; BS: Blood sugar; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; TG: Triglyceride; TCHOL: Total cholesterol; FFA: Free fatty acid.

Table 4. Metabolic follow-up values of the Atkins diet group.

	Baseline n=5	3rd month n=4	p	6th month n=4	p	12th month n=2	p
BW (kg)	104.83±3.8	96.6±7.3	**0.04**	94.6±3.6	**0.03**	99.4±1.6	0.3
BMI (kg/m^2)	39.7±2.2	37.7±2.3	**0.044**	35.8±1.8	**0.03**	38.9±0.05	0.29
BMR (kcal)	1713±47.52	1614±55.59	0.67	1587.5±55.94	**0.04**	1690.5±84.2	0.33
Waist circumference (cm)	115.8±3.8	111.5±2.5	**0.042**	109.5±3.82	0.092	109.5±6.5	0.65
Fat-free mass (kg)	63.03±2.04	56.44±2.14	0.09	58.0±3.63	**<0.001**	60.2±0.7	0.09
Blood gas pH	7.42±0.02	7.42±0.005	1.0	7.41±0.02	0.9	7.42±0.005	1.0

BW: Body weight; BMI: Body mass index; BMR: Basal metabolic rate.

Table 5. Metabolic follow-up values of the protein-rich mixed diet.

	Baseline n=17	3rd month n=13	p	6th month n=14	p	12th month n=9	p
BW (kg)	98.5±3.43	90.2±4.14	**<0.001**	92±3.4	**<0.001**	88.7±3.4	**<0.005**
BMI (kg/m^2)	35.1±1.5	31.7±1.7	**<0.001**	32.8±1.5	**<0.001**	31.5±1.2	**0.007**
BMR (kcal)	1,495.64±57.52	1,414.0±45.59	0.055	1,373.93±45.94	**0.005**	1,516±104.2	0.3
Waist circumference (cm)	114.1±3.1	104.7±3.8	**0.004**	105.9±3.08	**<0.001**	105.3±3.1	**0.036**
Fat-free mass (kg)	59.7±1.9	55.9±2.5	**0.001**	56.6±2.02	**0.002**	57.23±2.3	0.102
Blood gas pH	7.43±0.008	7.44±0.008	0.361	7.42±0.008	0.10	7.43±0.008	0.150

BW: Body weight; BMI: Body mass index; BMR: Basal metabolic rate.

Discussion

The incidence of obesity is increasing globally, and the associated comorbidities constitute major issues in each geographical area. In 2015, 107.7 million (98.7-118.4 million) children and 603.7 million (588.2-

619.8 million) adults were obese worldwide. The overall prevalence of obesity in children and adults was 5.0% and 12.0%, respectively (1,13). Despite the great variance among the countries, data indicate that the incidence of obesity has increased in the last thirty years in most of the populations (6). Large epidemiological studies have shown the association of obesity with diabetes mellitus, hypertension, dyslipidemia, heart disease, stroke, sleep apnea, cancer development, and increased mortality (14-16). Weight loss reduces obesity-associated morbidities and mortality (17).

When the patients in our study were analyzed, although there was a weight change of approximately 9-10% (Table 2), no changes were detected in inflammatory markers such as adiponectin and CRP. Fat may ectopically accumulate subcutaneously and in internal organs (liver, heart, pancreas, skeletal muscle). Ectopic fat accumulation leads to low-grade inflammation (18). In our study, ectopic fat accumulation could not be assessed. The absence of any changes in the inflammatory data in our study may be due to the fact that in patients, weight loss occurred largely in the subcutaneous tissue. However, there were statistically significant changes in waist circumference.This finding is probably due to the low number of cases included. Moreover, in previous studies, it was suggested that the variance in adiponectin, as well as other biological markers in response to weight loss, is not unimportant, and in order to obtain more significant results, either larger cohorts should be analyzed or more marked differences in weight should be ensured (19-22). The results presented in our study were obtained from a small group of eighteen people. Thus, statistically, more significant results can be expected if a higher number of patients are included in cohort studies.

Dietary change is the most important basis of obesity prevention and treatment. A balanced, moderate-fat, low-cholesterol, starchy, low-salt, fiber-rich, and moderate calorie-deficit dietary plan should ideally include three main meals and two snacks (23). Although Atkins diet is popular in social life, it has certain drawbacks. At the beginning of the dietary regimen, weight loss is rapid due to dehydration. This can cause the risk of vitamin, dietary fiber, and mineral deficiencies. This diet may also lead to increased purine intake and, consequently, increased cholesterol levels due to high-fat and high-salt nutrition. Increasing water intake is of vital importance in this diet. By this, kidneys can eliminate the generated ketone bodies and uric acid. In addition to the increased risk of kidney and liver diseases, this diet is associated with a high risk of atherosclerosis, cardiovascular diseases, and gout development. In the Atkins diet group, throughout our study, we did not observe any changes in the laboratory values that confirmed these concerns. This is probably because the patients attended group therapies regularly and were mandated to follow the necessary preventative measures.

One of the significant outcomes of our study was that although a significant change in weight occurred in the Atkins group in the sixth month compared with the baseline value, it was found that the patients gained weight in the twelfth month, and could not maintain the significant weight loss compared with the baseline value. However, in the protein-rich mixed diet group, it was found that weight loss continued during the last six months, and in the twelfth month, significant weight loss compared with the baseline values was attained. Our study has shown that a protein-rich mixed diet is a more sustainable weight loss program than the Atkins diet. Corroborating our findings, a meta-analysis of five studies has reported that the group that preferred a low-carbohydrate diet could not maintain the weight loss that occurred in the first six months into the twelfth month (24). In order to prevent long-term cardiovascular complications of obesity, weight loss programs must be sustainable.

Study Limitations

In our study, the dietary preference was left to the patients in order to increase the compliance of the participants. Since the initial design of the study was in this way, the groups were not evenly distributed. In addition, the withdrawal of participants from the study during the follow-up affected this irregularity further.

Conclusion

Multidisciplinary training programs in obesity are successful in ensuring and maintaining weight loss. Despite the successful weight loss, a slight change in metabolic parameters is observed. While weight loss ensures improvement in insulin resistance in the obese with metabolic syndrome, it does not do so in the obese without metabolic syndrome. Atkins diet and protein-rich mixed diet lead to similar rates of weight loss in the first six months, but a protein-rich mixed diet is more successful in the long-term maintenance of weight loss.

Data Availability

This study has been approved by Charite University's Medical Sciences Ethics Committee, and therefore performed in accordance with the ethical standards laid down by the 1964 Declaration of Helsinki and its later amendments.

Financially supporting

This research did not receive specific funding and was performed as part of the employment of the authors.

Informed consent

Informed consent of the participants was obtained.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Suzan Akpulat, Andreas Pfeiffer; Design: Suzan Akpulat, Andreas Pfeiffer; Control/Supervision: Nazlı Gülsoy Kırnap; Data Collection and/or Processing: Suzan Akpulat; Analysis and/or Interpretation: Andreas Pfeiffer; Literature Review: Nazlı Gülsoy Kırnap; Writing the Article: Nazlı Gülsoy Kırnap, Suzan Akpulat; Critical Review: Andreas Pfeiffer; References and Fundings: Nazlı Gülsoy Kırnap; Materials: Suzan Akpulat.

References

1. Afshin A, Forouzanfar MH, Sur P, Estep K, Lee A, et al. Health effects of overweight and obesity in 195 countries over 25 years. GBD 2015 Obesity Collaborators. N Engl J Med. 2017;377:13-27. [Crossref] [PubMed] [PMC]
2. Olshansky SJ, Passaro DJ, Hershow RC, Layden J, Carnes BA, Brody J, Hayflick L, Butler RN, Allison DB, Ludwing DS. A potential decline in life expectancy in the United States in the 21st century. N Engl J Med. 2005;17;352:1138-1145. [Crossref] [PubMed]
3. Vallis M. Quality of life and psychological well-being in obesity management: improving the odds of success by managing distress. Int J Clin Pract. 2016;70:196-205. [Crossref] [PubMed] [PMC]
4. Bray GA, Frühbeck G, Ryan DH, Wilding JPH. Management of obesity. Lancet. 2016;7;387:1947-1956. [Crossref] [PubMed]
5. Tsai AG, Wadden TA. In the clinic: obesity. Ann İntern Med. 2013;3;159:ITC3-1-ITC3-15; quiz ITC3-16. [Crossref] [PubMed]
6. Prospective Studies Collaboration; Whitlock G, Lewington S, Sherliker P, Clarke R, Emberson J, Halsey J, Qizilbash N, Collins R, Peto R. Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. Lancet. 2009;28;373:1083-1096. [Crossref] [PubMed]
7. Ryan D, Heaner M. Guidelines (2013) for managing overweight and obesity in adults. Preface to the full report. Obesity (Silver Spring). 2014;22 Suppl 2:S1-3. [Crossref] [PubMed]
8. Johnston BC, Kanters S, Bandayrel K, Wu P, Naji F, Siemieniuk RA, Ball GDC, Busse JW, Thorlund K, Guyatt G, Jansen JP, Mills EJ. Comparison of weight loss among named diet programs in overweight and obese adults: a meta-analysis. JAMA. 2014;3;312:923-933. [Crossref] [PubMed]
9. Hu T, Mills KT, Yao L, Demanelis K, Eloustaz M, Yancy WS Jr, Kelly TN, He J, Bazzano LA. Effects of low-carbohydrate diets versus low-fat diets on metabolic risk factors: a meta-analysis of randomized controlled clinical trials. Am J Epidemiol. 2012;1;176 Suppl 7:S44-54. [Crossref] [PubMed] [PMC]
10. Sacks FM, Bray GA, Carey VJ, Smith SR, Ryan DH, Anton SD, McManus K, Champagne CM, Bishop LM, Laranjo N, Leboff MS, Rood JC, de Jonge L, Greenway FL, Loria CM, Obarzanek E, Williamson DA. Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates. N Engl J Med. 2009;26;360:859-873. [Crossref] [PubMed] [PMC]
11. Gardner CD, Kiazand A, Alhassan S, Kim S, Stafford RS, Balise RR, Kraemer HC, King AC. Comparison of the Atkins, Zone, Ornish, and LEARN diets for change in weight and related risk factors among overweight premenopausal women: the A TO Z weight loss study: a randomized trial. JAMA. 2007;7;297:969-977. [Crossref] [PubMed]
12. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28:412-419. [Crossref] [PubMed]

13. NCD Risk Factor Collaboration (NCD-RisC). Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19.2 million participants. Lancet. 2016;387(10026):1377-1396. [Crossref]
14. McTigue K, Larson JC, Valoski A, Burke G, Kotchen J, Lewis CE, Stefanick ML, Horn LV, Kuller L. Mortality and cardiac and vascular outcomes in extremely obese women. JAMA. 2006;5;296:79-86. [Crossref] [PubMed]
15. Yan LL, Daviglus ML, Liu K, Stamler J, Wang R, Pirzada A, Garside DB, Dyer AR, Horn LV, Liao Y, Fries JF, Greenland P. Midlife body mass index and hospitalization and mortality in older age. JAMA. 2006;11;295:190-198. [Crossref] [PubMed]
16. Adams KF, Schatzkin A, Harris TB, Kipnis V, Mouw T, Ballard-Barbash R, Hollenbeck A, Leitzmann MF. Overweight, obesity, and mortality in a large prospective cohort of persons 50 to 71 years old. N Engl J Med. 2006;24;355:763-778. [Crossref] [PubMed]
17. Rueda-Clausen CF, Ogunleye AA, Sharma AM. Health benefits of long-term weight-loss maintenance. Annu Rev Nutr. 2015;35:475-516. [Crossref] [PubMed]
18. Schwartz MW, Seeley RJ, Zeltser LM, Drewnowski A, Ravussin E, Redman LM, Leibel RL. Obesity Pathogenesis: an endocrine society scientific statement. Endocr Rev. 2017;1;38:267-296. [Crossref] [PubMed] [PMC]
19. Yang WS, Lee WJ, Funahashi T, Tanaka S, Matsuzawa Y, Chao CL, Chen CL, Tai TY, Chuang LM. Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. J Clin Endocrinol Metab. 2001;86:3815-3819. [Crossref] [PubMed]
20. Ryan AS, Berman DM, Nicklas BJ, Elahi D. Adiponectin levels do not change with moderate dietary induced weight loss and exercise in obese postmenopausal women. Int J Obesity. 2003;27:1066-1071. [Crossref] [PubMed]
21. Monzillo LU, Hamdy O, Horton ES, Ledbury S, Mullooly C, Jarema C, Porter S, Ovalle K, Moussa A, Mantzoros CS. Effect of lifestyle modification on adipokine levels in obese subjects with insulin resistance. Obes Res. 2003;11:1048-1054. [Crossref] [PubMed]
22. Baratta R, Amato S, Degano C, Farina MG, Patane G, Vigneri R, Frittitta L. Adiponectin relationship with lipid metabolism is independent of body fat mass: evidence from both cross-sectional and intervention studies. J Clin Endocrinol Metab. 2004;89:2665-2671. [Crossref] [PubMed]
23. Esposito K, Giugliano D. Mediterranean diet and the metabolic syndrome: the end of the beginning. Metab Syndr Relat Disord. 2010;8:197-200. [Crossref] [PubMed]
24. Nordmann AJ, Nordmann A, Briel M, Keller U, Yancy WS, Jr., Brehm BJ, Bucher HC. Effects of low-carbohydrate vs low-fat diets on weight loss and cardiovascular risk factors: a meta-analysis of randomized controlled trials. Arch Intern Med. 2006;13;166:285-293. [Crossref] [PubMed]

Original Article
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The Effect of Falsely Highlighted Intestinal Intraluminal Areas and the Fat in Paraspinal Muscles on Abdominal Adipose Tissue Measurements Using Computed Tomography

İntestinal Lümen İçerisinde Hatalı Olarak Boyanan Alanların ve Paraspinal Kaslar İçerisindeki Yağ Alanlarının Bilgisayarlı Tomografi ile Abdominal Yağ Ölçümü Üzerindeki Etkisi

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Abstract

Objective: The measurement of abdominal fat using computed tomography (CT) is a reliable method for evaluating metabolic disorders. However, some limitations exist with the current CT measurement methods. One of them is falsely highlighted intestinal intraluminal areas and the other one is fat in paraspinal muscles. We aimed to investigate the effects of highlighted intestinal intraluminal areas and fat in paraspinal muscles on the measured values of abdominal fat. **Material and Methods:** Measurements were performed on 246 abdominal CT scans of 129 patients using dedicated quantitative CT software. Visceral and subcutaneous fats were measured at the level of L1-L2 disc space using two different methods. Method 1 included the highlighted intestinal intraluminal areas and fat in paraspinal muscles for measurements, whereas method 2 excluded them. The values measured using two methods were compared for a statistically significant difference. In addition, the correlation between anthropometric data and subcutaneous adipose tissue measurement methods was analyzed. **Results:** The mean age of patients was 53 years, and the mean body mass index was 29.73 kg/m^2. The waist circumference data were available of 91 patients, and the mean waist circumference was 94 cm. The Wilcoxon signed-rank sum test showed a statistically significant difference between methods 1 and 2 ($p<0.0001$). Although the measurements performed using methods 1 and 2 were strongly correlated ($r>0.9$), the Passing-Bablok regression analysis indicated a systematic and proportional error between measurements. **Conclusion:** Falsely highlighted intestinal intraluminal areas should be excluded for accurate visceral adipose tissue measurements, and the fat in paraspinal muscles affects subcutaneous fat measurement results.

Keywords: Multidetector computed tomography; visceral fat; subcutaneous fat; abdominal adipose tissue

Özet

Amaç: Bilgisayarlı tomografi (BT) ile abdominal yağ ölçümü, metabolik hastalık değerlendirmesi açısından oldukça güvenilir bir metottur. Mevcut BT ölçüm metotlarının bazı kısıtlılıkları vardır. Bu kısıtlılıklardan biri, intestinal lümen içerisinde yanıltıcı olarak boyanan alanlardır. Bir diğeri ise paraspinal kaslar içerisindeki yağ doku alanlarıdır. Çalışmamızın amacı, intestinal lümen içerisinde yanıltıcı olarak boyanan alanların ve paraspinal kaslar arasındaki yağ alanlarının abdominal yağ ölçümleri üzerindeki etkisinin araştırılmasıdır. **Gereç ve Yöntemler:** Yağ ölçümüne yönelik geliştirilmiş kantitatif BT yazılımı kullanılarak 129 hastanın 246 abdomen BT tetkikinde yağ ölçümleri gerçekleştirilmiştir. Viseral ve subkütan yağ ölçümleri, L1-L2 disk düzeyinden iki farklı yöntem kullanılarak gerçekleştirilmiştir. Metot 1'de, intestinal lümen içerisinde yanıltıcı olarak boyanan alanlar ve paraspinal kaslar arasındaki yağ alanları, abdominal yağ ölçümüne dâhil edilmiş, Metot 2'de ise edilmemiştir. İki metot ile ölçülen değerler istatistiksel anlamlı fark açısından karşılaştırılmıştır. Ayrıca antropometrik ölçümler ile subkütanöz yağ dokusu ölçüm metodu arasındaki korelasyon da değerlendirilmiştir. **Bulgular:** Ortalama hasta yaşı 53 yıl ve ortalama beden kitle indeksi 29,73 kg/m^2 olarak bulunmuştur. Bel çevresi ölçümleri 91 hastada mevcut olup, ortalama bel çevresi 94 cm'dir. Wilcoxon işaretli sıralar testi uygulandığında, Metot 1 ve Metot 2 arasında istatistiksel olarak anlamlı fark saptanmıştır ($p<0,0001$). İki metot ölçümleri arasında güçlü bir korelasyon bulunmakla birlikte ($r>0,9$), Passing-Bablok analizi ile iki metot arasında sistematik ve orantılı hata saptanmıştır. **Sonuç:** İntestinal lümen içerisinde yanıltıcı olarak boyanan alanlar viseral yağ ölçümü uygulanırken çıkartılmalıdır. Paraspinal kaslar içerisindeki yağ ise subkütan yağ ölçüm sonuçlarını etkileyecek düzeydedir.

Anahtar kelimeler: Multidedektör bilgisayarlı tomografi; viseral yağ; subkütanöz yağ; abdominal yağ dokusu

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Introduction

Body fat is distributed into two main compartments: subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT). These two tissue compartments have different metabolic characteristics. The VAT is of particular interest because of its unique biochemical characteristics that influence several normal and pathological processes. Moreover, it is associated with metabolic syndrome, cardiovascular disease, and several malignancies, including prostate, breast, and colorectal cancers (1-5). The quantitative assessment of VAT is crucial in evaluating the risk of developing these pathologic conditions and in providing an accurate prognosis (1).

Computed tomography (CT) is a useful and validated technique to quantitatively assess VAT and SAT (1,2,6-8). Compared with other techniques that evaluate VAT and SAT, CT generates the most accurate, specific, and comprehensive data (1).

However, the current CT quantification technique has its limitations and pitfalls, some of which are not yet addressed in the present literature. In CT, some areas within the intestinal lumen are falsely highlighted because of predefined Hounsfield unit (HU) ranges for fat measurement. These falsely highlighted areas are generally fecal material, liquid with intermediate density (bowel content), or an air-fluid interface with average density (9). To the best of our knowledge, only three studies exist in the literature that excluded falsely highlighted intestinal intraluminal areas (FHIIAs) while performing VAT measurement (9-11). However, neither these three studies nor the others have focused on VAT and addressed the effect of FHIIAs on measurements. In contrast, the majority of the studies measuring VAT did not exclude FHIIAs (2,7,8,12-15). Therefore, whether to include or exclude FHIIAs while performing VAT measurements is unclear. A similar confusion also exists with the fat within paraspinal muscles (FPM) while performing SAT measurements, as some of the studies in the literature include FPM, and some exclude them (7-11). The fatty infiltration of the FPM, secondary to muscle atrophy, obesity, or other reasons, is another drawback of the current CT quantification technique. The confusion about whether to include or exclude FPM in the SAT measurement method can be associated with this drawback.

However, whether these FHIIAs and FPM affect VAT and SAT measurements, respectively, remain unknown. The aim of this study was to investigate the effect of FHIIAs and FPM on VAT and SAT measurements, respectively.

Material and Methods

Study Participants

This retrospective study was approved by the institutional review board, and no informed consent was needed (Dokuz Eylül University Non-Interventional Research Ethics Committee, file no: 2017/19-13, date 27.07.2017). This study was conducted in accordance with the Helsinki Declaration. We included patients admitted to our university hospital between 2008 and 2017 who were evaluated using CT for the routine workup of their incidental adrenal lesions. Patients with malignancy were excluded from this study.

A flowchart of patient selection details is given in Figure 1. Given the alteration of bowel segment position and contents over time, more than one CT scans of any single patient were included. The VAT measurements were performed on 246 CT scans of 129 patients. In 11 scans, an accurate SAT measurement was excluded due to because of the inability to evaluate peripheral abdominal subcutaneous fat because of the limited visualization of these parts in the CT scans performed with an inadequate field of view. Therefore, the statistical analysis of SAT measurements was performed for 235 scans, excluding the 11 scans.

Fat Measurement Method

The VAT and SAT measurements were performed by two experienced radiologists. An axial view was used, with the patient lying in the supine position. Measurements were performed on a Philips Extended Brilliance Workspace (software version V3.5.0.22.54 Philips Medical Systems, Amsterdam, The Netherlands) using the quantitative CT software (Philips Medical Systems, Amsterdam, The Netherlands). The L1-L2 intervertebral disk level was selected for performing fat

measurements as described in the literature, indicating that VAT measurements at this level were more strongly associated with the metabolic syndrome than at other sites (16). A region of interest (ROI) of 100-200 mm^2 was drawn on pararenal visceral fat to determine the reference HU values for fat area measurements. An attenuation range of -60 to -165 HU in pararenal reference ROIs was achieved in line with the literature (2,3,7). Using this ROI as a fat density reference, the software automatically provided fat tissue mask values and fat area values.

The total adipose tissue (TAT) area was measured by drawing a contour around the skin on a CT scan image. To determine VAT, another contour was drawn around the visceral fat by identifying the innermost abdominal wall muscles and the anterior aspect of the vertebral column. The subcutaneous fat was defined as the area of adipose tissue between the skin and the outermost aspect of the abdominal wall muscles. These borders were defined in line with the literature (17). The SAT area was calculated by subtracting the VAT area from the TAT area. To account for FHIIAs in each VAT measurement (so as to exclude it from the total VAT measurement), ROIs were drawn around the intestinal lumen by identifying the innermost intestinal wall (Figure 2A). Using the same method, an additional ROI was drawn around the FPM to calculate FPM, so that it can be excluded from the total SAT measurement in the slice (Figure 2B).

Two measurement methods were used to evaluate the effect of FHIIAs and FPM on VAT and SAT, respectively. Method 1 included FHIIAs in VAT measurements and FPM in SAT measurements, whereas method 2 excluded them. The results of the two methods were statistically compared. In addition, Pearson's correlation test was performed to evaluate whether adding or removing FPM from SAT measurement was more correlated with clinical data (body mass index (BMI) and waist circumference).

Statistical Analysis

The differences between the results of the two methods (methods 1 and 2) were tested using the Passing-Bablok regression analysis using MedCalc software (MedCalc software Ltd.,Ostend, Belgium) and SPSS version 15.0 (SPSS Inc, Chicago, Illinois, USA). The correlation between the results of the two measurement methods was analyzed using SPSS version 15.0. The normality test (Kolmogorov-Smirnov) was performed to evaluate the normal distribution. The Wilcoxon signed-rank sum test and the Spearman's correlation test were used.

Intraobserver and interobserver variability between the two investigators were tested. To assess interobserver variability, additional 74 consecutive CT scans of another patient group were evaluated by both radiologists, and to assess intraobserver variability, each investigator performed fat area measurements in 18 consecutive CT scans six months apart.

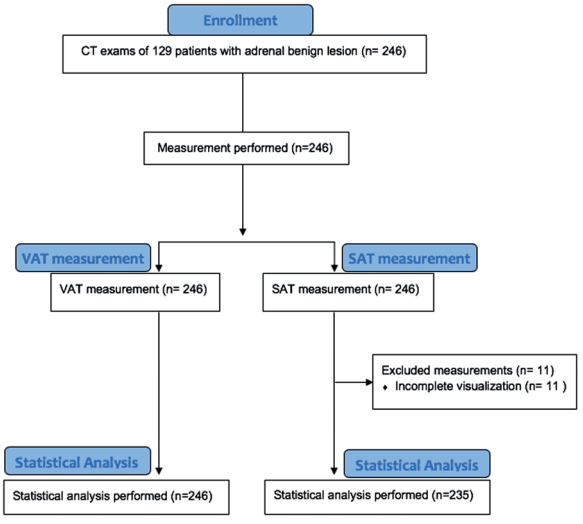

Figure 1: Patient enrollment, fat measurement, and statistical analysis flow can be reviewed in the flowchart.

CT: Computed tomography, VAT: Visceral adipose tissue, SAT: Subcutaneous adipose tissue.

Results

A total of 246 abdominal CT scans of 129 patients (99 women and 30 men) were statistically evaluated. The mean patient age was 53 years. The youngest patient aged 24 years and oldest 76 years. BMI data were available of 115 patients. Mean BMI was 29.73 kg/m^2 (minimum 17.48 and maximum 51.9). Waist circumference data were obtained for 91 patients. Mean waist circumference was 94 cm (minimum 64 cm and maximum 140 cm).

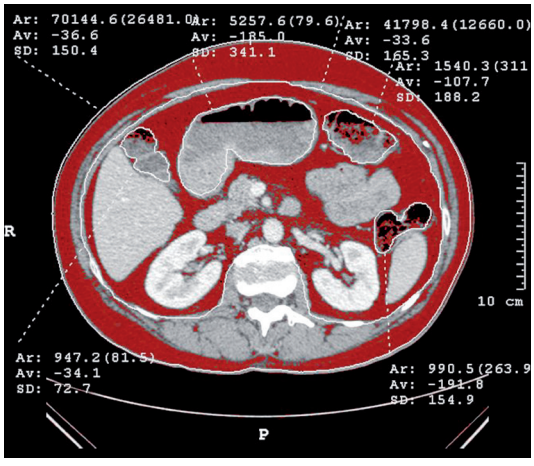

Figure 2A: VAT measurement; in this CT image, measurements of total adipose tissue area (TAT=264.81 cm^2), visceral adipose tissue area (VAT=126.60 cm^2) and falsely highlighted intestinal intraluminal areas (FHIIAs=0.81 cm^2, 0.79 cm^2, 3.11 cm^2, 2.63 cm^2) are shown. "Ar" means area inside of the drawn ROI. The numbers inside the bracket show the area measurement in pixels with fat density inside the selected ROI.
VAT: Visceral adipose tissue, TAT: Total adipose tissue, FHIIAs: Falsely highlighted intestinal intraluminal areas, ROI: Region of interest.

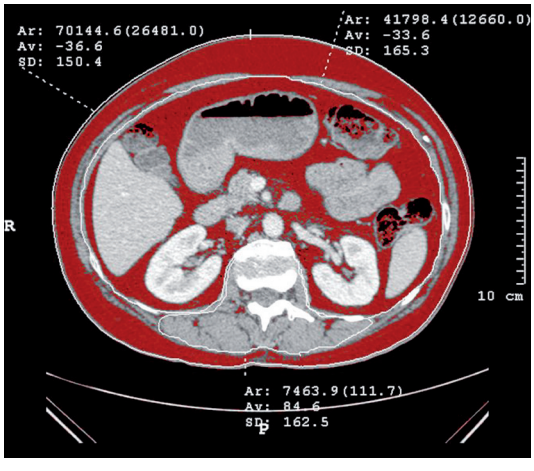

Figure 2B: SAT measurement; measurements of total adipose tissue area (TAT=264.81 cm^2), visceral adipose tissue area (VAT=126.60 cm^2) and fat in paraspinal muscles (FPM=1.11 cm^2) are shown. "Ar" means area inside the drawn ROI. The numbers inside the brackets show the area measurement in pixels with fat density inside the selected ROI.
SAT: Subcutaneous adipose tissue, TAT: Total adipose tissue, FPM: Fat in paraspinal muscles, ROI: Region of interest.

Moreover, 118 patients had two CT scans at different times, and only 11 had one CT scan. In three scans, FHIIA measurements were "zero."

The results of fat measurements are summarized in Table 1.

As the normality test did not show a normal distribution, the Wilcoxon signed-rank sum test was used, which showed a statistically significant difference between the two methods for VAT ($p<0.0001$) and SAT measurements ($p<0.0001$).

Although the results of methods 1 and 2 are strongly correlated ($r>0.9$), the Passing-Bablok regression analysis showed a systematic and proportional error between measurements; the 95% confidence interval (CI) for intercept A does not contain 0 and that for slope B does not contain 1 in the regression model (http://www.medcalc.org/manual/passing-bablok_regression.php). The Passing-Bablok regression analysis indicated that these two methods could not be substituted for each other.

A strong intraobserver and interobserver agreement was detected; the 95% CI for intercept A contains 0 and that for slope B contains 1 in all regression models. The regression coefficient was >0.9 in all regression models.

Pearson's correlation test performed to test whether adding or removing FPM in SAT measurement was more correlated with clinical data (BMI and waist circumference) showed similar results. Both the measurement methods were highly correlated with BMI (Method 1: $r=0.789$; Method 2: $r=0.787$) and waist circumference (Method 1: $r=0.682$; Method 2: $r=0.682$).

Discussion

CT is a validated technique for fat measurement (2). Shuster et al. have stated that the evaluation of the abdominal fat using CT generates the most accurate, specific, and comprehensive data than other techniques (1). However, CT has its own limitations because of predefined HU ranges for fat density mask, as seen in falsely highlighted areas of the intestinal lumen. Our study results demonstrated a statistically significant difference in VAT values depending upon the inclusion or exclusion of FHIIAs in the measurements. The minimum FHIIA was 0.03 cm^2, and maximum was 23.09 cm^2, indicating that the FHIIA measurement varies because of the density of intraluminal contents. The FHIIAs are very small com-

Table 1. Results of fat measurement.

		Min. (cm^2)/Max (cm^2)	Mean (cm^2)±sd;	Median (cm^2)/(25^{th}-75^{th})	p
VAT (n=246)	Method 1	7.84/551.37	130.60±90.04	112.13/(65.05-179.15)	p<0.0001
	Method 2	6.38/536.91	127.81±88.72	110.68/(62.79-175.60)	
SAT (n=235)	Method 1	21.30/560.88	171.54±95.82	150.84/(103.37-224.87)	p<0.0001
	Method 2	20.81/556.72	169.37±95.02	150.14/(99.84-223.14)	
FHIIA (n=246)		0.00/23.09	2.78±3.64	1.45/(0.67-3.19)	
FPM (n=235)		0.01/34.32	2.16±3.36	1.18/(0.61-2.53)	

FHIIA: Falsely highlighted intestinal intraluminal areas, FPM: Fat in paraspinal muscles, VAT: Visceral adipose tissue, SAT: Subcutaneous adipose tissue.

pared to VAT. However, if a larger number of patients are involved, the variability of FHIIA values widens, creating an overall inaccurate VAT measurement.

It is unclear whether adding or removing FHIIAs in VAT measurement was more correlated with clinical data. However, FHIIAs are definitely not a part of VAT; it is a misregistered area produced by fecal material, liquid with intermediate density, or an interface of air, fluid, and bowel content with average density (9). Although adding FHIIAs to VAT was found to be more correlated with clinical data, adding a misregistered area to a fat tissue measurement would be a methodological mistake. We can question whether is it really necessary to remove FHIIAs from VAT because it is a small amount of misregistered data. The answer to this question provided by this study was that it is necessary to remove FHIIA as it causes a statistically significant difference. Thus, FHIIAs should be excluded when calculating VAT regardless of its correlation with clinical data.

To the best of our knowledge, a few studies in the literature have excluded FHIIAs while measuring VAT (9-11). One of them was Hung et al.'s study, where they manually excluded FHIIAs to measure VAT (9). Moreover, Delivanis et al. have used semiautomated software to exclude FHIIAs to measure VAT (10). Lastly and recently, Akay et al. have used an oral contrast agent to prevent any intestinal involvement in the automated VAT measurement process and also excluded the remnant intestinal involvement manually, if any (11). In contrast, a majority of studies measuring VAT have not excluded FHIIAs (2,7,8-15). This methodological approach indicates that the effect of FHIIAs on the abdominal fat measurement is an underestimated problem in the literature, thus enhancing the importance of this study. This study was the first to show that FHIIAs may affect VAT measurement and highlight a problem that the majority of the studies missed.

Similarly, several previous studies have included FPM in SAT measurement (7,8). However, Hung et al., Delivanis et al., and Akay et al. have excluded FPM in their studies (9-11). In this study, a statistically significant difference was obtained between including and excluding FPM in SAT measurements. Although Pearson's correlation test showed that both the measurement methods for SAT measurement were correlated with clinical data at the same degree, the Passing-Bablok regression analysis indicated that these two methods could not be substituted for each other. Therefore, studies with larger patient groups are needed to reveal which measurement method is more correlated with the clinical data.

One minor limitation of this study was performing two-dimensional fat measurements on a single slice selected at a specific level rather than using three-dimensional measurements as the volume was measured across several slices. However, several studies have revealed that measuring VAT on a single CT or magnetic resonance imaging slice is a reliable method (2,8,11,15,16,18-20). Given the limitation of obtaining two-dimensional measurements in a three-dimensional patient, the validity of this method has been previously established by comparing the two-dimensional and three-dimensional VAT measurements in a different patient population (21). The association between the adipose tissue area and

the corresponding adipose tissue volume was significant (r=0.961; p<0.001) (21).
The main limitation of this study was the lack of data indicating that which the SAT measurement method is more consistent with anthropometric measurements and more accurately reflects metabolic disorders. However, this study was designed to highlight the drawbacks of measuring fat using CT and determine whether FHIIAs and FPM have any statistically significant effect on VAT and SAT measurements; therefore, the correlation with anthropometric SAT measurements was not studied. In addition, including FHIIAs in VAT measurement would be a methodological mistake because of its nonadipose origin, and its correlation with anthropometric measurements would make no sense. Moreover, this study is significant because it showed that measurements with methods 1 and 2 were different, and the methods cannot be substituted for each other.

Conclusion

In conclusion, this study was the first to demonstrate that including FHIIAs in VAT measurements and FPM in SAT measurements led to statistically significant differences in VAT and SAT measurements. FHIIAs should be excluded for accurate VAT measurements. FPM inclusion in SAT measurements should be considered only if supported by anthropometric measurements.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Mustafa M. Barış, Ahmet Peker; Design: Mustafa M. Barış, Ahmet Peker, Mustafa Seçil; Control/Supervision: Mustafa M. Barış, Ahmet Peker, Mustafa Seçil; Data Collection and/or Processing: Mustafa M. Barış, Ahmet Peker, Abdullah S. Yener; Analysis and/or Interpretation: Naciye S. Gezer, Mustafa Seçil, Abdullah S. Yener; Literature Review: Mustafa M. Barış, Naciye S. Gezer; Writing the Article: Mustafa M. Barış, Naciye S. Gezer; Critical Review: Mustafa Seçil, Abdullah S. Yener, Naciye S. Gezer; Materials: Abdullah S. Yener.

References

1. Shuster A, Patlas M, Pinthus JH, Mourstzakis M. The Clinical importance of visceral adiposity: a critical review of methods for visceral adipose tissue analysis. Br J Radiol. 2012;85:1-10. [Crossref] [PubMed] [PMC]
2. Yoshizumi T, Nakamura T, Yamane M, Islam AHMW, Menju M, Yamasaki K, Arai T, Kotani K, Funahashi T, Yamashita S, Matsuzawa Y. Abdominal fat: standardized technique for measurement at CT. Radiology. 1999;211:283-286. [Crossref] [PubMed]
3. Abraham TM, Pedley A, Massaro JM, Hoffmann U, Fox CS. Association between visceral and subcutaneous adipose depots and incident cardiovascular disease risk factors. Circulation 2015;132:1639-1647. [Crossref] [PubMed] [PMC]
4. Nicklas BJ, Penninx BWJH, Cesari M, Kritchevsky SB, Newman AB, Kanaya AM, Pahor M, Jingzhong D, Harris TB, Health, Aging and Body Composition Study. Association of visceral adipose tissue with incident myocardial infarction in older men and women: the health, aging and body composition study. Am J Epidemiol. 2004;160:741-749. [Crossref] [PubMed]
5. Kuk JL, Katzmarzyk PT, Nichaman MZ, Church TS, Blair SN, Ross R. Visceral fat is an independent predictor of all-cause mortality in men. Obesity (Silver Spring) 2006;14:336-341. [Crossref] [PubMed]
6. Greenfield JR, Samaras K, Chisholm DJ, Campbell LV. Regional intra-subject variability in abdominal adiposity limits usefulness of computed tomography. Obes Res. 2002;10:260-265. [Crossref] [PubMed]
7. Debono M, Prema A, Hughes TJB, Bull M, Ross RJ, Newell-Price J. Visceral fat accumulation and post-dexamethasone serum cortisol levels in patients with adrenal incidentaloma. J Clin Endocrinol Metab. 2013, 98:2383-2391. [Crossref] [PubMed]
8. Mazonakis M, Damilakis J. Computed tomography: what and how does it measure? Eur J Radiol. 2016;85:1499-1504. [Crossref] [PubMed]

9. Hung CS, Lee JK, Yang CY, Hsieh HR, Ma WY, Lin MS, Liu PH, Shih SR, Liou JM, Chuang LM, Chen MF, Lin JW, Wei JN, Li HY. Measurement of visceral fat: should we include retroperitoneal fat? PLoS One. 2014; 9:e112355. [Crossref] [PubMed] [PMC]
10. Delivanis DA, Iñguez-Ariza NM, Zeb MH, Moynagh MR, Takahashi N, McKenzie TJ, Thomas MA, Gogos C, Young WF, Bancos I, Kyriazopoulou V. Impact of hypercortisolism on skeletal muscle mass and adipose tissue mass in patients with adrenal adenomas. Clinical Endocrinology (Oxf). 2018;88: 209-216. [Crossref] [PubMed]
11. Akay S, Urkan, M, Balyemez U, Erşen M, Taşar M. Is visceral obesity associated with colorectal cancer? The first volumetric study using all CT slices. Diagn Interv Radiol. 2019;25:338-345. [Crossref] [PubMed] [PMC]
12. Sottier D, Petit JM, Guiu S, Hamza S, Benhamiche H, Hillon P, Cercueil JP, Krausé D, Guiu B. Quantification of the visceral and subcutaneous fat by computed tomography: interobserver correlation of a single slice technique. Diagn Interv Imaging. 2013;94:879-884. [Crossref] [PubMed]
13. Maurovich-Horvat P, Massaro J, Fox CS, Moselewski F, O'Donnell CJ, Hoffmann U. Comparison of anthropometric, area- and volume-based assessment of abdominal subcutaneous and visceral adipose tissue volumes using multi-detector computed tomography. Int J Obes (Lond). 2007;31:500-506. [Crossref] [PubMed]
14. Yener S, Baris M, Secil M, Akinci B, Comlekci A, Yesil S. Is there an association between non-functioning adrenal adenoma and endothelial dysfunction? J Endocrinol Invest. 2011;34:265-270. [Crossref] [PubMed]
15. Rosenquist KJ, Pedley A, Massaro JM, Therkelsen KE, Murabito JM, Hoffman U, Fox CS. Visceral and subcutaneous fat quality and cardiometabolic risk. JACC Cardiovasc Imaging logy. 2013;6:762-771. [Crossref] [PubMed] [PMC]
16. Kuk JL, Church TS, Blais SN, Ross R. Does measurement site for visceral and abdominal subcutaneous adipose tissue alter associations with the metabolic syndrome? Diabetes Care. 2006; 29:679-684. [Crossref] [PubMed]
17. Kuk JL, Nichaman MZ, Church TS, Blair SN, Ross R. Liver fat is not a marker of metabolic risk in lean premenopausal women. Metabolism. 2004;53: 1066-1071. [Crossref] [PubMed]
18. Sjöstrom L, Kvist H, Cederblad A, Tylén U. Determination of total adipose tissue and body fat in women by computed tomography, 40K, and tritium. Am J Physiol. 1986; 250:E736-745. [Crossref] [PubMed]
19. Kvist H, Chowdhury B, Sjöström L, Tylén U, Cederblad A. Adipose tissue volüme determination in males by computed tomography and 40K. Int J Obes. 1988;12:249-266. [PubMed]
20. Shen W, Punyanitya M, Wang Z, Gallagher D, St-Onge MP, Albu J, Heymsfield SB, Heshka S. Visceral adipose tissue: relations between single-slice areas and total volume. Am J Clin Nutr. 2004;80: 271-278. [Crossref] [PubMed] [PMC]
21. Yener S, Baris M, Peker A, Demir O, Ozgen B, Secil M. Autonomous cortisol secretion in adrenal incidentalomas and increased visceral fat accumulation during follow-up. Clin Endocrinol (Oxf). 2017;87: 425-432. [Crossref] [PubMed]

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Factors Affecting Survival in Adrenocortical Cancers: Single-Center Experience

Adrenokortikal Kanserlerde Sağ Kalıma Etki Eden Faktörler: Tek Merkez Deneyimi

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Abstract

Objective: Adrenocortical cancer (ACC) is one of the rare endocrine system cancers that are aggressive. Despite surgical treatment, the mortality rate is quite high. This study aimed to examine prognostic factors affecting survival in patients with ACC, the role of dehydroepiandrosterone sulfate (DHEA-S), and the maximum standard uptake (SUV_{max}) values on predicting mortality through the single-center data. **Material and Methods:** A total of 21 patients who were diagnosed with adrenal cancer and followed from a single center were included in the study. Patients who survived follow-ups were included in the survived group (n=6), and those who died were included in the dead group (n=15). The demographic, anatomical, pathological, and clinical characteristics of the patients were analyzed. Positron emission tomography-computerized tomography imaging and SUV_{max} values of adrenal masses were compared. The effect of all these data on survival was examined. **Results:** The mortality rate among patients with ACC was 71%. According to the Kaplan-Meier survival analysis, the average life expectancy was 23.66±2.79 (95% CI=18.18-29.13) months. The mass size of the survived and dead groups was 9.2±3.82 cm and 10.84±4.74 cm, respectively. The production rate of adrenal hormone was higher in the dead group ($p<0.01$; 80%). Moreover, the DHEA-S level and SUV_{max} values were statistically significantly higher in the dead group ($p<0.001$; $p<0.05$, respectively). Although no metastases were observed in the survived group during follow-ups after the operation, distant metastases were observed in 8 people from the dead group (53%; $p<0.05$). **Conclusion:** The mortality rate was very high in ACC despite surgical and medical treatments. The higher DHEA-S and SUV_{max} values may indicate that the overall survival duration was low.

Keywords: Adrenocortical cancer; mortality; DHEA-S; positron emission tomography

Özet

Amaç: Adrenokortikal kanser [adrenocortical cancer (ACC)], agresif seyreden nadir görülen endokrin sistem kanserlerindendir. Cerrahi tedaviye rağmen mortalitesi oldukça yüksektir. Bu çalışmada, ACC hastalarda sağ kalıma etki eden prognostik faktörleri dehidroepiandrosteron sülfat (DHEA-S) ve maksimum standart uptake (SUV_{max}) değerlerinin mortaliteyi ön görmedeki rolünü, tek merkez verileri üzerinden araştırmayı amaçladık. **Gereç ve Yöntemler:** Adrenal kanser tanısı almış tek merkezden takipli 21 hasta çalışmaya alındı. Takiplerde hayatta olanlar sağ olan grup (n=6), ölenler ölü olan grup (n=15) olarak kabul edildi. Hastaların demografik, anatomik, patolojik ve klinik özelliklerine bakıldı. Adrenal kitlelerin, pozitron emisyon tomografi görüntülemesi ile SUV_{max} değerleri karşılaştırıldı.Tüm bu verilerin sağkalım üzerine etkileri araştırıldı. **Bulgular:** ACC'li hastalarda mortalite oranı %71 olarak bulundu. Kaplan-Meier yaşam analizine göre ortalama yaşam süresi 23,66±2,79 (%95 GA=18,18-29,13) ay olarak bulundu. Sırasıyla, sağ olan ve ölü olan grubun kitle boyutu 9,2±3,82 cm ve 10,84±4,74 cm olarak bulundu. Adrenal hormon üretimi, ölü olan grupta daha yüksek oranda izlendi ($p<0,01$; %80). Ayrıca DHEA-S seviyesi ve SUV_{max} değeri ölü olan grupta istatistiksel olarak yüksek bulundu (sırasıyla $p<0,001$; $p<0,05$). Operasyon sonrası takiplerde sağ olan grupta hiç metastaz yokken, ölü olan grupta 8 kişide uzak metastaz saptandı (%53; $p<0,05$). **Sonuç:** ACC'de mortalite oranı, cerrahi ve medikal tedavilere rağmen oldukça yüksektir. DHEA-S seviyesinin ve SUV_{max} değerinin yüksek olması, genel sağkalım süresinin düşük olduğunu öngörebilir.

Anahtar kelimeler: Adrenokortikal kanser; mortalite; DHEA-S; pozitron emisyon tomografi

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Introduction

Together with the widespread use of radiological imaging in clinics, the possibility of imaging an adrenal mass incidentally began to increase. Moreover, most of these adrenal masses are benign. Despite its rare occurrence, they are sometimes found to be adrenocortical cancer (ACC).

ACC, situated on the adrenal cortex, progresses aggressively with an average annual incidence of 1-2/1,000,000 (1). It is the second most aggressive malignant cancer of the endocrine system after anaplastic thyroid cancer (2). It can occur at any age, even in early childhood and the fourth and fifth decades of life.

ACC can be active or inactive hormonally. It can secrete corticosteroids, mineralocorticoids, or androgens from steroid hormones in 50%-70% of cases. More than half of the active hormone-producing ACCs lead to Cushing syndrome (3). A high level of dehydroepiandrosterone sulfate (DHEA-S), a marker of adrenal androgen release in the evaluation of the adrenal masses detected incidentally, suggests adrenocortical carcinoma (4). However, the DHEA-S level was lower in benign adrenal masses. Although the DHEA-S level is considered an indicator for ACC, its role in predicting the mortality rate has not yet been studied in the literature.

Computerized tomography (CT) and magnetic resonance imaging (MRI) detect changes in adrenal masses after the mass grows and becomes visible. Metabolic changes occur prior to canceration in the adrenal gland before the adrenal mass is formed. 18-Fluorodeoxyglucose (FDG) positron emission tomography (PET) shows these metabolic changes early and provide an early diagnosis (5).

As ACC is a rare endocrine tumor, researchers have not yet studied its clinical characteristics and prognostic factors affecting survival. This study aimed to examine demographic and clinical features of the ACC, factors affecting the prognosis, and the overall survival over single-center data.

Material and Methods

This retrospective study was approved by an institutional Ethics Committee of Dicle University Faculty of Medicine (Declaration No: 225, Approval Date: 04.07.2018). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Study Protocol and Patients

A total of 21 patients who were histopathologically diagnosed with ACC during January 2005-May 2018 from the Dicle University Faculty of Medicine were included in this study. Patient data and medical follow-up were recorded retrospectively from the Dicle University Hospital Database System. The demographic characteristics of patients (age and gender), localization, size and volume of the tumor, whether the tumor was operated, tumor stage, hormonal activity (production of glucocorticoid, mineralocorticoid, androgen, and catecholamine), total Weiss scores of the pathology material of patients who were operated, preoperative DHEA-S level, maximal standardized uptake value (SUV_{max}) of adrenalin mass in pre-op PET-CT imaging, metastasis status, recurrence status, chemotherapy, and general follow-up period were all recorded. The status of patients survival and death was collected from the database system of public health directorate; the patients who died because of ACC were included in the study. Patients with ACC who died because of other reasons were excluded from the study. The overall survival time was calculated from the date of tissue diagnosis to the date of death or the last follow-up. Then, the patients were divided into two groups, including survival (n=6) and dead groups (n=15).

Calculation of the Size and Volume of the Adrenal Mass

The measurements of width, length, and depth were taken from the radiological imaging of the adrenal masses. The largest measurement of the mass was accepted as a dimension in centimeter. The volume of adrenalin masses was calculated in cubic centimeter using the prolate ellipse formula (widthxlengthxdepthxπ/6).

Evaluation of Hormonal Activity

a. Evaluation of glucocorticoid activity

1 mg dexamethasone suppression test

Preoperative basal adrenocorticotropic hormone (ACTH) and cortisol levels of patients

were recorded. Then, 1 mg dexamethasone tablets were administered to patients at 23:00, and the cortisol level was recorded the next morning. Patients with a cortisol value of less than 1.8 µg/dL after the intake of 1 mg dexamethasone were considered suppressed. Patients with a cortisol value of ≥1.8 µg/dL underwent the 2-day 2 mg dexamethasone test.

2-Day 2 mg dexamethasone suppression test

Patients who were not suppressed by 1 mg dexamethasone were given a 0.5 mg dexamethasone tablet every six hours for two days (with a total daily dose of 2 mg dexamethasone). The first dose was administered at 09:00 on the morning of the first day. After 6 h of administering the final dose at 03:00 on the second day, the blood was taken for the measurement of cortisol level. Patients with a cortisol value of <1.8 µg/dL were considered suppressed, and those with ≥1.8 µg/dL were considered not suppressed.

Free cortisol levels in 24-hour urine

In the morning, when the urine was collected, the residue urine in the bladder was emptied using micturition when a patient wakes up. All urine during the day and night was then collected in a container and stored in a cool place. When the patient wakes up again the next morning, the first urine was also collected and added, and all collected urine was examined.
If patients were not suppressed after administering 2-day 2 mg dexamethasone and had a higher level of 24-hour urine cortisol, the presence of hypercorticosomy was considered.

Evaluation of mineralocorticoid activity

Blood samples were collected from the patient at 08.00 in the morning for evaluating levels of aldosterone (ng/dL) and renin (ng/mL/hour). Patients with a rate of the plasma aldosterone level to plasma renin level ≥30 were considered positive.

Evaluation of androgen production

Patients with a high DHEA-S level because of the adrenal-induced androgen precursor or those with a high level of total testosterone were considered positive.

Evaluation of catecholamine production

Patients were provided with a diet deprived of food and beverages such as bananas, coffee, and vanilla-containing phenolic acid for three days. Later in the morning, after the first urine was ejected, all the urine urinated during the day, and overnight was collected in the same container. Urine was acidified with boric acid to a pH between 2 and 3. Urine samples collected for 24 h were kept in the dark and cool place. Urinary catecholamine levels were determined using high-performance liquid chromatography with electrochemical detection.

Analysis of Total Weiss Score

Pathologists evaluated the histologic changes according to the Weiss criteria (nuclear atypia; atypical mitoses; frequent mitoses; a small percentage of clear cells; diffuse architecture; necrosis; and the invasion of venous, sinusoidal, or capsular structures). Total Weiss scores were calculated on the basis of the number of positive results of these nine parameters.

Tumor Staging

Patients were staged on the basis of tumor node metastasis classification in the sample European Network for the Study of Adrenal Tumors (Ensat) in 2004 (6). Thus, the stage was classified as follows:
Stage 1: Tumor ≤5 cm
Stage 2: Tumor >5 cm
Stage 3: Lymph node involvement and/or tumor infiltration into surrounding tissue and/or a tumor thrombus in the vena cava and/or renal vein
Stage 4: Metastatic disease

Statistical Analysis

Data analyses were performed using the Statistical Package for Social Sciences (SPSS), Version 18.0 for Windows (SPSS Inc., Chicago, IL, USA). The Shapiro-Wilk test was used for the normal distribution of data. The Mann-Whitney U test among non-parametric tests was used as the 24-hour free urine cortisol did not conform to the normal distribution among groups. Normally distributed variables were presented using means and standard deviations. The independent two-sample t-test was used to compare continuous variables between two

groups. The chi-square test was used to compare categorical data between groups. Pearson's correlation was used for the simple regression analysis. Kaplan-Meier survival curves and the log-rank test were used for determining the overall survival and specific probability of survival for each of the observed variables, respectively. A p-value of <0.05 was considered statistically significant for all analyses.

Results

Out of 21 patients, six were in the survived group and 15 in the dead group. The survived group included two men and four women; the dead group consisted of three men and 12 women. Overall, 76% of patients with ACC diagnosis were women. The average age of patients in the survived group was 54.67±13.36 years, and in the dead group was 42.07±18.07 years. The average age of the two groups was not statistically significant (p>0.05). Moreover, 14 adrenal masses were located on the right and seven on the left. The mass size was 9.2±3.82 mm, and 10.84±4.74 mm in the survived and dead groups, respectively; and the mass volume was 264.50±299.66 cm^3, and 493.61±679.51 cm^3 in the survived and dead groups, respectively (p>0.05). Demographic and anatomical data for the two groups are given below in Table 1.

Basal ACTH among patients examined was 13.31±8.16 pg/mL in the survived group and 11.93±11.99 pg/mL in the dead group. On the other hand, basal cortisol was 20.97±17.21 µg/dL, and 24.78±12.76 µg/dL in the survived and dead groups, respectively. Basal ACTH and basal cortisol levels in both groups were not significant (p>0.05). The results of 1 mg dexamethasone suppression test showed that three and nine patients from the survived and dead groups were not suppressed, respectively. The results of a two-day 2 mg dexamethasone suppression test showed that there was no cortisol suppression in one and seven patients from the survived and dead groups, respectively. As 24-hour urine cortisol values were not normally distributed, the Mann-Whitney U test among nonparametric tests was used. The median 24-hour urine cortisol in the survived and dead groups was 136 nm/day (min-max: 78-1106) and 148 nm/day (min-max: 80-2052), respectively. The 24-hour free urine cortisol values between the two groups were not significant, according to the Mann-Whitney U test (p>0.05). Moreover, one patient in the survived group and seven in the dead group had hypercortisolism. Considering the mineralocorticoid activity, hyperaldosteronism was detected in two patients in the dead group and no patient in the survived group. The 24-hour urine catecholamines were in the normal range in the survived group and higher in a patient in the dead group. Hyperandrogenemia was never observed in the survived group and was detected in six patients from the dead group. In general, the total adrenal endocrine hormone production was observed in one patient in the survived group and 12 patients from the dead group (p<0.01). Patient's status of secreting adrenal endocrine hormone is shown below in Table 2.

All patients in the survived group were operated, whereas five patients from the dead group were not operated because four of them had distant metastasis, and one had a low-performance status. According to the total Weiss scores calculated in the pathol-

Table 1. Demographic and anatomical data of groups.

Parameters	Survived group (n=6)	Dead group (n=15)	p
Male	2	3	>0.05
Female	4	12	>0.05
Age (years)	54.67±13.36	42.07±18.07	>0.05
Localization-right	3	11	>0.05
Localization-left	3	4	>0.05
Mass size (cm)	9.2±3.82	10.84±4.74	>0.05
Mass volume (cm^3)	264.50±299.66	493.61±679.51	>0.05

ogy for the patients operated, the average score was 5.33±1.21, and 5.3±1.94 in the survived and dead groups, respectively. Total Weiss scores were not significant between the two groups (p>0.05). DHEA-S levels measured when patients were diagnosed with the disease were 371.13±33.21 µg/dL in the survived group and 763.05±229.74 µg/dL in the dead group. A significant correlation was found between DHEA-S levels of the two groups (p<0.001). No significant elevation of the DHEA-S level was observed in the postoperative follow-up in the six patients who were operated in the survived group. In the dead group, ten patients who were operated survived for 5.15±1.65 months and had DHEA-S levels of 544±216.9 ug/dL when DHEA-S started to increase after the operation.

According to Pearson's correlation analysis, a negative-oriented relationship was observed between the DHEA-S level and the general survival rate (p<0.05; r=-0.45). The relationship between DHEA-S and general survival is shown below in Figure 1.

In the PET-CT scans taken before the patients were operated, the SUV_{max} value of the adrenal mass was 7.78±2.66 in the survived group and 14.26±5.67 in the dead group (p<0.05). According to Pearson's correlation analysis, a powerful negative-oriented relationship was observed between SUV_{max} values and general survival rate (p<0.001; r=-0.65). The relationship between SUV_{max} values and general survival is shown in Figure 2.

According to the ACC classification of ENSAT study group, five patients were at stage 2 and one at stage 3 in the survived group, whereas eight patients were at stage 2, three at stage 3, and four at stage 4 in the dead group. Metastasis was not observed in the survived group at the time of diagnosis, whereas four patients from the dead group had metastasis at the time of diagnosis. Moreover, metastasis was not developed in follow-ups after full resection of the tumor in the survived group; however, new metastasis developed in eight patients in the dead group (p<0.05). ACC-related metastases were most commonly observed in the lymph nodes, liver, lung, and bones. One patient from the survived group and seven patients from the dead group received chemotherapy. No recurrences were observed during the follow-ups of patients from the survived group; five patients who were operated had recurrences in the dead group (p>0.05). Appraisements related to groups are shown below in Table 3.

The average survival duration of patients according to the Kaplan-Meier survival analysis was 23.66±2.79 (95% CI=18.18-29.13) months, and the median survival duration was 19.1 (95% CI=13.01-25.18) months. The general survival chart of the patients is shown below in Figure 3.

Discussion

Information on ACC is limited as they are rarely observed. The incidence rate among women is 1.2 to 1.5 times more than men (7). ACC more frequently occurred between

Table 2. Adrenal endocrine hormone secretion status of the groups.

Parameters	Survived group (n=6)	Dead group (n=15)	p
Basal ACTH (pg/mL)	13.31±8.16	11.93±11.99	>0.05
Basal cortisol (µg/dL)	20.97±17.21	24.78±12.76	>0.05
1 mg DST non-suppression	3	9	>0.05
2 mg DST non-suppression	1	7	>0.05
Median 24-hour urine cortisol (nM/day)	136 (min-max: 78-1106)	148 (min-max: 80-2052)	>0.05
Hypercortisolism	1	7	>0.05
Hyperaldosteronism	0	2	>0.05
Hyperandrogenemia	0	6	>0.05
Elevation of 24-hour urine fractionated catecholamines	0	1	>0.05
Total adrenal endocrine hormone production	1 (%16)	12 (%80)	**<0.01**

* DST: Dexamethasone suppression test.

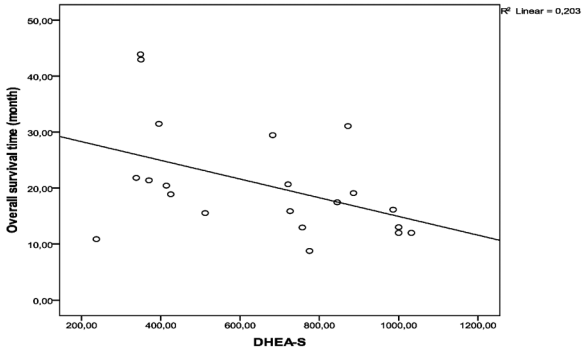

Figure 1: There was a negative correlation between dehydroepiandrosterone sulfate (DHEA-S), and overall survival time.

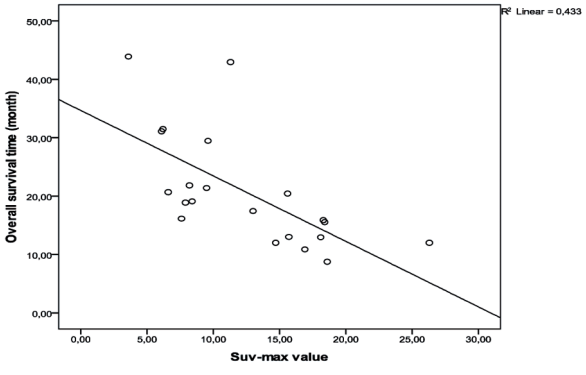

Figure 2: Negative-oriented relationship between standardized uptake value (SUV_{max}) values and overall survival time.

the fourth and sixth decades of life (8). Women's dominance was remarkable in this study, as 76% of ACC patients included were women. The average age of patients was between 40 and 50 years. More tumors were localized in the right adrenal gland.

When previous studies were analyzed, the diameter of the adrenal mass in patients with ACC was ≥10 cm (7,17). In this study, the average mass size was 10.37±4.47 cm (min-max=4.2-22 cm). The size of the adrenal mass among deceased patients was higher than those who survived. The survival rate of patients with a greater mass size was lower. However, it was not found statistically significant. In previous studies, tumor stage was found to be a strong prognostic factor in predicting mortality (6). As the staging was conducted on the basis of the mass size, the mass size affects the prognosis. However, the fact that the mass size was not statistically significant in this study may be because of the limited number of patients.

The most crucial stage in the treatment of ACC is complete resection of the tumor. The mortality rate was higher in patients who were not fully resected or who were inoperable because of being metastatic (8). In this study, only five deceased patients could not be operated because of the advanced stage. Steroid hormone production was observed among 50%-75% of patients with ACC. Mostly, cortisol was secreted. ACCs that are hormonally active have poor prognosis (9,16). The harmful effects of excessive steroid hormone production on the body and the increasing complications after the operation may be the reason for high mortality. In this study, eight of 21 (38%) patients

Table 3. Evaluations of the groups and comparison of over-all survival times.

Parameters	Survived group (n=6)	Dead group (n=15)	p
Operation	100%	66%	>0.05
Total Weiss score	5.33±1.21	5.3±1.94	>0.05
DHEA-S (µg/dL)	371.13±33.21	763.05±229.74	<0.001
SUV_{max} value	7.78±2.66	14.26±5.67	<0.05
Stage-1	0	0	>0.05
Stage-2	5	8	>0.05
Stage-3	1	3	>0.05
Stage-4	0	4	>0.05
Recurrence	0	5	>0.05
Metastasis in diagnosis	0	4	>0.05
Metastasis in follow-up	0	8	<0.05

DHEA-S: Dehydroepiandrosterone sulfate.

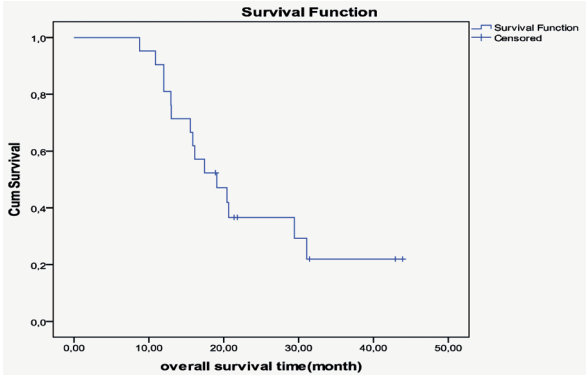

Figure 3: Kaplan-Meier survival curve for adrenocortical cancer.

showed extreme cortisol secretion. Seven of these patients were in the dead group. Moreover, two patients had hyperaldosteronism, and one had a high level of catecholamine. The fact that patients with hormone-producing ACC were dominant in the dead group and a statistically strong relationship was found may conclude that endocrine hormone secretion is an indicator of bad prognosis ($p<0.01$).

The adrenocortical adenoma and adrenocortical carcinomas can be histologically separated precisely using Weiss scoring. Patients with a total Weiss score of 3 or above were diagnosed with ACC. In previous studies, high Weiss scores were associated with recurrence and mortality (10-12). The Weiss scores in the patients operated in the study did not differ between the survived and the dead groups.

A higher level of DHEA-S may be observed in ACC than adrenocortical adenomas (13). Although the high level of DHEA-S was used as a parameter for diagnoses, it was not adequately examined by researchers in predicting mortality. The DHEA-S level was significantly higher in the dead group in this study ($p<0.001$). No correlation was observed between the size of the adrenal mass and the DHEA-S level. A negative-oriented correlation was observed between the DHEA-S level and the general survival, according to Pearson's correlation analysis. Considering this relationship, a high level of DHEA-S can be said to show poor prognosis. In this study, the low levels of DHEA-S noted in the patients after operation were markedly increased in the average clinical follow-up of 5.15±1.65 months. Therefore, it is crucial that clinicians follow these patients for the first six months postoperatively. The early diagnosis of this cancer that has high mortality can increase the survival of patients.

It is sometimes very difficult for clinicians to decide in favor of malignancy in adrenal masses. The F-18 FDG PET-CT plays a crucial role in the differentiation of the adrenal mass from benign, malignant types. The SUV_{max} value was higher in malignant adrenal masses (5,14). The relationship between the SUV_{max} value and mortality is not adequately studied. In this study, the SUV_{max} value was significantly higher in the dead group than the survived group ($p<0.05$). According to Pearson's correlation analysis, a strong negative-oriented relationship was observed between SUV_{max} values and the general survival rate. Thus, it is estimated that the mortality rate of patients with ACC with high SUV_{max} value is high.

In patients with ACC, lymph node invasion is typically observed at the time of diagnosis. It is most commonly observed in the lungs and liver as distal metastases (17). Metastasis in follow-ups, lymph node invasion was noted in three patients; lung and liver metastasis in three patients; lung and peritoneal metastasis in two patients. In follow-ups, ACC recurred at the operation site in five patients in the dead group. Relapse or metastasis development increased mortality (18). Despite surgical treatment, ACC relapse was noted frequently (23%).

Chemotherapy regimens are recommended in patients who are metastatic, progressive, or recurrent (18,19). The two most commonly used chemotherapy regimens are etoposide, doxorubicin, cisplatin, and mitotane (EDP-M) and streptozotocin and mitotane (Sz-M). In this study, nine patients were administered EDP-M in the dead group who were at an advanced stage and had a recurrence. However, despite surgery and chemotherapy regimens, ACC still had a high mortality rate (71%).

Bilimoria et al. found that the median survival was 32 months, and 5-year survival rate was 38% in patients with ACC (7). Tritos et al. reported a median survival of 17 months in patients with ACC (20). Tauchmanova et al. found that general survival

was 41 months in patients with ACC (3). Similar to the previous findings, this study showed that the median survival was 19.1 months, and the average survival was 23.66 months in patients with ACC.

Study Limitations

As this was a single-center study, a small number of patients were included. Thus, studies including large patient populations from multiple centers can help in better understanding ACC.

Conclusion

ACC still has high mortality rates despite surgical and medical treatments (71%). Moreover, it is observed 3.2 times more in women than men. The mass size and volume were not effective in mortality. The presence of adrenal steroid hormone production increased mortality. In follow-ups, the development of metastases was considered a poor prognostic factor. A high level of DHEA-S and SUV_{max} value at the time of diagnosis may predict lower overall survival. Closely following up with these patients is very important as relapses are common in the first six months postoperation.

Despite all the treatments in patients with ACC, the average survival was 23 months. Further large-scale studies are warranted to treat ACC, which has high mortality and is rarely seen.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Mehmet Güven; Design: Mehmet Güven; Control/Supervision: Alpaslan Kemal Tuzcu, Mehmet Güven; Data Collection and/or Processing: Mehmet Şimşek, Mehmet Güven; Analysis and/or Interpretation: Alpaslan Kemal Tuzcu, Mehmet Güven; Literature Review: Mehmet Şimşek; Writing the Article: Mehmet Güven; Critical Review: Mehmet Güven, Alpaslan Kemal Tuzcu.

References

1. Kerkhofs TMA, Verhoeven RHA, Van der Zwan JM, Dieleman J, Kerstens MN, Links TP, Van de Poll-Franse LV, Haak HR. Adrenocortical carcinoma: a population-based study on incidence and survival in the Netherlands since 1993. Eur J Cancer. 2013;49:2579-2586. [Crossref] [PubMed]
2. Schteingart DE, Doherty GM, Gauger PG, Giordano TJ, Hammer GD, Korobkin M, Worden FP. Management of patients with adrenal cancer: recommendations of an international consensus conference. Endocr Relat Cancer. 2005;12:667-680. [Crossref] [PubMed]
3. Tauchmanovà L, Colao A, Marzano LA, Sparano L, Camera L, Rossi A, Palmieri G, Marzano E, Salvatore M, Pettinato G, Lombardi G, Rossi R. Adrenocortical carcinomas: twelve-year prospective experience. World J Surg. 2004;28:896-903. [Crossref] [PubMed]
4. Osella G, Terzolo M, Borretta G, Magro G, Alí A, Piovesan A, Paccotti P, Angeli A. Endocrine evaluation of incidentally discovered adrenal masses (incidentalomas). J Clin Endocrinol Metab. 1994;79:1532-1539. [Crossref] [PubMed]
5. Elaini AB, Shetty SK, Chapman VM, Sahani DV, Boland GW, Sweeney AT, Maher MM, Slattery JT, Mueller PR, Blake MA. Improved detection and characterization of adrenal disease with PET-CT. Radiographics. 2007;27:755-767. [Crossref] [PubMed]
6. Fassnacht M, Johanssen S, Quinkler M, Bucsky P, Willenberg HS, Beuschlein F, Terzolo M, Mueller HH, Hahner S, Allolio B; German Adrenocortical Carcinoma Registry Group. Limited prognostic value of the 2004 International Union Against Cancer staging classification for adrenocortical carcinoma: proposal for a revised TNM classification. Cancer. 2009;115:243-250. [Crossref] [PubMed]
7. Bilimoria KY, Shen WT, Elaraj D, Bentrem DJ, Winchester DJ, Kebebew E, Sturgeon C. Adrenocortical carcinoma in the United States: treatment utilization and prognostic factors. Cancer. 2008;113:3130-3136. [Crossref] [PubMed] [PMC]
8. Icard P, Goudet P, Charpenay C, Andreassian B, Carnaille B, Chapuis Y, Cougard P, Henry JF, Proye C. Adrenocortical carcinomas: surgical trends and results of a 253-patient series from the French Association of Endocrine Surgeons study group. World J Surg. 2001;25:891-897. [Crossref] [PubMed]

9. Abiven G, Coste J, Groussin L, Anract P, Tissier F, Legmann P, Dousset B, Bertagna X, Bertherat J. Clinical and biological features in the prognosis of adrenocortical cancer: poor outcome of cortisol-secreting tumors in a series of 202 consecutive patients. J Clin Endocrinol Metab. 2006;91:2650-2655. [Crossref] [PubMed]
10. Lucon AM, Pereira MA, Mendonça BB, Zerbini MC, Saldanha LB, Arap S. Adrenocortical tumors: results of treatment and study of Weiss's score as a prognostic factor. Rev Hosp Clin Fac Med Sao Paulo. 2002;57:251-256. [Crossref] [PubMed]
11. Weiss LM. Comparative histologic study of 43 metastasizing and nonmetastasizing adrenocortical tumors. Am J Surg Pathol. 1984;8:163-169. [Crossref] [PubMed]
12. Weiss LM, Medeiros LJ, Vickery Jr AL. Pathologic features of prognostic significance in adrenocortical carcinoma. Am J Surg Pathol. 1989;13:202-206. [Crossref] [PubMed]
13. Michalkiewicz E, Sandrini R, Figueiredo B, Miranda ECM, Caran E, Oliveira-Filho AG, Marques R, Pianovski MAD, Lacerda L, Cristofani LM, Jenkins J, Rodriguez-Galindo C, Ribeiro RC. Clinical and outcome characteristics of children with adrenocortical tumors: a report from the International Pediatric Adrenocortical Tumor Registry. J Clin Oncol. 2004;22:838-845. [Crossref] [PubMed]
14. Chong S, Lee KS, Kim HY, Kim YK, Kim BT, Chung MJ, Yi CA, Kwon GY. Integrated PET-CT for the characterization of adrenal gland lesions in cancer patients: diagnostic efficacy and interpretation pitfalls. Radiographics. 2006;26:1811-1824; discussion 1824-1826. [Crossref] [PubMed]
15. Margonis GA, Kim Y, Tran TB, Postlewait LM, Maithel SK, Wang TS, Glenn JA, Hatzaras I, Shenoy R, Phay JE, Keplinger K, Fields RC, Jin LX, Weber SM, Salem A, Sicklick JK, Gad S, Yopp AC, Mansour JC, Duh QY, Seiser N, Solorzano CC, Kiernan CM, Votanopoulos KI, Levine EA, Poultsides GA, Pawlik TM. Outcomes after resection of cortisol-secreting adrenocortical carcinoma. Am J Surg. 2016;211:1106-1113. [Crossref] [PubMed] [PMC]
16. Keskin S, Taş F, Vatansever S. Adrenocortical carcinoma: clinicopathological features, prognostic factors and outcome. Urol Int. 2013;90:435-438. [Crossref] [PubMed]
17. Dy BM, Strajina V, Cayo AK, Richards ML, Farley DR, Grant CS, Harmsen WS, Evans DB, Grubbs EG, Bible KC, Young WF, Perrier ND, Que FG, Nagorney DM, Lee JE, Thompson GB. Surgical resection of synchronously metastatic adrenocortical cancer. Ann Surg Oncol. 2015;22:146-151. [Crossref] [PubMed]
18. Berruti A, Baudin E, Gelderblom H, Haak HR, Porpiglia F, Fassnacht M, Pentheroudakis G; ESMO Guidelines Working Group. Adrenal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2012;23:vii131-138. [Crossref] [PubMed]
19. Baudin E, Leboulleux S, Al Ghuzlan A, Chougnet C, Young J, Deandreis D, Dumont F, Dechamps F, Caramella C, Chanson P, Lanoy E, Borget I, Schlumberger M. Therapeutic management of advanced adrenocortical carcinoma: what do we know in 2011? Horm Cancer. 2011;2:363-371. [Crossref] [PubMed]
20. Tritos NA, Cushing GW, Heatley G, Libertino JA. Clinical features and prognostic factors associated with adrenocortical carcinoma: Lahey Clinic Medical Center experience. Am Surg. 2000;66:73-79. [PubMed]

Original Article
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The Relationship Between TSH Level and Stage of Differentiated Thyroid Carcinoma

Diferansiye Tiroid Karsinomunun Evresi ile TSH Düzeyi Arasındaki İlişki

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Abstract

Objective: The aim of this study was to determine whether thyroid-stimulating hormone (TSH) levels during the diagnosis of patients with differentiated thyroid carcinoma could be used for the prediction of cancer behavior. **Material and Methods:** The records of 329 patients with differentiated thyroid carcinoma who did not use levothyroxine at the time of diagnosis were reviewed retrospectively. The demographic and clinical characteristics of the cases and serum TSH levels were recorded at the time of diagnosis and statistically analyzed. **Results:** A total of 329 cases with 322 papillary carcinomas and 7 follicular carcinomas were included in the study. The median age of the participants at the time of diagnosis was 45 (17-76) years. Eighty-three percent of the cases were diagnosed in stage 1, 6.7% in stage 2, 3.3% in stage 3, and 7.0% in stage 4. The median serum TSH level at the time of diagnosis of the cases was 1.34 (0.01-9.97) mIU/mL. We did not observe any statistically significant relationship between the serum TSH level and the stage of differentiated thyroid carcinoma, although higher serum TSH level was associated with lymph node metastasis and higher risk group in the American Thyroid Association (ATA) classification. **Conclusion:** The relationship between serum TSH level and thyroid cancer has not been clearly determined, but high TSH levels at the time of diagnosis were found to be associated with lymph node metastasis and medium-high ATA risk score.

Keywords: Thyroid cancer; tumor burden; lymphatic metastasis; neoplasm staging

Özet

Amaç: Bu çalışmanın amacı, diferansiye tiroid karsinomlu hastaların tanı anındaki tiroid stimüle edici hormon (TSH) düzeylerinin kanser davranışını tahmin etmek için kullanılıp kullanılamayacağını belirlemekti. **Gereç ve Yöntemler:** Çalışmada tanı anında levotiroksin kullanmayan diferansiye tiroid karsinomlu 329 hastanın kayıtları retrospektif olarak incelendi. Olguların demografik ve klinik özellikleri ile tanı anındaki serum TSH düzeyleri kaydedildi ve istatistiksel olarak analiz edildi. **Bulgular:** Çalışmaya, toplam 329 (322'si papiller, 7'si foliküler karsinomlu) olgu dâhil edildi. Katılımcıların tanı anındaki ortanca yaşı 45 (17-76) yıl idi. Olguların %83'ü evre 1, %6,7'si evre 2, %3,3'ü evre 3 ve %7,0'ı evre 4 olarak teşhis edildi. Olguların tanı anında medyan serum TSH düzeyi 1,34 (0,01-9,97) mIU/mL idi. Serum TSH düzeyi ile diferansiye tiroid karsinomunun evresi arasında istatistiksel olarak anlamlı bir ilişki gözlemlemedik, ancak yüksek serum TSH düzeyi, "American Thyroid Association (ATA)" sınıflandırmasına göre lenf nodu metastazı ile ilişkili bulunmuştur. **Sonuç:** Serum TSH düzeyi ile tiroid kanseri arasındaki ilişki net olarak belirlenmemiştir, ancak tanı anındaki yüksek TSH düzeyleri lenf nodu metastazı ve orta-yüksek ATA risk skoru ile ilişkili bulunmuştur.

Anahtar kelimeler: Tiroid kanseri; tümör yükü; lenfatik metastaz; tümör evrelemesi

The study was presented as a poster at the European Endocrinology Congress held in Lisbon-Portugal on 20–23 May 2017.

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Introduction

Thyroid cancers account for about 1% of all malignancies and are the most common malignancy of the endocrine system. The incidence of thyroid cancer, which has been reported to be 2-3/100,000 individuals worldwide in the last century, has witnessed a 2 to 5-fold increase in the last 20 years (1). In Turkey, according to the data of 2014 Cancer Statistics, the incidence of thyroid cancer was 20.7/100,000 in women and 5.5/100,000 in men (2). In countries where iodine intake is sufficient, 95% of thyroid cancers are differentiated thyroid carcinoma (DTC), and among these, papillary cancers constitute the majority (3).

For DTC staging, several scoring systems such as MACIS, MSKCC, AMES, EORTC, and TNM are used. The most commonly used TNM Classification of Malignant Tumors (TNM) staging system developed by the American Joint Committee on Cancer is staging based on tumor size, lymph node, and distant metastasis. However, the TNM classification does not fully evaluate the risk factors and recurrence associated with thyroid cancer; therefore, a separate risk scoring system was developed by the American Thyroid Association (ATA), grouped as low, moderate, and high risk (4).

Thyroid-stimulating hormone (TSH), growth factors, and some cytokines are primarily responsible for the growth of the thyroid gland (5). TSH receptor is expressed on the DTC cell membrane, and TSH stimulation increases the synthesis and growth of proteins such as thyroglobulin and increases the activity of sodium-iodine symporter (6). The underlying mechanism for thyroid hyperplasia is chronic stimulation caused by slightly elevated TSH levels, which may lead to carcinomatous changes in the case of iodine deficiency (4). Long-term TSH stimulation is known to contribute to neoplastic growth and a worse prognosis (7).

In this study, the effect of TSH on thyroid gland growth was examined, and any possible relationship between the level of TSH at the time of diagnosis and the aggression of cancer in DTC patients was determined.

Material and Methods

Case Features

Between January 2006 and January 2016, a total of 329 cases were diagnosed for pathological DTC in the Necmettin Erbakan University Meram Medical Faculty of Endocrinology and Metabolic Diseases Department. The data of the patients were retrospectively evaluated. The inclusion criteria were a new histopathological diagnosis of DTC, and age 18 years or older. The exclusion criteria were a history of use of levothyroxine therapy and insufficient data. Approval was obtained from Necmettin Erbakan University Meram Medical Faculty, Ethics Committee for Drug and Non-Medical Device Research (No:2016/572/33) for the study and it was carried out in accordance with the patient rights regulation of the Helsinki Declaration. Cases were evaluated by examining identification information, laboratory results, pathology reports, and radiology reports from the hospital automation systems. Sex, age, cancer types, histopathological subtypes of cancer, serum TSH levels at the time of diagnosis, tumor diameters, lymph node metastases, distant metastases, and tumor multifocality situations were recorded. Patients' TNM cancer stages and ATA risk scores were also determined. The relationship between serum TSH level at the time of diagnosis and tumor diameters, lymph node metastasis, tumor multifocality, TNM stages, and ATA risk scores were analyzed statistically.

Statistical Analysis

The data obtained from the research were statistically analyzed by IBM SPSS 23.0 (IBM SPSS Statistics, Version 23.0 Armonk, NY: IBM Corp.). Descriptive statistics are provided using the median (minimum-maximum) and percentage distribution. Normality analysis was performed by the Kolmogorov-Smirnov test. Kruskal-Wallis variance analysis was used to compare continuous data between multiple groups, and the Mann-Whitney U test was used to compare between two groups. Pearson's correlation analysis was used to determine the relationship between numerical data. A p-value of <0.05 was accepted for statistical significance.

Results

Among the 329 cases included in the study, 261 (79.3%) were female, and 68 (20.7%) were male. The median age of the participants at the time of diagnosis was estimated to be 45 (18-76) years; 155 (47.1%) cases were under 45 years of age, and 174 (52.9%) were 45 years and older.

Three hundred twenty-two (97.9%) patients had papillary carcinoma and 7 (2.1%) had follicular carcinoma. The median tumor diameter was 1.2 (0.1-12) cm. In addition, among 322 papillary carcinoma cases, 126 (39.1%) cases were microcarcinomas. Additionally, 120 (36.5%) cases had multifocal tumors. At the time of diagnosis, the number of cases without lymph node metastasis was 276 (83.9%), and the number of cases with lymph node metastasis was 53 (16.1%). Only 1 (0.3%) case had distant metastasis, and no distant metastasis was detected in the remaining 328 (99.7%) cases at the time of diagnosis.

Considering the TNM staging at the time of diagnosis, 273 (83.0%) cases were in stage 1, 22 (6.7%) were in stage 2, 11 (3.3%) were in stage 3, 19 (5.8%) were in stage 4A, 3 (0.9%) were in stage 4B, and one (0.3%) case was in stage 4C. According to the risk classification, 232 (70.5%) cases were in the low-risk group, 93 (28.3%) cases were in the intermediate-risk group, and four (1.2%) cases were in the high-risk group. The distribution of the histopathological subtypes of the tumors and other tumor characteristics are shown in Table 1.

The median serum TSH levels at the time of diagnosis were found to be 1.34 (0.01-9.97) mIU/mL. The distribution of serum TSH level of the participants is presented in Figure 1.

No statistically significant correlation was observed between the tumor diameters at the time of diagnosis and serum TSH levels (p=0.300). The distribution and correlation curve of serum TSH levels based on tumor diameters are presented in Figure 2.

Table 2 presents the median serum TSH levels according to the presence of lymph node metastasis, ATA risk score, tumor multifocality status, and TNM stages. Serum TSH level at the time of diagnosis was found to be significantly higher in patients with lymph

Table 1. Some tumor characteristics of differentiated thyroid carcinoma cases.

		n (%)
Cancer type	Papillary carcinoma	322 (97.9)
	Follicular carcinoma	7 (2.1)
	Classic type	239 (72.7)
Histopathological subtype	Follicular type	70 (21.3)
	Other	20 (6.0)
Tumor multifocality	No	209 (63.5)
	Yes	120 (36.5)
Lymph node metastasis	No	276 (83.9)
	Yes	53 (16.1)
Distant metastases	No	328 (99.7)
	Yes	1 (0.03)
	Stage 1	273 (83.0)
	Stage 2	22 (6.7)
TNM stage	Stage 3	11 (3.3)
	Stage 4A	19 (5.8)
	Stage 4B	3 (0.9)
	Stage 4C	1 (0.3)
	Low	232 (70.5)
ATA risk scores	Intermediate	93 (28.3)
	High	4 (1.2)
	Total	329 (100)

ATA: American Thyroid Association.

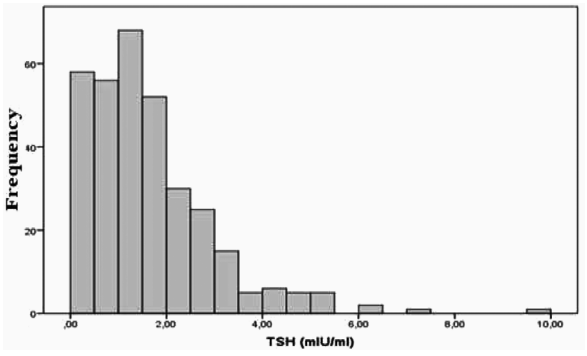

Figure 1: Distribution of serum thyroid-stimulating hormone (TSH) levels in patients with differentiated thyroid carcinoma.

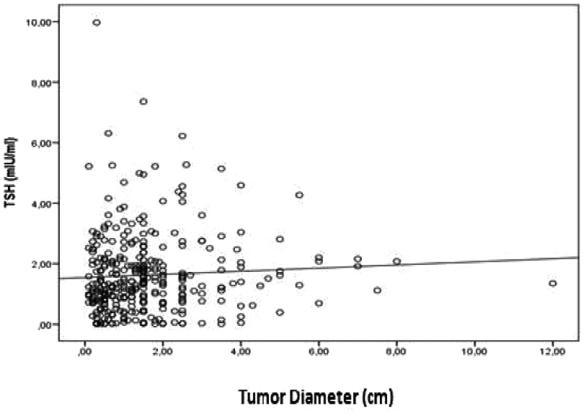

Figure 2: Distribution and correlation curves of thyroid-stimulating hormone (TSH) levels according to tumor diameters of patients with differentiated thyroid carcinoma.

node metastasis than those with non-lymph node metastases (p<0.001) and in patients with intermediate-high ATA risk than those with low risk (p=0.031). No statistically significant difference was observed between serum TSH levels at the time of diagnosis in patients with and without multifocal tumors (p=0.950). There was also no statistically significant difference between the serum TSH levels at the time of diagnosis according to the stage of the cases (p=0.052).

Discussion

The majority of thyroid cancers are DTCs with papillary and follicular subtypes. Donald et al. reported that 93.2% of patients with DTC had papillary thyroid carcinoma, and 6.8% had follicular thyroid carcinoma (8). In the current study, among 329 patients with DTC, 97.9% of the patients had papillary thyroid carcinoma, and 2.1% had follicular thyroid carcinoma.

In our study, 79.3% of the cases were female, and 20.7% were male. Likewise, in a study conducted by Mazurat et al. in 2013, including 2125 DTC cases, 76.6% of the patients were female, and 23.4% were male (9). In another study conducted by Lily et al. in 2012, including 55,995 DTC cases, 77.5% of the patients were female, and 22.5% were male (10). The earlier available reports indicated that thyroid cancers are 2-4 times more common in women than men.

Table 2. Serum TSH levels according to lymph node metastasis status, ATA risk score, tumor multifocality status and TNM stages of differential thyroid carcinoma cases.

		Serum TSH level (mIU/mL) Median (Range)	p
Lymph node metastasis	No	1.26 (0.01-9.97)	<0.001
	Yes	1.89 (0.05-6.22)	
ATA risk score	Low	1.25 (0.01-9.97)	0.031
	Intermediate-high	1.55 (0.01-6.22)	
Tumor multifocality	No	1.35 (0.01-9.97)	0.950
	Yes	1.31 (0.01-6.22)	
	Stage 1	1.37 (0.01-9.97)	
	Stage 2	0.69 (0.01-3.02)	
TNM stages	Stage 3	1.83 (0.53-4.38)	0.052
	Stage 4A	1.35 (0.05-4.56)	
	Stage 4B	2.08 (1.12-2.16)	
	Stage 4C	1.92 (1.92-1.92)	

TSH: Thyroid-stimulating hormone; ATA: American Thyroid Association.

Accordingly, in our study, the female/male ratio with DTC was found to be 3.83/1, like that in earlier literature.

Merhy et al. conducted a study in the USA and observed that the median age at diagnosis of DTC patients was 49 years (11). In this study, we found that the age of diagnosis was between 18 and 76 years and the median age of diagnosis was 45 years in accordance with the literature.

In earlier reports, the tumor diameter was found in the range of 0.1-12 cm and the median tumor diameter was 1.2 cm in our study. Pietro et al. conducted a study on 215 patients and reported a mean tumor diameter of 1.53 cm (12). In another study by Giovanni et al., the average tumor diameter was 1.36 cm (13). Papillary microcarcinomas with a tumor diameter of less than 1 cm constitute approximately 30% of all papillary cancers and are less aggressive (14,15). In our study, the rate of papillary microcarcinoma was 39.1%, which is higher than that reported in earlier studies.

In our study, tumor multifocality was found in 36.5% of the patients with DTC, and this rate is congruent with earlier reports. For instance, in the meta-analysis study published by Guo and Wang in 2014, this rate was estimated to be 36.6% (16).

We also classified the 329 cases according to TNM staging system and found that 83.0% of patients were in stage 1, 6.7% were in stage 2, 3.3% were in stage 3, 5.8% were in stage 4A, 0.9% were in stage 4B, and 0.3% were in stage 4C. In addition, at the time of diagnosis, 16.1% of the cases presented with lymph node metastasis, while only 0.3% of the cases presented with distant metastasis. In a study conducted by Kocak et al., 69.3% of the patients were found to be in stage 1, 23% in stage 2, 6.3% in stage 3, and 1.3% in stage 4 (17). In another study on 75 DTC cases, Karacavus et al. found that 21.6% of the patients had lymph node metastasis, and 9.5% had distant metastasis (18). While the incidence of lymph node metastasis in our study is congruent with the findings of earlier studies, the incidence of distant metastases in stage 1 patients is higher, and the incidence of distant metastasis is less than that reported in the literature; this can be attributed to the high rate of microcarcinoma cases in our study.

The patients in our study were categorized according to the ATA risk stratification system into low, intermediate, and high-risk groups. A total of 70.5% of the cases were in the low-risk group, 28.3% were in the intermediate-risk group, and 1.2% were in the high-risk group. Kocak et al. conducted a study on 300 patients with DTC and found that 54.7% of the patients were in low risk, 39.7% were in intermediate-risk, and 5.7% were in high-risk group (17), while Tuttle et al. studied 588 patients with DTC, and found that 23% of the patients were in low, 50% were in intermediate, and 27% in the high-risk groups (19). Deviating from earlier reports, more patients in our study were in the low-risk group. This can be attributed to the non-aggressive tumor behavior and low distant metastasis rate in the cases in this study.

Considering serum TSH levels, Tuna et al. conducted a study in 201 patients with DTC and found that their preoperative serum TSH level was in the range of 0.01-9.6 mIU/mL and median serum TSH level was 1.66 mIU/mL (20). In our study, serum TSH levels at the time of diagnosis were in the range of 0.01-9.97 mIU/mL, and the median serum TSH level was 1.34 mIU/mL.

We then investigated the relationship between serum TSH levels at the time of diagnosis and tumor size, tumor multifocal status, lymph node metastasis status, ATA risk score, and TNM stages. No statistically significant difference was found between serum TSH levels at the time of diagnosis according to the TNM staging of the cases. There was also no statistically significant correlation between tumor diameter and serum TSH levels. In addition, no statistically significant difference, based on the TSH level, was observed between the patients with and without multifocal tumors at the time of diagnosis. Similarly, Tuna et al. also reported that there was no correlation between tumor diameter and serum TSH levels, and that serum TSH levels did not change according to tumor multifocality or lymph node metastasis status (20). The findings of our study support the findings of Tuna's study in terms of tumor diameter and multifocality. However, we observed that serum TSH levels were significantly higher in patients with lymph node metastasis at

the time of diagnosis than those without lymph node metastasis. In addition, the serum TSH level at the time of diagnosis was significantly higher in moderate-high ATA risk group patients than in low ATA risk group patients.

Not many studies have investigated the relationship between thyroid cancer and serum TSH levels. Recently, Haymart et al., reported that high serum TSH level increased the incidence of thyroid cancer and also associated with advanced-stage cancer (21). In another study, the same researchers reported that high serum TSH levels related to the extrathyroidal spread of cancer (22). On the other hand, some studies report no correlation between serum TSH levels and the incidence of DTC and poor prognostic factors. For instance, in two different studies conducted by Kim et al., no significant relationship was observed between serum TSH level and tumor size (23,24). Likewise, we observed no direct correlation between serum TSH levels at the time of diagnosis and the stage of DTC.

This is one of the rare studies investigating the relationship between thyroid cancer and serum TSH level in our country. However, a limitation of this study is that the majority of patients had microcarcinomas and low-grade carcinomas.

Conclusion

Although no direct correlation was observed between serum TSH level and cancer stage, high serum TSH levels were found to be associated with the presence of lymph node metastasis, and high ATA risk group. The relationship between serum TSH level and thyroid cancer has not been clearly identified in the literature, and this deficiency should be overcome by repeating similar studies by determining the effect of TSH on thyroid cancer aggressiveness in more homogeneous case groups.

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Conflict of Interest

There are no conflicts of interest between the authors and/or family members of the scientific and medical committee members, or members of the potential conflicts of interest, counseling, expertise, working conditions, shareholding, and similar situations in any firm.

Authorship Contributions

Idea/Concept: Mustafa Kulaksızoğlu, Esra Tunçez; Design: Esra Tunçez; Control/Supervision: Mustafa Kulaksızoğlu; Data Collection and/or Processing: Esra Tunçez, İsmail Hakkı Tunçez; Analysis and/or Interpretation: Esra Tunçez, İsmail Hakkı Tunçez; Literature Review: Esra Tunçez; Writing the Article: Esra Tunçez; Critical Review: Mustafa Kulaksızoğlu; References and Fundings: Esra Tunçez; Materials: Esra Tunçez.

References

1. Burinicardi FC. Schwartz's principels of surgery. In: Lal G, Clark HO, eds. Thyroid, Parathyroid, Adrenal. (9th ed). Newyork: McGraw Hill Company; 2010:1343-1408.
2. Şencan İ, Keskinkılıç B. Türkiye Kanser İstatistikleri. Ankara: T.C. Sağlık Bakanlığı Türkiye Halk Sağlığı Kurumu; 2017. p.48.
3. Jameson JL, De Groot LJ. Endocrinology. (6th ed). Philadelphia: Elsevier Health Sciences; 2010. p.3064.
4. Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri EL, McIver B, Pacini F, Schlumberger M, Sherman SI, Steward DL, Tuttle RM. Revised American Thyroid Association management guidelines for patients with thyroid nodules and differantiated thyroid cancer. Thyroid. 2009;19:1167-1214. [Crossref] [PubMed]
5. Jameson JL, Weetman AP. Thyroid cancer. In: Braunwald E, Fauci AS, Kasper DL, Hauser SL, Longo DL, Jameson JL, eds. Harrison's Principles of Internal Medicine. (15th ed). St. Louis: McGraw-Hill; 2001:2079-2083.
6. Bulotta S, Celano M, Costante G, Russo D. Emerging strategies for managing differentiated thyroid cancers refractory to radioiodine. Endocrine. 2016;52:214-221. [Crossref] [PubMed]
7. Schneider AB, Ron E. Carcinoma of follicular epithelium. In: Braverman LE, Utiger RD, eds. Werner and Ingbar's the Thyroid: A Fundamental and Clinical Text. (8th ed). Philadelphia: Lippincott Williams and Wilkins; 2000:878-886.
8. McLeod DSA, Cooper DS, Ladenson PW, Ain KB, Brierley JD, Fein HG, Haugen BR, Jonklaas J, Magner J, Ross DS, Skarulis MC, Steward DL, Maxon HR, Sherman SI; The National Thyroid Cancer Treatment Cooperative Study Group. Prognosis of differentiated thyroid cancer in relation to serum thyrotropin and thyroglobulin antibody status at time of diagnosis. Thyroid. 2014;24:35-42. [Crossref] [PubMed] [PMC]

9. Mazurat A, Torroni A, Hendrickson-Rebizant J, Benning H, Nason RW, Pathak KA. The age factor in survival of a population cohort of well-differentiated thyroid cancer. Endocr Connect. 2013;2:154-160. [Crossref] [PubMed] [PMC]
10. Johnston LE, Cao HST, Chang DC, Bouvet M. Sociodemographic predictors of survival in differentiated thyroid cancer: results from the SEER database. ISRN Endocrinol. 2012;2012:384707. [Crossref] [PubMed] [PMC]
11. Merhy J, Driscoll HK, Leidy JW, Chertow BS. Increasing incidence and characteristics of differentiated thyroid cancer in Huntington, West Virginia. Thyroid. 2001;11:1063-1069. [Crossref] [PubMed]
12. Calò PG, Medas F, Pisano G, Boi F, Baghino G, Mariotti S, Nicolosi A. Differentiated thyroid cancer: indications and extent of central neck dissection--our experience. Int J Surg Oncol. 2013;2013:625193. [Crossref] [PubMed] [PMC]
13. Conzo G, Docimo G, Pasquali D, Mauriello C, Gambardella C, Esposito D, Tartaglia E, Della Pietra C, Napolitano S, Rizzuto A, Santini L. Predictive value of nodal metastases on local recurrence in the management of differentiated thyroid cancer. Retrospective clinical study. BMC Surgery. 2013;13:S3. [Crossref] [PubMed]
14. Pelizzo MR, Boschin IM, Toniato A, Piotto A, Bernante P, Pagetta C, Rampin L, Rubello D. Papillary thyroid microcarcinoma (PTMC): prognostic factors, management and outcome in 403 patients. Eur J Surg Oncol. 2006;32:1144-1148. [Crossref] [PubMed]
15. Chow SM, Law SCK, Chan JKC, Au SK, Yau S, Lau WH. Papillary microcarcinoma of the thyroid-prognostic significance of lymph node metastasis and multifocality. Cancer. 2003;98:31-40. [Crossref] [PubMed]
16. Guo K, Wang Z. Risk factors influencing the recurrence of papillary thyroid carcinoma: a systematic review and meta-analysis. Int J Clin Exp Pathol. 2014;7:5393-5403. [PubMed]
17. Kocak M, Koseoglu R, Sonmez B, Turkyilmaz S, Dogan I, Ersoz HO, Erem C. Evaluatıon of recurrence risk in differentiated thyroid cancer after treatment. In: Arlt W, Visser J, Beuschlein F, eds. Dublin: Bioscientifica; Endocrine Abstracts. 2015. p.416. [Crossref]
18. Karacavus S, Caglayan K, Sahin S, Sipahi M, Bal A, Arslan E, Seckin S, Suher M. Yozgat bölgesinde diferansiye tiroid kanseri nedeniyle takip edilen hastaların klinik ve demografik özellikleri. Bozok Med J. 2014;4:26-30. [Crossref]
19. Tuttle RM, Tala H, Shah J, Leboeuf R, Ghossein R, Gonen M, Brokhin M, Omry G, Fagin JA, Shaha A. Estimating risk of recurrence in differentiated thyroid cancer after total thyroidectomy and radioactive iodine remnant ablation: using response to therapy variables to modify the initial risk estimates predicted by the new American Thyroid Association staging system. Thyroid. 2010;20:1341-1349. [Crossref] [PubMed] [PMC]
20. Tuna MM, Başaran MN, Karakılıç E, Ayçicek Doğan B, Arduç A, Işık S, Berker D, Güler S. Diagnostic and prognostic value of TSH levels in differentiated thyroid cancers. Turk Jem. 2014;1:1-4. [Crossref]
21. Haymart MR, Repplinger DJ, Leverson GE, Elson DF, Sippel RS, Jaume JC, Chen H. Higher serum thyroid stimulating hormone level in thyroid nodule patients is associated with greater risks of differentiated thyroid cancer and advanced tumor stage. J Clin Endocrinol Metab. 2008;93:809-814. [Crossref] [PubMed] [PMC]
22. Haymart MR, Glinberg SL, Liu J, Sippel RS, Jaume JC, Chen H. Higher serum TSH in thyroid cancer patients occurs independent of age and correlates with extrathyroidal extension. Clin Endocrinol (Oxf). 2009;71:434-439. [Crossref] [PubMed] [PMC]
23. Kim HS, Lee SJ, Park JK, Jo CH, Shon HS, Jung ED. Association between serum thyroid stimulating hormone level and papillary thyroid microcarcinoma in Korean euthyroid patients. Endocrinol Metab. 2011;26:297-302. [Crossref]
24. Kim KW, Park YJ, Kim EH, Park SY, Park DJ, Ahn SH, Park DJ, Jang HC, Cho BY. Elevated risk of papillary thyroid cancer in Korean patients with Hashimoto's thyroiditis. Head Neck. 2011;33:691-695. [Crossref] [PubMed]

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Effects of Isotretinoin Treatment on Levels of Hormones Involved in the Etiopathogenesis of Acne

İzotretinoin Tedavisinin Akne Etiyopatogenezinde Yer Alan Hormon Düzeyleri Üzerindeki Etkileri

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Abstract

Objective: This study was performed to measure the effect of isotretinoin treatment on hormonal responses and insulin resistance in acne patients. **Material and Methods:** A total of 30 acne vulgaris patients and 30 control group volunteers were examined between February 2015 and June 2015. Firstly, the basal insulin resistance and endocrine hormone levels were measured in both groups. A daily dose of 120–150 mg/kg oral isotretinoin was administered to the patient group for three months. Following this, insulin resistance and endocrine hormone levels were re-evaluated in both groups. **Results:** Age, waist circumference, and body mass index were similar between the patient and control groups. Liver transaminase, low-density lipoprotein (LDL), adrenocorticotropic hormone, cortisol, 17-hydroxyprogesterone, and total testosterone levels were different in the patient group compared to the control group ($p<0.05$). The levels of dehydroepiandrosterone sulfate (DHEA-S), liver transaminase, LDL, and triglycerides increased after three months of isotretinoin administration ($p<0.05$). The changes in blood triglyceride levels were correlated with the changes in insulin growth factor-1, DHEA-S, total testosterone, progesterone, LDL, and estradiol levels ($p<0.05$). C**onclusion:** Isotretinoin might not affect pituitary gland hormones, adrenal hormones, and insulin resistance significantly. Increased blood triglyceride levels may be expected in patients whose testosterone and progesterone hormone levels are high.

Keywords: Acne vulgaris; isotretinoin; insulin resistance; hormones

Özet

Amaç: Bu çalışma, akne hastalarında izotretinoin tedavisinin hormonal yanıt ve insülin direnci üzerindeki etkisini ölçmek amacıyla planlanmıştır. **Gereç ve Yöntemler:** Şubat 2015-Haziran 2015 tarihleri arasında toplam 30 akne vulgaris hastası ve 30 gönüllü kontrol grubu incelendi. İlk olarak, her iki grupta bazal insülin direnci ve endokrin hormon düzeyleri ölçüldü. Üç ay boyunca hasta grubuna günde 120-150 mg/kg oral izotretinoin uygulandı. Bunu takiben her iki grupta da insülin direnci ve endokrin hormon düzeyleri yeniden değerlendirildi. **Bulgular:** Hasta ve kontrol grubu arasında yaş, bel çevresi ve beden kitle indeksi benzerdi. Hasta grubunda karaciğer transaminazları, "low-density lipoprotein (LDL)", adrenokortikotropik hormon, kortizol, 17-hidroksiprogesteron ve total testosteron düzeyleri kontrol grubuna göre farklıydı ($p<0,05$). Üç aylık izotretinoin uygulamasından sonra dihidroepiandrosteron sülfat (DHEA-S), karaciğer transaminazları, LDL ve trigliserid düzeyleri artmıştı ($p<0,05$). Kan trigliserid düzeylerindeki değişiklik, insülin benzeri büyüme faktörü-1, DHEA-S, total testosteron, progesteron, LDL ve östradiol düzeylerindeki değişikliklerle korele idi ($p<0,05$). **Sonuç:** İzotretinoin hipofiz bezi hormonlarını, adrenal hormonları ve insülin direncini anlamlı bir şekilde etkilemeyebilir. Testosteron ve progesteron hormon seviyeleri yüksek olan hastalarda kan trigliserid düzeylerinde artış beklenebilir.

Anahtar kelimeler: Akne vulgaris; izotretinoin; insülin direnci; hormonlar

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Introduction

Acne vulgaris is a chronic inflammatory disease of the pilosebaceous unit, including hair follicles, associated with the sebaceous glands (1). Although, generally it is known as a disorder of adolescents, it may affect adults (2).

Androgen hormones play an important role in the pathogenesis of acne vulgaris (1). However, several factors, besides androgens, are also involved in the development and differentiation of normal sebaceous glands. Thus, abnormal expression of one or more of these factors leads to acne formation (3). The sebaceous gland activity is also affected by the growth hormone (GH), insulin-like growth factor-1 (IGF-1), insulin, corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), melanocortin, and glucocorticoid hormones (1). Insulin enhances acne development in many patients with polycystic ovary syndrome (PCOS) (4).

Isotretinoin, a synthetic retinoid, is the most effective treatment option for acne and is used in moderate or severe acne patients non-responsive to conventional treatments (5). Due to its severe side effects, patients must be monitored regularly by clinicians. However, the mechanism of action of isotretinoin therapy in healing acne vulgaris remains unclear (6). There are limited studies about the effects of isotretinoin on hormones involved in the etiopathogenesis of acne (7,8).

Here, we aimed to understand hormonal disorders in patients with acne vulgaris and to observe changes in hormone and insulin resistance levels after isotretinoin treatment.

Material and Methods

Study Design

A total of 30 female patients between the ages of 18 and 45 were admitted to Ankara University, Faculty of Medicine, Endocrinology and Metabolism Diseases and Dermatology outpatient clinics. These patients clinically diagnosed as acne vulgaris with the severity of 2-4 were included in the study between February 2015 and June 2015. The control group consisted of 30 healthy volunteers. Since hormonal changes are gender-dependent, evaluation of both genders was not possible. Thus, females, with more frequent acne vulgaris, were included in this study. In this group, volunteers were healthy individuals without acne or other inflammatory skin diseases. In this group, the median ages were consistent with the patient group.

Inclusion criteria were:

- Female
- Between 18 and 45 years old
- Having acne vulgaris with clinical severity of 2-4
- Providing written consent to participate in the study

Exclusion criteria were:

- Younger than 18 years old.
- Pregnant
- Having one or more disorders (or diseases) in addition to acne vulgaris
- Having a history of any endocrinopathy (diabetes mellitus, thyroid function disorder, PCOS, adrenal disorder, hypophyseal disorder)
- Dyslipidemia, liver function disorder
- Medications affecting hormone levels and insulin resistance
- Previously received isotretinoin treatment

Clinical Assessment

A form was established in which the anamnesis, physical examination, and laboratory tests were included. According to this form, the age of the patients, age of onset of acne, age of menarche, body height-weight, waist circumference, and smoking history were recorded. The body mass index (BMI) was calculated by dividing body weight in kilograms to height in meters squared. Post-adolescence acne was defined as the acne first seen in the fifth year after menarche. Acne patients were included in this study according to the location of their acne and clinical severity. According to the examination, the following grades were determined:

1. Grade 1: Comedonal acne
2. Grade 2: Mild papulopustular acne
3. Grade 3: Severe papulopustular acne
4. Grade 4: Nodulocystic acne

The modified Ferriman-Galwey (mF-G) scoring system was used for the assessment of hirsutism.

The grade 2-4 patients were treated with isotretinoin. Fasting blood glucose, fasting insulin, lipid profile, alanine transaminase

(ALT), aspartate transaminase (AST), thyroid-stimulating hormone (TSH), free T3 (fT3), free T4 (fT4), follicle-stimulating hormone (FSH), luteinizing hormone (LH), progesterone, estradiol, prolactin, morning ACTH, morning cortisol, dehydroepiandrosterone sulfate (DHEA-S), total testosterone, 17-hydroxyprogesterone (17-OHP), GH, and IGF-1 levels were evaluated before starting the drug treatment and at the end of three months (120-150 mg/kg/day oral isotretinoin). Patients with high cortisol levels were given 1 mg dexamethasone suppression test. Insulin resistance was evaluated by the homeostatic model assessment of insulin resistance (HOMA-IR) score. Values of 2.5 and above were accepted as positive for insulin resistance.

Examination of Serum Samples

Serum samples were collected from the venous blood of patients at around 9 AM. Patients were in their early follicular phase (at 2-5 days of menstruation) and fasted for at least 8 h before blood collection.

Fasting blood glucose levels were evaluated by glucose oxidase technique using a spectrophotometer (UniCel DxC SYNCHRON, United States) in the Biochemistry Lab of the Faculty of Medicine at Ankara University. Fasting insulin, GH, 17-OHP, and IGF-1 levels were also determined. The radioimmunoassay (RIA) method was used with ready-made kits (DIAsource, Belgium), and the serum samples were stored at 2-8°C after centrifugation.

Lipid profiles were measured. Samples were prepared for the direct quantitative method (Beckman Coulter, United States) with the storage of serum samples at 2-8°C after centrifugation.

ALT and AST levels were quantified. The samples were prepared for an enzymatic kinetic method and stored at 2-8°C after centrifugation.

FSH, LH, progesterone, estradiol, cortisol, prolactin, total testosterone, TSH, fT3, and fT4 levels were measured. The samples were prepared for the magnetic particle-based chemiluminescence enzyme immunoassay method (Access Immunoassay Systems, Beckman Coulter, United States), and the serum samples were stored at 2-8°C after centrifugation.

The quantity of ACTH was detected. The samples were prepared for an electrochemiluminescence immunoassay (ECLIA) method (Roche Diagnostics GmbH, Mannheim, Germany). Serum samples were centrifuged after collection in EDTA tubes and were stored at -20°C according to the storage conditions of the Biochemistry Laboratory.

Serum DHEA-S levels were specified. The samples were prepared for the ECLIA method (Roche Diagnostics GmbH, Mannheim, Germany) and were stored at 2-8°C after centrifugation.

Statistical Analysis

Descriptive data were demonstrated as mean±standard deviation in the normal distribution, median (min-max) in non-normal distribution. Also, the number of cases and percent of nominal variables were shown.

The significance of the differences between the mean values of groups was determined by the t-test and the Mann-Whitney U test in terms of normally-distributed or not. Categorical variables were assessed by Pearson's chi-squared test or Fisher's exact test.

The relationship between variables was assessed by Pearson's correlation test for normal distribution and Spearman's correlation test for non-normal distribution.

Risk coefficients were determined by performing a multivariate logistic regression analysis. Risk factors independent of the parameters (including hirsutism) affecting the formation of acne were defined.

SPSS 22.0 (SPSS Inc.) was used for all statistical analyses. A value of $p<0.05$ was considered statistically significant.

Ethical Issues

The study was carried out following the Declaration of Helsinki and was approved by the Ethics Committee of the School of Medicine, Ankara University in Ankara, Turkey (Ethics Committee Approval No:02-57-15 and Approval Date:15 February 2015). Informed consent was obtained from each participant.

Results

Demographic Characteristics of the Patients

A total of 30 female patients of acne vulgaris administered 120-150 mg/kg/day of isotretinoin for three months were assessed

as the patient group. Another 30 healthy female volunteers with no inflammatory skin disease were enrolled as the control group. The mean age of the patient group was 23.2±3.7 years, while the mean age of the control group was 23.4±5.2 years. There was no significant difference between the ages of the groups (p=0.592). When both groups were compared, no significant differences were found for menarche age, body height/weight, BMI and waist circumference (Table 1). Also, no significant difference was found in mF-G scoring between the groups. The mF-G score was 8 or above in 30% of the patient group, and 10% of the control group with the scoring system not statistically significant (p=0.053).

Comparison of Laboratory Data

When the patient (pre-treatment with isotretinoin) and control groups were compared, the values of ALT, AST, and fT4 were higher in the control group than in the patient group. The values of low-density lipoprotein (LDL), TSH, ACTH, cortisol, prolactin, progesterone, 17-OHP, and total testosterone were higher in the patient group than in the control group. There was no statistically meaningful difference between the two groups when we compared other values (Table 2).

Evaluation After Isotretinoin Treatment

Serum levels of ALT, AST, LDL, triglyceride, and DHEA-S were significantly increased, and serum levels of fT4 were significantly decreased in the patient group after three months of isotretinoin treatment. There were no statistically significant differences in the other markers between the two groups (Table 3). There were no statistically significant differences between the BMI and waist circumference values between pre- and post-treatment in the patient group.

Effects of Severity of Acne in Hormonal Parameters

When thirty acne vulgaris patients were evaluated according to the clinical severity of acne, four were at clinical stage 2 (13.3%), nineteen at clinical stage 3 (63.3%), and seven at clinical stage 4 (23.4%). There was no statistically meaningful correlation between the severity of acne and HOMA-IR, fasting insulin, LDL, triglyceride, TSH, cortisol, IGF-1, and testosterone levels.

Correlation Graph and Tables

The correlation analysis of relationships between hormones in acne vulgaris patients before drug administration are summarized in Table 4. There was a strong negative correlation between high-density lipoprotein (HDL) and triglyceride. There were positive correlations of IGF-1 with both DHEA-S and total testosterone. When the correlations between variables were evaluated separately for the patient and control groups, a correlation of prolactin levels with ACTH and cortisol was observed only in the patient group.

Statistically significant changes in markers before and after the treatment were compared with baseline values of these markers. It was observed that the change in LDL was correlated positively with DHEA-S (r=0.458; p=0.012). The change in DHEA-S was not correlated with any of the other parameters. However, the change in triglyceride (ΔTg) was correlated with LDL, ALT, estradiol, progesterone, DHEA-S, and total testosterone (Table 5).

Table 1. Demographic and clinical characteristics according to groups before the drug treatment.

Variables	Patient Group (n=30)	Control Group (n=30)	p
Age	23.2±3.7	23.4±5.2	0.592
Age of menarche	13.3±0.85	13.07±0.98	0.293
Height (cm)	163.03±6.3	164.83±4.7	0.198
Weight (kg)	58.3±8.19	62.2±7.01	0.051
BMI (kg/m^2)	21.9±2.9	22.9±3.1	0.169
Waist circumference (cm)	72.4±10.1	74.1±9.7	0.373

BMI: Body Mass Index.

Table 2. Comparison of laboratory values of patient and control groups at basal level.

Variables	Patient Group n=30	Control Group n=30	p
Fasting blood glucose (mg/dL)	82.6±7.03	86.8±11.7	0.124
Fasting insulin (microIU/mL)	10.1±4.8	10.5±10.8	0.436
HOMA-IR score	2.07±1.06	2.24±2.6	0.412
ALT (U/L)	14.4±3.02	17.07±4.8	0.013
AST (U/L)	18.4±3.3	20.8±5.4	0.048
LDL (mg/dL)	90.8±21.8	75.6±12.4	0.002
Triglyceride (mg/dL)	59.6±26.6	57.3±23.5	0.728
HDL (mg/dL)	55.6±12.2	54.7±13.06	0.662
TSH (microIU/mL)	2.34±1.6	1.37±1.16	0.001
Free T3 (pmol/L)	4.8±0.59	4.71±0.77	0.304
Free T4 (pmol/L)	10.6±1.65	11.8±1.8	0.02
ACTH (pg/mL)	30.1±18.7	12.3±7.4	<0.001
Cortisol (microgram/dL)	22.8±8.6	11.6±4.8	<0.001
Growth hormone (ng/mL)	2.3±2.6	1.3±2.1	0.078
IGF-1 (ng/mL)	322.2±153.5	283.1±106.9	0.264
Prolactin (ng/mL)	18.3±7,3	11.7±4,8	<0.001
LH (mIU/mL)	8.78±6.1	12.1±14.6	0.859
FSH (mIU/mL)	5.38±2.21	6.69±3.5	0.181
Estradiol (pg/mL)	97.43±58.5	93.83±84.9	0.178
Progesterone (ng/mL)	2.9±3.1	0.8±0.9	<0.001
17-OH progesterone (ng/mL)	1.26±0.68	0.92±0.39	0.023
DHEA-S (microgram/dL)	322.2±153.5	283.1±106.9	0.264
Total testosterone (ng/dL)	53.9±15.9	41.06±17.02	0.04

HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; LDL: Low-Density Lipoprotein; HDL: High-Density Lipoprotein; TSH: Thyroid-Stimulating Hormone; ACTH: Adrenocorticotropic Hormone; IGF-1: Insulin-like Growth Factor 1; LH: Luteinizing Hormone; FSH: Follicle Stimulating Hormone; 17-OH progesterone: 17-hydroxyprogesterone; DHEA-S: Dehydroepiandrosterone Sulfate.

When linear regression analysis was used to evaluate the factors affecting the change in triglyceride levels, one-unit increase in testosterone and progesterone levels was observed to cause triglyceride increase of 1.48% and 8.47-fold, respectively (p=0.05 and p=0.048, respectively).

Discussion

In the present study, ALT, AST, LDL, and DHEA-S levels changed with isotretinoin treatment at the end of the third month of treatment in adult women. DHEA-S and testosterone were possibly associated with IGF-1 in acne etiopathogenesis. DHEA-S, testosterone, and progesterone were correlated with changes in triglyceride levels after isotretinoin treatment.

Acne is generally known as a disease of the adolescent period. However, recent epidemiological studies have shown that the development of acne in the post-adolescent period has increased and lasts until the mid-forties and is seen more often among women (2,9). In our study, the mean age of the patients was 23.23±3.7 years, and all participants had post-adolescent acne. Acne is frequently seen between the ages of 20-29 (10). When the site of acne was evaluated, we detected acne on the face of all patients and the torso in 33%. Dreno et al. identified acne on the face in 89.8% of patients and on the torso in 48.4% (11). Furthermore, the age of individuals with post-adolescent acne, age of onset, and site of acne show similarity with the literature.

Androgens play a key role in the etiology of acne by causing sebum production and follicular hyperkeratinization (12). Therefore, acne and hirsutism are common clinical

Table 3. Comparison of laboratory findings between basal evaluation and three months after isotretinoin treatment in the patient group.

Variables	Before isotretinoin Mean±SD	After isotretinoin Mean±SD	p
BMI (kg/m^2)	21.9±2.9	21.6±2.7	0.679
Waist circumference (cm)	72.4±10.1	72.1±9.8	0.907
mF-G score (>8) (%)	30.0	27.3	0.818
HOMA-IR score	2.07±1.06	2.22±0.88	0.206
ALT (U/L)	14.4±3.02	17.8±8.4	0.017
AST (U/L)	18.4±3.33	22.17±5.8	<0.001
LDL (mg/dL)	90.8±21.8	108.4±23.9	<0.001
Triglyceride (mg/dL)	59.6±26.6	89.2±41.3	<0.001
TSH (microIU/mL)	2.34±1.66	2.12±1.11	0.405
Free T4 (pmol/L)	10.6±1.6	10.1±1.3	0.04
ACTH (pg/mL)	30.1±18.7	30.06±21.9	0.478
Cortisol (microgram/dL)	22.8±8.6	24.8±10.5	0.417
IGF-1 (ng/mL)	326.7±146	264.1±73.6	0.184
Prolactin (ng/mL)	18.3±7.3	16.9±9.8	0.289
LH (mIU/mL)	8.7±6.1	10.5±7.04	0.153
FSH (mIU/mL)	5.38±2.21	6.33±2.4	0.128
Estradiol (pg/mL)	97.4±58.5	90.4±52.7	0.614
Progesterone (ng/mL)	2.98±3.12	1.7±1.3	0.120
17-OH progesterone (ng/mL)	1.26±0.68	1.09±0.45	0.344
DHEA-S (microgram/dL)	322.2±153.5	341.9±106.1	0.002
Total testosterone (ng/dL)	53.9±15.9	57.6±15.8	0.088

SD: Standard Deviation; BMI: Body mass index; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; LDL: Low-Density Lipoprotein; TSH: Thyroid-Stimulating Hormone; ACTH: Adrenocorticotropic Hormone; IGF-1: Insulin-like Growth Factor 1; LH: Luteinizing Hormone; FSH: Follicle Stimulating Hormone; 17-OH progesterone: 17-hydroxyprogesterone; DHEA-S: Dehydroepiandrosterone Sulfate.

Table 4. Correlation analysis of basal hormone levels of the patient group before drug treatment.

Correlation Coefficient (r)	ALT	ACTH	Cortisol	HDL	IGF-1	DHEA-S	Free T3
Triglyceride	n/s	0.259*	n/s	-0.472**	n/s	n/s	n/s
AST	0.631**	-0.301*	n/s	n/s	n/s	n/s	n/s
Prolactin	n/s	0.480**	0.465**	n/s	n/s	n/s	n/s
Progesterone	-0.260*	0.374**	0.409**	n/s	0.283*	n/s	n/s
DHEA-S	n/s	n/s	n/s	n/s	0.415**	-	0.343**
Testosterone	n/s	n/s	0.370**	n/s	0.512**	0.689**	0.399**
Free T3	n/s	n/s	n/s	n/s	0.406**	0.343**	-
Cortisol	n/s	0.709**	-	n/s	0.316*	n/s	n/s

ALT: Alanine Aminotransferase; ACTH: Adrenocorticotropic Hormone; HDL: High-Density Lipoprotein; IGF-1: Insulin-like Growth Factor 1; DHEA-S: Dehydroepiandrosterone Sulfate; AST: Aspartate Aminotransferase; n/s: not significance.
*p <0.05 **p<0.011.

Table 5. Correlation analysis of the change in triglyceride level with the hormonal parameters.

Correlation Coefficient (r)	LDL	ALT	DHEA-S	Total testosterone	Progesterone	Estradiol	IGF-1
(ΔTg)	-0.592**	-0.36*	0.423*	0.445**	0.495**	0.425*	0.365*

LDL: Low Density Lipoprotein; ALT: Alanine Aminotransferase; DHEA-S: Dehydroepiandrosterone Sulfate; IGF-1: Insulin-like Growth Factor 1.
*p<0.05 **p<0.01.

symptoms of hyperandrogenism among women. When hirsutism was evaluated with modified Ferriman-Gallwey scoring, there were nine individuals in the patient group (30%), and three subjects in the control group (9%) who had an mF-G score over 8 in our study. However, there was no relationship between acne severity and mF-G score and between HOMA-IR and BMI. The hirsutism rate was high in the group with acne, although it was not statistically meaningful (p=0.052). A study by Borgia et al., including 129 women with acne reported a hirsutism rate of 19.38%, unrelated to acne severity (13). Also, the mF-G score was not different pre- and post-treatment in this study.

We highlighted that neither acne severity nor isotretinoin treatment had effects on insulin and glucose levels. In another study, when pre- and post- isotretinoin treatment levels were evaluated in women diagnosed with severe post-adolescent acne, basic parameters were similar in the control and patient groups; likewise, both androgenic hormones and insulin resistance were unchanged post-treatment in the patient group (14). Retinoic acid (RA) was subcutaneously given to non-obese rats, and there was no change in insulin and glucose levels after injection in an experimental study. Although there were some changes in white adipose tissue after RA administration, the brown adipose tissue distribution was unchanged (15). A decrease in brown adipose tissue is associated with type 2 diabetes mellitus and obesity.

The increase of sebum excretion in acne vulgaris, changes in lipid composition, and differences in oxidant/antioxidant levels of lipids on the surface of the skin lead to acne growth (16). Our study showed higher LDL levels in the patient group than in the control group. Triglyceride and HDL levels were not different between the control and patient groups. A previous study indicated higher total cholesterol and LDL levels than the control group, while the HDL levels were lower in non-obese female patients with acne vulgaris who did not have hirsutism (17). Another study, with 184 acne patients and 82 healthy people, showed no difference in LDL and triglyceride levels between the groups; however, HDL levels were lower in the acne patient group (18). Generally, there is no consensus in the literature regarding acne vulgaris and the lipid profile. Additionally, isotretinoin treatment leads to increased ALT and AST levels. In light of this information, the lipid profile and liver enzymes should be checked in acne patients. Our study highlights that isotretinoin treatment causes significantly increased triglyceride and LDL levels. In a previous study, it was demonstrated that triglyceride levels increased after short-term isotretinoin application, not related to insulin resistance (19). In our study, we also found out that hypertriglyceridemia and insulin resistance were not relevant. We found out that triglyceride and androgens had a strong correlation with IGF-1 levels. There might be other effective factors beyond isotretinoin. IGF-1 sebocytes play an important role in increasing lipid synthesis (20). For SEB-1 sebocytes, IGF-1 increases lipogenesis by increasing sterol response element-binding protein-1 (SREBP-1) levels via the PI3 k/Akt and MAPK/ERK signal transduction pathways (21). SERBP-1 expression regulates fatty acid synthesis genes and is increased by androgens (22). Molecular-lev1el relationships between these markers are supported by our study. In our study, changes in triglyceride levels were related to basic DHEA-S, total testosterone, oestradiol, and progesterone levels. There might be different unknown factors playing a role in hypertriglyceridemia.

For acne vulgaris etiology, IGF-1 affects the stage of sebum production. The maximum sebum production starts at puberty, and this state correlates with peak values of IGF-1 and GH levels in the intermediate phase of puberty (23). Thus, increased GH and IGF-1 levels play an important role in acne progression. As the oscillation of GH is rapid and distant, evaluating serum levels is not accurate. However, evaluating IGF-1 in serum tests is more convenient because the oscillation of IGF-1 is stable and reflects the cumulative effect of GH. In a previous study, 82 female patients between the ages of 20-25 years with post-adolescent acne had higher IGF-1 levels than the control group (24). Parallel to the levels of GH and IGF-1, DHEA-S levels progressively increase at puberty. DHEA-S and other adrenal androgens may be an important factor for sebum se-

cretion in the prepubertal period and during the pubertal period (25). As an increase in both IGF-1 and DHEA-S corresponds to the same period, a relationship between these hormones may exist. Aizawa et al. showed an increase in DHEA-S and IGF-1 levels in post-adolescent women with acne, but there was no correlation between them (24). However, another study found a relationship between IGF-1 level and dihydrotestosterone (DHT) in 8 women with acne, while IGF-1 was related to DHEA-S and androstenedione in 8 men with acne and IGF-1 levels were not different from the control group (26). IGF-1 and other factors lead to acne growth in women, although it can occur in women with normal IGF-1 levels (26). Serum DHEA-S and total testosterone levels are related to the presence of acne in the prepubertal period, and adolescent girls aged 14-17 years (27). Many studies have pointed out a complex relationship between IGF-1 and androgens. In a previous study, the relationship between serum androgens and IGF-1 was shown through an increase in IGF-1 after applying oral DHEA-S in postmenopausal women (28). Similarly, another study monitored oophorectomized women administered with testosterone and showed a linear increase in IGF-1 levels (29). IGF-1 and androgen might play a role in acne pathogenesis. Forkhead box O1 (FOXO1) regulation of androgen receptor activity on the genetic level may explain the relationship between IGF-1, androgens, and androgen receptors. These play important roles in sebum production, sebocyte growth, and keratinocyte proliferation (30). IGF-1 levels were decreased in a study after isotretinoin treatment, due to declining gene expression related to the androgen receptor and an increasing FOXO1 nuclear concentration (31). In our study, IGF-1 levels were similar between the patient and control groups. After isotretinoin treatment, there was no statistical change in IGF-1 levels. However, in the correlation analysis, IGF-1 levels were strongly related to both DHEA-S and testosterone levels.

Hyperandrogenism is related to sebum production and severe acne growth (32). A previous study showed that total testosterone, DHT, androstenedione, sex hormone-binding globulin (SHBG), free testosterone, and free DHT are increased in patients with acne (33). In the literature, it has been found that total testosterone and DHEA-S levels are decreased after three months of isotretinoin treatment (8). However, in another study, it was stated that there was no change in androgen levels after six months of isotretinoin treatment, and there was no relationship between acne severity and androgens (20). While we observed a higher total testosterone level in the acne group than in the control group, an increase in the DHEA-S level was identified three months after isotretinoin treatment. A total of three patients complained about hirsutism in the third month of treatment. As isotretinoin may increase DHEA-S levels, these data emphasize the evaluation of patients in this respect. Also, our study confirmed a strong positive correlation between basic free T3, DHEA-S, and total testosterone. Thyroxin-binding globulin, along with SHBG, plays an important role in acne vulgaris etiology, and further studies are suggested from this point of view.

In the present study, when we assessed correlations between other hormones, cortisol level was found to be significantly correlated with prolactin, progesterone, and IGF-1 levels. The role of progesterone in acne pathogenesis remains unclear. However, it is known that glucocorticoid, progesterone, and IGF-1 levels can increase in acne vulgaris (34). We also observed a significant increase in progesterone in the acne group compared to the control group. The influence of progesterone on acne is more complicated, and the fluctuation of sebum production in women during the menstrual cycle is attributed to progesterone effect (1). Progestins may cause an increase in pro-inflammatory cytokine production, such as IL-6 (1). Therefore, the correlation between progesterone, cortisol, and acne vulgaris could be dedicated to the pro-inflammatory process. CRH genes are intensely involved in the skin, and CRH is known to trigger lipogenesis (35). Acne flares have been seen during a period of stress, associated with the strong effect of CRH on the sebaceous glands (36). Additionally, CRH increases the level of prolactin during stress periods in animal models (37). IGF-1 plays a role in acne pathogen-

esis by increasing the pro-inflammatory cytokine release via the NF-κB pathway through its receptor in sebocytes (38). It can be hypothesized that the positive correlation of IGF-1 and cortisol is due to this pro-inflammatory effect of IGF-1 in the study.

This study has some limitations. First, the sample size was small; second, this study was single-centered. Third, patients with acne vulgaris were not of the same clinical severity. Fourth, isotretinoin treatment did not reach a cumulative dose. Fifth, it was not asked whether all participants had menstrual disorders. Concurrently, this study has several strengths. First, only women in the reproductive period were included. Second, the assessments were made in comparison to a control group. Third, in this study, a broader hormone profile was evaluated compared to previous studies.

Conclusion

In conclusion, interactions between hormones and interactions at the molecular level play essential roles in the etiopathogenesis of acne vulgaris. Isotretinoin had no conspicuous effect on hypophyseal function, adrenal hormones, or insulin resistance. For a deeper understanding of acne vulgaris etiopathogenesis and the effects of isotretinoin, further studies are necessary. Thus, we aim to contribute to the literature with our findings to enlighten future studies in this respect.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Mustafa Şahin, Süleyman Emre Koçyiğit; Design: Süleyman Emre Koçyiğit; Control/Supervision: Mustafa Şahin, Demet Çorapçıoğlu; Data Collection and/or Processing: Süleyman Emre Koçyiğit, atma Sena Dost Günay, Yousef Houshyar; Analysis and/or Interpretation: Mustafa Şahin, Süleyman Emre Koçyiğit; Literature Review: Mustafa Şahin; Writing the Article: Süleyman Emre Koçyiğit; Critical Review: Süleyman Emre Koçyiğit, Fatma Sena Dost Günay; References and Fundings: Süleyman Emre Koçyiğit, Yousef Houshyar; Materials: Süleyman Emre Koçyiğit, Mustafa Şahin.

References

1. Arora MK, Yadav A, Saini V. Role of hormones in acne vulgaris. Clin Biochem. 2011;44:1035-1040. [Crossref] [PubMed]
2. Goulden V, Stables GI, Cunliffe WJ. Prevalence of facial acne in adults. J Am Acad Dermatol. 1999;41:577-580. [Crossref] [PubMed]
3. Azmahani A, Nakamura Y, McNamara KM, Sasano H. The role of androgen under normal and pathological conditions in sebaceous glands: the possibility of target therapy. Curr Mol Pharmacol. 2016;9:311-319. [Crossref] [PubMed]
4. Balta I, Ekiz O, Ozuguz P, Ustun I, Karaca S, Kacar SD, Eksioglu M. Insulin resistance in patients with post-adolescent acne. Int J Dermatol. 2015;54:662-666. [Crossref] [PubMed]
5. Akman A, Durusoy C, Senturk M, Koc CK, Soyturk D, Alpsoy E. Treatment of acne with intermittent and conventional isotretinoin: a randomized, controlled multicenter study. Arch Dermatol Res. 2007;299:467-473. [Crossref] [PubMed] [PMC]
6. Cooper AJ, Harris VR. Modern management of acne. Med J Aust. 2017;206:41-45. [Crossref] [PubMed]
7. Karadag AS, Takci Z, Ertugrul DT, Bilgili SG, Balahoroglu R, Takir M. The effect of different doses of isotretinoin on pituitary hormones. Dermatology. 2015;230:354-359. [Crossref] [PubMed]
8. Karadag AS, Ertugrul DT, Tutal E, Akin KO. Isotretinoin influences pituitary hormone levels in acne patients. Acta Derm Venereol. 2011;91:31-34. [Crossref] [PubMed]
9. Goulden V, Clark SM, Cunliffe WJ. Post-adolescent acne: a review of clinical features. Br J Dermatol. 1997;136:66-70. [Crossref] [PubMed]
10. Collier CN, Harper JC, Cafardi JA, Cantrell WC, Wang W, Foster KW, Elewski BE. The prevalance of acne in adults 20 years and older. J Am Acad Dermatol. 2008;58:56-59. [Crossref] [PubMed]
11. Dréno B, Thiboutot D, Layton AM, Berson D, Perez M, Kang S, Global Alliance to Improve Outcomes in Acne. Large-scale international study enhances understanding of an emerging acne population: adult females. J Eur Acad Dermatol Venereol. 2015;29:1096-1106. [Crossref] [PubMed]

12. Cibula D, Hill M, Vohradnikova O, Kuzel D, Fanta M, Zivny J. The role of androgens in determining acne severity in adult women. Br J Dermatol. 2000;143:399-404. [Crossref] [PubMed]
13. Borgia F, Cannavò S, Guarneri F, Cannavò SP, Vaccaro M, Guarneri B. Correlation between endocrinological parameters and acne severity in adult women. Acta Derm Venereol. 2004;84:201-204. [Crossref] [PubMed]
14. Cetinözman F, Aksoy DY, Elcin G, Yildiz BO. Insulin sensitivity, androgens and isotretionin therapy in women with severe acne. J Dermatolog Treat. 2014;25:119-122. [Crossref] [PubMed]
15. Mercader J, Ribot J, Murano I, Felipe F, Cinti S, Bonet ML, Palou A. Remodeling of white adipose tissue after retinoic acid administration in mice. Endocrinology. 2006;147:5325-5332. [Crossref] [PubMed]
16. Makrantonaki E, Ganceviciene R, Zouboulis C. An update on the role of the sebaceous gland in the pathogenesis of acne. Dermatoendocrinol. 2011;3:41-49. [Crossref] [PubMed] [PMC]
17. Bakry OA, El Shazly RMA, El Farargy SM, Kotb D. Role of hormones and blood lipids in the pathogenesis of acne vulgaris in non-obese, non-hirsute females. Indian Dermatol Online J. 2014;5:S9-S16. [Crossref] [PubMed] [PMC]
18. Ekiz O, Balta I, Unlu E, Bulbul Sen B, Rifaioglu EN, Dogramaci C. Assessment of thyroid function and lipid profile in patients with postadolescent acne in a Mediterranean population from Turkey. Int J Dermatol. 2015;54:1376-1381. [Crossref] [PubMed]
19. Stoll D, Binnert C, Mooser V, Tappy L. Short-term administration of isotretinoin elevates plasma triglyceride concentrations without affecting insulin sensitivity in healthy humans. Metabolism. 2004;53:4-10. [Crossref] [PubMed]
20. Makrantonaki E, Vogel K, Fimmel S, Oeff M, Seltmann H, Zouboulis CC. Interplay of IGF-I and 17beta-estradiol at age-specific levels in human sebocytes and fibroblasts in vitro. Exp Gerontol. 2008;43:939-946. [Crossref] [PubMed]
21. Smith TM, Gilliland K, Clawson GA, Thiboutot D. IGF-1 induces SREBP-1 expression and lipogenesis in SEB-1 sebocytes via activation of the phosphoinositide 3-kinase/Akt pathway. J Invest Dermatol. 2008;128:1286-1293. [Crossref] [PubMed] [PMC]
22. Rosignoli C, Nicolas JC, Jomard A, Michel S. Involvement of the SREBP pathway in the mode of action of androgens in sebaceous glands in vivo. Exp Dermatol. 2003;12:480-489. [Crossref] [PubMed]
23. Rosenfield RL, Deplewski D. Role of androgens in the developmental biology of the pilosebaceous unit. Am J Med. 1995;98:80S-88S. [Crossref] [PubMed]
24. Aizawa H, Niimura M. Elevated serum insulin-like growth factor-1 (IGF-1) levels in women with postadolescent acne. J Dermatol. 1995;22:249-252. [Crossref] [PubMed]
25. Stewart ME, Downing DT, Cook JS, Hansen, JR, Strauss JS. Sebaceous gland activity and serum dehydroepiandrostreone sulfate levels in boys and girls. Arch Dermatol. 1992;128:1345-1348. [Crossref] [PubMed]
26. Cappel M, Mauger D, Thiboutot D. Correlation between serum levels of insulin-like growth factor 1, dehydroepiandrosterone sulfate, and dihydrotestosterone and acne lesion counts in adult women. Arch Dermatol. 2005;141:333-338. [Crossref] [PubMed]
27. Lucky AW, Biro FM, Simbartl LA, Morrison JA, Sorg NW. Predictors of severity of acne vulgaris in young adolescent girls: results of a five-year longitudinal study. J Pediatr. 1997;130:30-39. [Crossref] [PubMed]
28. Genazzani AD, Stomati M, Strucchi C, Puccetti S, Luisi S, Genazzani AR. Oral dehydroepiandrosterone supplementation modulates spontaneous and growth hormone-releasing hormone-induced growth hormone and insulin-like growth factor-1 secretion in early and late postmenopausal women. Fertil Steril. 2001;76:241-248. [Crossref] [PubMed]
29. Azziz R, Deal CL, Potter HD, Gargosky SE, Rosenfeld RG. Regulation of extragonadal insulin-like growth factor-binding protein-3 by testosterone in oophorectomized women. J Clin Endocrinol Metab. 1994;79:1747-1751. [Crossref] [PubMed]
30. Yanase T, Fan W. Modification of androgen receptor function by IGF-1 signaling implications in the mechanism of refractory prostate carcinoma. Vitam Horm. 2009;80:649-666. [Crossref] [PubMed]
31. Melnik BC. Isotretinoin and FoxO1: a scientific hypothesis. Dermatoendocrinol. 2011;3:141-165. [Crossref] [PubMed] [PMC]
32. Chen HC, Smith SJ, Tow B, Elias PM, Farese Jr RV. Leptin modulates the effects of acyl CoA:diacylglycerol acyltransferase deficiency on murine fur and sebaceous glands. J Clin Invest. 2002;109:175-181. [Crossref] [PubMed]
33. Lawrence D, Shaw M, Katz M. Elevated free testosterone concentration in men and women with acne vulgaris. Clin Exp Dermatol. 1986;11:263-273. [Crossref] [PubMed]
34. Ju Q, Tao T, Hu T, Karadağ AS, Al-Khuzaei S, Chen W. Sex hormones and acne. Clin Dermatol. 2017;35:130-137. [Crossref] [PubMed]
35. Zouboulis CC, Seltmann H, Hiroi N, Chen W, Young M, Oeff M, Scherbaum WA, Orfanos CE, McCann SM, Bornstein SR. Corticotropin-releasing hormone: an autocrine hormone that promotes lipogenesis in human sebocytes. Proc Natl Acad Sci USA. 2002;99:7148-7153. [Crossref] [PubMed] [PMC]
36. Thiboutot D, Gollnick H, Bettoli V, Dréno B, Kang S, Leyden JJ, Shalita AR, Lozada VT, Berson D, Finlay A, Goh CL, Herane MI, Kaminsky A, Kubba R, Layton A, Miyachi Y, Perez M, Martin JP, Ramos-E-Silva M, See JA, Shear N, Wolf Jr J, Global Alliance to Improve Outcomes in Acne. New insights into the management of acne: an update from the Global Alliance to Improve Outcomes in Acne group. J Am Acad Dermatol. 2009;60:S1-50. [Crossref] [PubMed]
37. Akema T, Chiba A, Oshida M, Kimura F, Toyoda J. Permissive role of corticotropin-releasing factor in the acute stress-induced prolactin release in female rats. Neurosci Lett. 1995;198:146-148. [Crossref] [PubMed]
38. Cong TX, Hao D, Wen X, Li XH, He G, Jiang X. From pathogenesis of acne vulgaris to anti-acne agents. Arch Dermatol Res. 2019;311:337-349. [Crossref] [PubMed]

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Thrombotic Microangiopathy After Spontaneous Pheochromocytoma Rupture: A Rare MEN 2A Case

Spontan Feokromasitoma Rüptüründen Sonra Gelişen Trombotik Mikroanjiyopati: Nadir Bir MEN 2A Olgusu

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Abstract

Pheochromocytoma is an adrenal medulla-derived tumor originating from the chromaffin cells that produce and secrete catecholamines. These tumors usually occur sporadically, but they may also be associated with genetic diseases, such as multiple endocrine neoplasia syndrome type 2 (MEN 2). A hypertensive crisis that occurs after the spontaneous rupture of pheochromocytoma, is a rare clinical complication with a high mortality rate. In this article, we present a male case who developed hypertensive crisis and thrombotic microangiopathy (TMA) after a spontaneous pheochromocytoma rupture due to MEN 2A.

Keywords: Pheochromocytoma; thrombotic microangiopathy; MEN 2A

Özet

Feokromasitoma, katekolamin üreten ve salgılayan kromaffin hücrelerden kaynaklanan adrenal medulla kaynaklı bir tümördür. Bu tümörler genellikle sporadik olarak ortaya çıkar, ancak multipl endokrin neoplazi sendromu tip 2 (MEN2) gibi genetik hastalıklarla da ilişkili olabilir. Spontan feokromasitoma rüptüründen sonra gelişen hipertansif kriz, nadir görülen ve mortalitesi yüksek olan klinik bir durumdur. Bu yazıda, MEN 2A'ya bağlı spontan feokromasitoma rüptürü sonrası hipertansif kriz ve trombotik mikroanjiyopati (TMA) gelişen erkek bir olgu sunmayı amaçladık.

Anahtar kelimeler: Feokromasitoma; trombotik mikroanjiyopati; MEN 2A

Introduction

Tumors that originate from chromaffin cells in the adrenal medulla and secrete catecholamine are called pheochromocytoma (1). Catecholamine producing tumors are rare, and their incidence is 2 to 8 cases/million people yearly (2). This disease commonly affects people in their 40s and 50s, but it occurs earlier in people with disease-associated germline mutations. Although these tumors are typically sporadic, they are also associated with genetic disorders, including multiple endocrine neoplasia syndrome type 2 (MEN 2).

Their symptoms may occur in episodes or paroxysmally and involves a broad clinical spectrum due to the high circulating catecholamine level in plasma. Typical symptoms are tachycardia, pallor, headache, and sweating (3). Hypertension occurs in ap-

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proximately 80-90% of pheochromocytoma patients, where 50% sustain hypertension, 45% have paroxysmal hypertension, and 5 to 15% are normotensive (4,5). Episodes may occur either spontaneously or due to medications (e.g., anesthetic agents, radiocontrast media, decongestants, beta-adrenergic antagonists, metoclopramide), intra-abdominal pressure increasing maneuvers (eg., weight lifting, defecation, exercise), posture changes, and anxiety (6). Spontaneous pheochromocytoma rupture may cause malignant hypertension, though its exact mechanism is unknown. A massive catecholamine release is probably associated with the tumor's vasoconstriction, which is followed by necrosis and bleeding (7). Another rare, devastating, and fatal event is spontaneous hemorrhage within the pheochromocytoma, resulting in the capsule rupture.

Thrombotic microangiopathy (TMA) is a rare disease, where endothelial damage causes thrombosis in capillaries and arterioles. Manifestations, such as thrombocytopenia, anemia, purpura, and renal failure, can be seen in TMA (8). Malignant hypertension causes TMA and may present with symptoms similar to thrombotic thrombocytopenic purpura (TTP) (9).

In this report, we present a patient who developed malignant hypertension and TMA, due to the rupture of pheochromocytoma and recovered through plasmapheresis.

Case Report

A 32-year-old male patient was admitted to the emergency department with a sudden onset of left-flank abdominal pain, headache, and palpitation. There was no known chronic illness, drug, alcohol, or illicit substance use, and the family's medical history revealed that his 37-year-old brother died of pheochromocytoma-induced hypertensive pulmonary edema. During the first evaluation in the emergency department, his blood pressure was 190/135 mmHg, and heart rate was 140 beats/min (sinus tachycardia). There was an increased tenderness in the left upper quadrant. Emergency laboratory tests were normal except for leukocytosis (WBC=15.8x10^3/μL) and minimal abnormalities in the liver function test. Abdominal computed tomography (CT) (Figure 1A, B) revealed a 43 mm mass lesion in the left adrenal gland with perirenal hematoma and right adrenal hematoma. The patient was admitted to the intensive care unit with a preliminary diagnosis of pheochromocytoma rupture and hypertensive crisis. Along with the routine examination, a 24-hour urine catecholamine level was planned to explain the etiology. The urology department consulted the patient for an emergency surgical treatment but did not recommend it since his vital signs were unstable, increasing the risk of mortality. Nitroprusside and doxazosin treatment was provided to control blood pressure. On the second day of the follow-up in the intensive

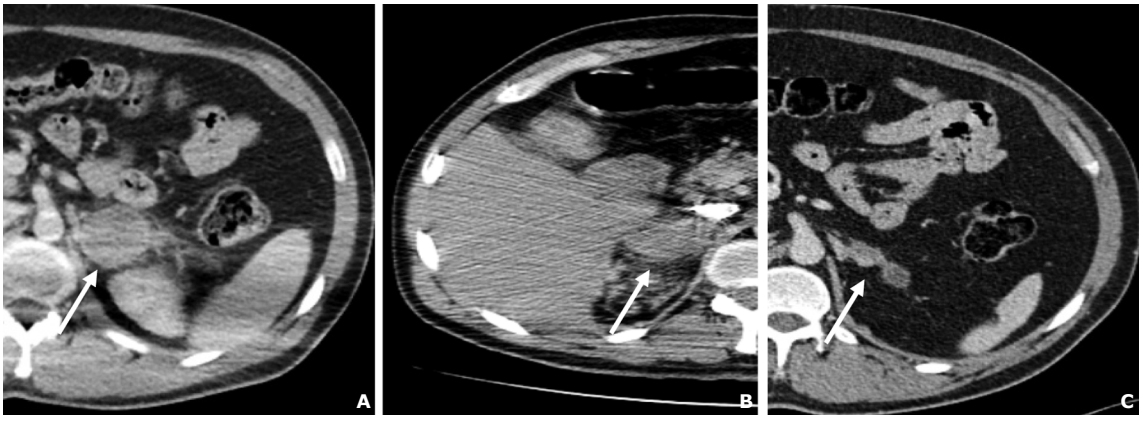

Figure 1: A) Adrenal computed tomography (CT) at admission to the emergency department (43 mm left adrenal mass, perirenal hemorrhage). **B)** Adrenal CT at admission to the emergency department (right adrenal hematoma). **C)** Adrenal CT taken one year later (7 mm nodular lesion in left adrenal).

care unit, the patient developed fever (38.8ºC), blurred consciousness, shortness of breath, and loss of strength in his left arm. Laboratory tests (Table 1) showed deterioration in coagulation markers and renal function tests (azotemia), hemolytic anemia (coombs negative), and thrombocytopenia. Anticoagulant therapy (low molecular weight heparin) and pulse steroid therapy were initiated while investigating the etiology of TMA. Plasmapheresis treatment was provided in 5 sessions. Daily plasma volume of approximately 40 mL/kg was used as a replacement dose for plasma exchange. Fresh frozen plasma was used as the exchange fluid. In the samples, taken during the patient's admission to the intensive care unit, urinary metanephrine and the normetanephrine levels were 7 and 4 times higher than the upper limit of normal, respectively. However, with the plasmapheresis treatment, the patient's platelet value and liver function test values returned to the normal level (Figure 2). The ADAMTS13 activity was found to be 35% (40-130), and ADAMTS13 inhibitor was <2 U/mL (<12). TTP was excluded from the diagnosis since the diagnosis of TTP is only supported by the ADAMTS13 activity level of less than 5%. Catastrophic Anti-Phospholipid antibody syndrome (CAPS) was ruled out, and the examinations related to connective tissue diseases were negative as well. The patient was diagnosed with TMA after detecting pheochromocytoma hemorrhagic rupture by the clinical and laboratory values. His urine catecholamine levels were normal before the discharge (Table 2). The patient had low cortisol and elevated adrenocorticotropic hormone levels and was diagnosed with primary adrenal failure and discharged with oral hydrocortisone replacement therapy. High calcium and parathyroid hormone (PTH) levels (calcium 10.73 mg/dl (8.4-10.2), PTH 72 pg/mL (1-65)) were detected in the follow-up and he was diagnosed with

Table 1. Laboratory findings of the patient.

		Emergency admission	Before plasma exchange	After plasma exchange	Normal range
Complete blood cell counts	WBC	15.8	18.2	16.5	4-10 (x10³/uL)
	RBC	5.6	3.3	4.3	3.6-5.7 (x10⁶/µL)
	Hemoglobin	16.9	9.8	12.7	12.1-17.2 g/dL
	Hematocrit	46.4	26	37.3	36.1-50.3%
	Platelets	248	37	338	150-400 (x10³/uL)
	MCV	82.7	79.8	84.1	82.2-99 fL
	MCH	30.1	30.1	29.8	27.6-33.3 pg
	MCHC	36.4	37.7	35.4	32-36 g/dL
Blood chemistry	Total protein	8	4.5	6	6.4-8.3 g/dL
	Albumin	4.3	2.7	3.4	3.5-5 g/dL
	Aspartate aminotransferase	44	629	35	5-34 u/L
	Alanine aminotransferase	74	407	98	0-55 u/L
	Creatinine	0.61	1.1	0.72	0.72-1.25 mg/dL
	Urea	20.1	65	32.7	15-44 mg/dL
	Plasma glucose	119	175	135	70-105 mg/dL
	Sodium	139	127	136	136-145 mmol/L
	Potassium	4	4.8	4.6	mmol/L
	Calcium	8.4	7.6	8.87	8.4-10.2 mg/dL
Coagulation tests	PT	15.5	19.6	13.4	11-15 sec
	PT%	65	50	98	70-120%
	INR	1.24	1.65	1.03	1-1.5 INR
	aPTT	30.1	31.2	29	26.5-40 sec

WBC: White blood cell; RBC: Red blood cell; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; PT: Prothrombin time; INR: International normalized ratio; aPTT: Activated partial thromboplastin time.

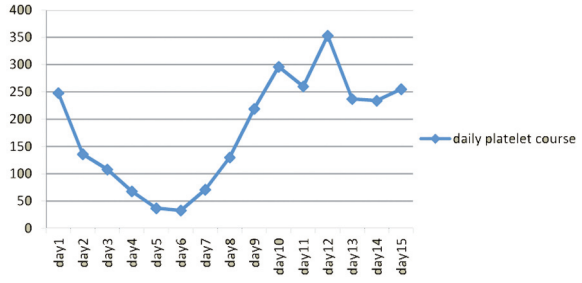

Figure 2: Daily platelet course.

primary hyperparathyroidism. The thyroid fine-needle aspiration cytology was reported suspicious for malignancy with serum calcitonin level of 2730 pg/mL (0-30) Therefore, total thyroidectomy and parathyroid surgeries were performed. Before the surgery, 24-h urinary catecholamine levels of the patient were normal, and imaging showed that the adrenal mass had almost disappeared. Since primary adrenal insufficiency was developed in the patient, he was operated on stress dose steroids. Final pathology was reported as medullary thyroid carcinoma and parathyroid gland hyperplasia. RET proto-oncogene mutation was found positive and MEN 2A syndrome was diagnosed. After the pheochromocytoma rupture, the patient was followed up for one year, and TMA relapse was not observed. Also, the 24-hour urine catecholamines did not increase, and, in the adrenal CT control (Figure 1C), the mass had reduced significantly.

Discussion

The patient had no typical pheochromocytoma symptoms (e.g., hypertension, headache, palpitations, and sweating episodes). We suspected pheochromocytoma rupture primarily due to the presence of hypertensive crisis on admission to the emergency department. Also, the 43 mm mass in the left adrenal region, perirenal hemorrhage in the abdominal CT, and the history of brother's death because of hypertensive pulmonary edema due to pheochromocytoma lead to the diagnosis. Paroxysmal hypertension usually occurs in patients with elevated plasma epinephrine levels and is very typical for MEN 2-associated pheochromocytoma (10). Spontaneous, non-traumatic hemorrhage of pheochromocytoma is a complication that can rarely occur in these patients, and is often associated with anticoagulant medications or severe sepsis, with most common symptoms being abdominal pain and hypertensive crisis. In this context, previously, there are only 54 similar cases reported (11). The patient had no known disease history, and he was presented with severe left upper quadrant pain and high blood pressure, which started immediately at rest without any stimulating factor. The patient was admitted for the hypertensive crisis due to excessive catecholamine release after pheochromocytoma rupture, and bilateral adrenal apoplexy due to vasospasm of bilateral adrenal circulation with hypoxia, which may have developed too. Based on this, coagulation tests and platelet levels are expected to be normal. Moreover, since pheochromocytoma originates from the adrenal medulla, the cortex is not expected to be affected by the rupture. However, even after the patient's clinical condition improved, primary adrenal insufficiency remained permanent with a need to continue steroid replacement therapy while the catecholamine levels decreased to the normal range. This suggested that the bilateral adrenal cortex was permanently affected.

Table 2. Endocrinological tests of the patient.

	A/D	At the time of discharge	A year after discharge	Normal range
Urinary metanephrine	2244	94	33	52-341 µ/24 h
Urinary normetanephrine	1629	213	277	88-444 µ/24 h
Urinary epinephrine	249	1,7		2-22 µ/24 h
Urinary norepinephrine	769	26		20-81 µ/24 h
Urinary dopamine	274	69		40-400 µ/24 h

Focal neurological deficits may develop in TTP/hemolytic-uremic syndrome (HUS)/TMA. These are usually transient and can last up to 24 h, such as transient ischemic attacks. These neurological deficits can be in the form of monoparesis or hemiparesis in one half of the body or bilaterally. The patient had a loss of strength in his left arm. No pathology could explain the clinical picture of the cranial imaging, and his neurological symptoms improved in a short time.

Surgery is the final treatment option for pheochromocytoma, and pharmacological treatment is still vital in preoperative and intraoperative blood pressure control. The patient was evaluated by the urology department for the surgical treatment, but it was postponed because of his unstable vital signs.

TMA, which causes thrombosis in capillaries and arterioles, may be hereditary or acquired. Hereditary TTP, also called Upshaw-Schulman syndrome, is caused by hereditary TMA (12) whereas the acquired TMA is caused by the following: Shiga toxin-dependent TMA (also known as a HUS), classical ADAMTS13 deficiency acquired TTP, complement-mediated TMA (also called atypical HUS), and drug-mediated TMA. Apart from the aforementioned disorders, autoimmune diseases such as systemic sclerosis and systemic lupus erythematosus, severe preeclampsia, disseminated cancer, systemic infections, malignant hypertension, hematopoietic stem cell transplantation, and organ transplantation can also cause TMA. Additionally, there is another disease similar to TTP that develops after surgery, known as postoperative TTP (13). The type of TMA determines the success of treatment and prognosis. Plasma infusion or exchange allows the replacement of proteins necessary for the complement cascade and helps in treating the patients with atypical HUS and TTP (14).

In literature, some cases have been presented with TMA and diagnosed with pheochromocytoma. However, unlike these cases, our case was presented with a TMA that developed as a result of pheochromocytoma rupture (15-17).

In our case, pulse steroid and plasma exchange treatments were started once the sample was taken for ADAMTS13 activity because the clinical and laboratory findings were compatible with TTP. Plasma exchange is recommended for the patients diagnosed with primary TMA, or suspected TTP (18). Considering the secondary causes of TMA in the patient, necessary examinations were performed, such as tests for antiphospholipid antibody syndrome and other collagen tissue diseases, especially for systemic lupus erythematosus. The results excluded the patient from the diagnoses, and ADAMTS13 activity was also found normal.

After excluding the primary TMA and the possible secondary causes, the patient was evaluated for secondary TMA due to malignant hypertension after the rupturing of pheochromocytoma. Although plasma exchange is recommended for primary TMA, some studies show that plasma exchange therapy is beneficial in secondary TMA as well (19-22). Since the platelet count of the patient increased above 200 ($x10^3$/uL), along with a sufficient increase in hemoglobin level, plasma exchange therapy was discontinued.

Our case study is the first reported case of TMA associated with malignant hypertension, secondary to pheochromocytoma, where the patient was benefitted from plasmapheresis. Plasmapheresis should be performed empirically in patients with TMA, which is caused by malignant hypertension until ADAMTS13 activity is assessed. Possible benefits of plasmapheresis should be considered in patients advancing to multiple organ failure, even if ADAMTS13 activity is normal.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and

medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: İlker Çordan; Design: Melia Karaköse; Control/Supervision: Mustafa Kulaksızoğlu, Feridun Karakurt; Data Collection and/or Processing: Hatice Çalışkan Burgucu; Analysis and/or Interpretation: İlker Çordan; Literature Review: Seda Yılmaz; Writing the Article: Muhammet Kocabaş, İlker Çordan; Critical Review: Muhammet Kocabaş, Mustafa Can; References and Fundings: Mustafa Can; Materials: İlker Çordan.

References

1. DeLellis RA, Lloyd RV, Heitz PU, Eng C: Pathology and Genetics of Tumours of Endocrine Organs. 2004, IARC Press Lyon, France.
2. Stenström G, Svärdsudd K. Pheochromocytoma in Sweden 1958-1981. An analysis of the national cancer registry data. Acta Med Scand. 1986;220:225-232. [Crossref] [PubMed]
3. Lenders JWM , Duh QY, Eisenhofer G, Gimenez-Roqueplo AP, Grebe SKG, Hassan Murad M, Naruse M, Pacak K, Young Jr WF, Endocrine Society: Pheochromocytoma and paraganglioma: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2014;99:1915-1942. [Crossref] [PubMed]
4. Manger WM. The protean manifestations of pheochromocytoma. Horm Metab Res. 2009;41:658-663. [Crossref] [PubMed]
5. Zelinka T, Eisenhofer G, Pacak K. Pheochromocytoma as a catecholamine producing tumor: Implications for clinical practice. Stress. 2007;10:195-203. [Crossref] [PubMed]
6. Young Jr WF, Maddox DE. Spells: in search of a cause. Mayo Clin Proc. 1995;70:757-765. [Crossref] [PubMed]
7. Takana K, Nogushi S, Shuin T, Kinoshita Y, Kubota Y, Hosaka M. Spontaneous rupture of adrenal pheochromocytoma: a case report. J Urol. 1994;151:120-121. [Crossref] [PubMed]
8. Benz K, Amann K.Thrombotic microangiopathy: new insights. Curr Opin Nephrol Hypertens. 2010;19:242-247. [Crossref] [PubMed]
9. Khanal N, Dahal S, Upadhyay S, Bhatt VR, Bierman PJ. Differentiating malignant hypertension-induced thrombotic microangiopathy from thrombotic thrombocytopenic purpura. Ther Adv Hematol. 2015;6:97-102. [Crossref] [PubMed] [PMC]
10. Manger W, Gifford RW. The clinical and experimental pheochromocytoma. Malden (MA): Blackwell Science, 1996.
11. Habib M, Tarazi I, Batta M. Arterial embolization for ruptured adrenal pheochromocytoma. Curr Oncol. 2010;17:65-70. [Crossref] [PubMed] [PMC]
12. George JN, Nester CM. Syndromes of thrombotic microangiopathy. N Engl J Med. 2014;6;371:1847-1848. [Crossref] [PubMed]
13. Eskazan AE, Buyuktas D, Soysal T. Postoperative thrombotic thrombocytopenic purpura. Surg Today. 2015;45:8-16. [Crossref] [PubMed]
14. Tsai HM. The molecular biology of thrombotic microangiopathy. Kidney Int. 2006;70:16-23. [Crossref] [PubMed] [PMC]
15. Gillett MJ, Arenson RV, Yew MK, Thompson IJ, Irish AB. Diagnostic challenges associated with a complex case of cystic phaeochromocytoma presenting with malignant hypertension, microangiopathic haemolysis and acute renal failure. Nephrol Dial Transplant. 2005;20:1014. [Crossref] [PubMed]
16. Schweizer H, Boehm J, Winterer JT, Wild D, Neumann HPH, Wiech T, Stubanus M, Kuehn EW. Phaeochromocytoma and thrombotic microangiopathy: favourable outcome despite advanced renal failure. J Clin Pathol. 2010;63:754-756. [Crossref] [PubMed]
17. Sakai C, Takagi T, Oguro M, Wakatsuki S, Kuwahara T. Malignant pheochromocytoma accompanied by microangiopathic hemolytic anemia: a case report. Jpn J Clin Oncol. 1994;24:171-174. [PubMed]
18. Rock GA, Shumak KH, Buskard NA, Blanchette VS, Kelton JG, Nair RC, Spasoff RA. Comparison of plasma exchange with plasma infusion in the treatment of thrombotic thrombocytopenic purpura. Canadian apheresis study group. N Engl J Med. 1991;325:393-397 [Crossref] [PubMed]
19. Nguyen TC, Han YY, Kiss JE, Hall MW, Hassett AC, Jaffe R, Orr RA, Janosky J, Carcillo JA. Intensive plasma exchange increases a disintegrin and metalloprotease with thrombospondin motifs-13 activity and reverses organ dysfunction in children with thrombocytopenia-associated multiple organ failure. Crit Care Med. 2008;36:2878-2887. [Crossref] [PubMed] [PMC]
20. Minami T, Akazawa M, Kanda E, Nonomura Y. [13-year history of scleroderma complicated by renal crisis and thrombotic microangiopathy treated with plasma exchange: A case report]. Nihon Rinsho Meneki Gakkai Kaishi. 2015;38:116-120. [Crossref] [PubMed]
21. Flombaum CD, Mouradian JA, Casper ES, Erlandson RA, Benedetti F. Thrombotic microangiopathy as a complication of long-term therapy with gemcitabine. Am J Kidney Dis. 1999;33:555-562. [Crossref] [PubMed]
22. Rock GA, Shumak KH, Buskard NA, Blanchette VS, Kelton JG, Nair RC, Spasoff RA. Comparison of plasma exchange with plasma infusion in the treatment of thrombotic thrombocytopenic purpura. Canadian apheresis study group. N Engl J Med. 1991;8;325:393-397. [Crossref] [PubMed]