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Research Article

Impact of Vascular Endothelial Growth Factor (VEGF) rs2010963 Polymorphism on Painful Crisis Frequency in Sickle Cell Anemia

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Abstract

Objectives: Sickle cell anemia is a hemoglobinopathy characterized by painful crises. Vascular endothelial growth factor (VEGF) has been implicated in vaso-occlusive pathogenesis. This study aimed to investigate the relationship between the VEGF-A rs2010963 gene polymorphism and the frequency of vaso-occlusive painful crises in patients with sickle cell anemia (SCA).

Methods: In this case-control study, biochemical blood analysis and VEGF-A rs2010963 genotyping were performed using real-time polymerase chain reaction (PCR) in 89 patients with SCA and 100 healthy volunteers. The relationship between the frequency of painful crises and genotype distribution was investigated in the patient group.

Results: In the patient group, the mean values of white blood cell count, platelet count, ferritin, C-reactive protein (CRP), and lactate dehydrogenase were significantly higher than those in the control group ($p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, and $p<0.001$, respectively). The genotype distribution of VEGF-A rs2010963 did not differ significantly between the patient and control groups ($p=0.164$). No relationship was found between genotype distribution and the frequency of painful crises in the patient group ($p=0.536$).

Conclusion: The VEGF-A rs2010963 polymorphism alone does not appear to predict vaso-occlusive crisis frequency in SCA, highlighting the polygenic nature of pain susceptibility in this disease. Broader genetic and functional analyses are warranted.

Keywords: Gene, painful crisis, polymorphism, sickle cell anemia

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Sickle cell disease (SCD) affects approximately 7.7 million individuals worldwide, with more than 500,000 affected births occurring annually; the majority of cases, approximately 80%, are concentrated in Africa.[1] Sickle cell anemia (SCA) is a clinically severe phenotype of SCD, characterized by significant morbidity and mortality, with erythrocyte sickling representing the fundamental underlying pathophysiological mechanism.

Acute and chronic pain are among the most common complications of SCA. While acute painful episodes are generally more frequent, the prevalence of chronic pain increases progressively with advancing age.[2-4] Painful vaso-occlusive crises

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(VOCs) typically arise from tissue infarction caused by sickled erythrocytes, most often affect a single anatomical site, and are usually characterized by severe intensity. VOCs are the most common acute complications of SCA and can contribute to the development of chronic pain, tissue-organ damage, and early mortality.[5-9] Acute VOCs frequently necessitate hospitalization and substantially impair quality of life.[10-14]

Vascular endothelial growth factor (VEGF), a glycoprotein closely linked to hypoxic signaling, plays a pivotal role in the generation of painful episodes by increasing vascular permeability, driving angiogenic remodeling, and enhancing endothelial cell adhesion through the upregulation of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1, acting as a potent mitogen for the vascular endothelium.[15-17] Beyond its proliferative effects, VEGF modulates endothelial integrin expression and promotes chemotaxis, thereby amplifying local inflammatory responses at the vessel wall.[18-20] This cycle of endothelial activation and injury is integral to the emergence of SCA-related complications.

The VEGF gene is highly polymorphic, and specific variants within its regulatory regions have been proposed as potential modulators of vaso-occlusive susceptibility in SCA. Despite the biological plausibility of a VEGF-VOC axis, empirical data directly linking VEGF gene polymorphisms to vaso-occlusive painful crisis frequency in SCA remain sparse. Against this background, the present study was undertaken to evaluate the potential relationship between the VEGF-A rs2010963 polymorphism and the frequency of vaso-occlusive painful crises in a cohort of adult patients with SCA.

Materials and Methods

This study was designed as a prospective investigation conducted by the Hematology Department of Hatay Mustafa Kemal University Research Hospital. A total of 189 subjects were enrolled, including 89 patients diagnosed with sickle cell anemia (SCA) carrying the HbSS genotype and aged 18 years or older who presented to the Hematology Clinic, and 100 healthy individuals serving as the control cohort. Ethical approval was obtained from the Ethical approval was obtained from the Ethics Committee of Hatay Mustafa Kemal University (Approval No: 01, dated 03/09/2020). Exclusion criteria included individuals younger than 18 years, persons with disabilities, pregnant women, and individuals lacking the legal capacity to provide informed consent. All participants underwent detailed medical history taking and physical examination, and the annual frequency of painful crises was recorded for patients with SCA. Biochemical analyses, including complete blood count, CRP, LDH, ferritin, vitamin B12, and folate, and genotyping were performed on peripheral blood samples. Comparative analyses were conducted to elucidate differences between the SCA cohort and healthy controls.

DNA Extraction and Genotyping

Genomic DNA isolation was performed from peripheral blood samples using a commercially available purification kit (GeneJET Genomic DNA Purification Kit; Thermo Fisher Scientific, Catalog No: K0721) in strict accordance with the manufacturer's instructions (Table 1). All samples were processed in 2 mL microcentrifuge tubes.

The extraction procedure involved sequential lysis, Proteinase K digestion at 55°C, ethanol precipitation, and spin-column purification with two wash steps, followed by elution in 50 µL of buffer. DNA concentration and purity were assessed spectrophotometrically (Multiskan GO; Thermo Fisher Scientific) using 260/280 nm absorbance ratios, and working stocks were prepared at 4 ng/µL in nuclease-free water. Genotyping of the VEGF-A rs2010963 variant was subsequently performed using allele-specific TaqMan SNP Genotyping Assays (Thermo Fisher Scientific, Catalog No: 4351379.rs2010963), as detailed in Table 2.

Statistical Analysis

All continuous variables were first evaluated for normality of distribution using the Kolmogorov-Smirnov test, and variance homogeneity was assessed using Levene's test. Demographic characteristics and laboratory parameters were summarized using descriptive statistics and frequency distributions. Given the non-normal distribution of continuous data, between-group comparisons were performed using the Mann-Whitney U test. Genotype frequencies were compared between groups and across clinical subgroups using Pearson's chi-square test. A two-tailed p-value of less than 0.05 was considered statistically signifi-

Table 1. Components of the DNA extraction kit

GeneJET Genomic DNA Purification Kit	#K0721 50 preps
Proteinase K Solution	1.2 ml
RNase A Solution	1 ml
Digestion Solution	11 ml
Lysis Solution	24 ml
Wash Buffer I	10 ml
Wash Buffer II	10 ml
Elution Buffer (10 mM Tris-Cl, PHT 9.0, 0.5 mM EDTA)	30 ml
Collection Tubes (2 ml)	50
Collection Tubes (1.5 ml)	50

Table 2. Contents of the Real- time PCR kit used

Component	20µL/reaction
5X HOT FIREPol Probe Universal qPCR Mix	4 µL
Prob	0.4 µL
Template DNA	5 µL
dH_2O	11 µL
Total	20 µL

cant. All analyses were conducted using IBM SPSS Statistics, version 23.0 (IBM Corp., Armonk, NY, USA).

Results

The SCA patient cohort consisted of 48 male and 41 female individuals, whereas the control group comprised 55 males and 45 females. The mean age was 34.83±10.71 years among patients and 38.35±16.69 years among controls (Table 3). A summary of laboratory parameters is provided in Table 3. Leukocyte counts were elevated in the patient group (13.11±8.87×10^3/µL) relative to controls (7.55±3.43×10^3/µL; p=0.001). Platelet counts were higher in patients (388.16±213.72×10^3/µL) than in healthy subjects (246.96±92.89×10^3/µL; p=0.001). Serum ferritin concentrations were greater in the SCA cohort (1215.28±1679.20 ng/mL) compared with controls (120.91±206.63 ng/mL; p=0.001). Inflammatory markers were also elevated in patients: CRP levels averaged 14.28±30.81 mg/L versus 3.17±3.93 mg/L in controls (p=0.001), and LDH values were 612.65±442.52 IU/L compared with 207.77±73.05 IU/L (p=0.001). Serum vitamin B12 concentrations did not differ between the two groups (442.18±283.32 pg/mL in patients vs. 491.16±282.22 pg/mL in controls; p=0.316). Folate levels, however, were higher in patients with SCA (15.75±14.47 ng/mL) than in controls (10.47±8.73 ng/mL; p=0.007).

Genotypic analysis of the VEGF rs2010963 polymorphism revealed that the heterozygous GC genotype was the most prevalent variant in both the SCA and control groups, followed by the homozygous mutant CC genotype and the wild-type GG genotype. The distribution of genotypes did not differ between patients and healthy controls (p=0.164) (Table 4). Furthermore, when patients with SCA were stratified according to their annual painful crisis frequency, no

Table 4. Distribution of VEGF-A (rs2010963) genotypes in patients and controls

		Patient n (%)	Control n (%)	p
VEGF-A (rs2010963)	Wild type (GG)	22 (24.7)	14 (14.0)	0.164
	Mutant (CC)	29 (32.6)	35 (35.0)	
	Heterozigous (GC)	38 (42.7)	51 (51.0)	

Table 5. VEGF rs2010963 polymorphism and painful crisis frequency

		Wild Type n (%)	Mutant n (%)	Heterozigous n (%)	p
Annual number of painful crises	No	8 (27.6)	10 (34.5)	11 (37.9)	0.536
	1-2	5 (15.6)	10 (31.3)	17 (53.1)	
	>2	9 (32.1)	9 (32.1)	10 (35.7)	

Table 3. Characteristics of the patient and control groups

	Patient	Control	
	n (%)	n (%)	
Sex			
Male	48 (53.9)	55 (55)	p
Female	41 (46.1)	45 (45)	
Age (Mean±SD)	34.83±10.71	38.35±16.69	
	Mean±SD (min-max)	Mean±SD (min-max)	
WBC (10^3/µl)	13.11±8.87 (1.54 – 73.31)	7.55±3.43 (0.44 – 25.29)	**<0.001**
Platelet (10^3/µl)	388.16±213.72 (33.00 – 1192.00)	246.96±92.89 (60.00 – 547.00)	**<0.001**
Ferritin(ng/ml)	1215.28±1679.2 (36.00 – 7947.00)	120.91±206.63 (3.50 – 1164.00)	**<0.001**
CRP (mg/L)	14.28±30.81(2.00 – 171.00)	3.17±3.93(0.00 – 24.00)	**<0.001**
LDH (IU/L)	612.65±442.52 (218.00 – 2724.00)	207.77±73.05 (118.00 – 548.00)	**<0.001**
Vitamin B12 (pg/ml)	442.18±283.32 (114.00 – 2010.00)	491.16±282.22 (158.00 – 1613.00)	0.316
Folat (ng/ml)	15.75±14.47 (22.50 – 95.00)	10.47±8.73 (2.00 – 64.00)	**0.007**

statistically significant association was identified between rs2010963 genotype and VOC frequency (p=0.536) (Table 5).

Discussion

The present study examined whether the VEGF rs2010963 polymorphism exerts a measurable influence on the frequency of vaso-occlusive crises (VOCs) in adult patients with sickle cell anemia (SCA). Our analyses did not reveal a relationship between rs2010963 genotype distribution and VOC occurrence, a finding that both diverges from and aligns with various lines of evidence in the existing literature, collectively underscoring the complex nature of genotype-phenotype correlations in SCA.

Although a mechanistic basis for linking aberrant VEGF expression to VOC development in SCA is biologically plausible, direct empirical evidence connecting VEGF polymorphisms or circulating VEGF concentrations to VOC pathogenesis remains relatively limited. Antwi-Boasiako et al. reported elevated serum VEGF levels in Ghanaian patients with SCA compared with healthy volunteers, with further increases noted during active VOC episodes.[21] Amle et al.[22] described an 18-base pair insertion-deletion polymorphism within the VEGF promoter, in which the deletion allele was associated with higher VEGF expression, greater microalbuminuria risk, and accelerated nephropathy progression in SCA. Farhan et al. reported an association between the AA genotype of the VEGF 2578 C/A (rs699947) polymorphism and elevated VOC risk in patients with SCA.[23] In a study by Al-Habboubi et al.[24], higher frequencies of the rs2010963 C allele, rs833068 A allele, and rs3025020 C allele were documented in patients with SCA experiencing VOC relative to those in steady state. Moreover, they reported differences in genotype distributions of rs2010963, rs833068, and rs3025020 between these groups. The risk of VOC was increased among heterozygous and, more prominently, homozygous carriers of the rs2010963 polymorphism, whereas the association with VOC was observed only in homozygous carriers for rs833068 and rs3025020.[24] Although no differences in serum VEGF levels were found between VOC and steady-state controls, a decline in serum VEGF levels was observed in heterozygous and homozygous genotypes of rs2010963 and rs833068, while the opposite trend was seen in patients with VOC. Additionally, rs2010963, rs833068, and rs3025020 polymorphisms were associated with VOC type, and rs3025020 was further linked to hospitalization, VOC treatment, and crisis duration.[24] Elevated VEGF levels have been demonstrated during vaso-occlusive painful crisis episodes.[25] In pediatric patients, the VEGFA −583C>T (rs3025020) polymorphism has been linked to the development of acute chest syndrome, with the minor −583T allele and the −583T/T genotype increasing the risk of acute chest syndrome.[26] Several considerations may account for these discrepant findings. The multifactorial etiology of VOC suggests that no single genetic variant is likely to function as a robust independent predictor of crisis susceptibility. Painful episodes in SCA emerge from a complex interplay among genetic determinants, particularly variants modulating fetal hemoglobin levels, environmental exposures such as infection and dehydration, dysregulated inflammatory pathways, oxidative stress, and hydration status.[27] Within this intricate pathophysiological matrix, the phenotypic contribution of rs2010963 may be attenuated or masked by other, more dominant genetic or environmental influences, rendering its effect size too modest to detect without adequately powered studies. Sample size represents another pertinent limitation; genetic association studies typically require large cohorts to reliably identify variants with small-to-moderate effect sizes, and the present study may have lacked sufficient statistical power to detect such associations. Genetic heterogeneity further complicates interpretation, as the functional impact of specific polymorphisms often varies across ethnic and geographic populations, implying that rs2010963 may behave differently depending on the ancestral background of the cohort under investigation.

The genetic architecture of acute pain in SCA appears to be considerably more complex than previously appreciated. A meta-analysis identified polymorphisms across multiple pain-related and inflammatory genes as contributors to acute pain susceptibility in SCA, while variants in several globin-regulatory loci were associated with attenuated pain responses.[28] Complementing these observations, Rampersaud et al. constructed a polygenic risk score for acute pain crises in a pediatric SCA population, integrating 21 variants distributed across 9 independent genomic loci.[29] Taken together, these findings reinforce the notion that pain susceptibility in SCA is governed by a polygenic framework, within which the contribution of any individual variant, including rs2010963, is likely to be modest and context-dependent.

Several limitations inherent to this study warrant acknowledgment. The scope of genetic analysis was confined to the rs2010963 variant, precluding a broader evaluation of other potentially relevant VEGF or inflammatory gene polymorphisms. The absence of serum VEGF measurements limits our ability to draw inferences regarding the functional consequences of rs2010963 on protein expression in this cohort. The ethnic homogeneity of the study population may restrict the generalizability of our findings to other demographic groups. Finally, important clinical covariates

known to modulate VOC frequency, including hydroxyurea use, infection status, and lifestyle-related factors, were not comprehensively controlled, introducing potential confounding that may have influenced the observed results.

Future investigations should consider haplotype-based analyses incorporating rs2010963 alongside other functionally relevant VEGF SNPs, as well as multigene panels targeting inflammatory and pain-related pathways. Functional studies designed to assess VEGF transcription, circulating protein levels, and vascular responsiveness in relation to genotype would substantially advance mechanistic understanding. Expanding recruitment to ethnically diverse, multicenter cohorts will be essential for disentangling the effects of genetic background. Integrating pharmacogenetic dimensions, particularly with respect to hydroxyurea efficacy and opioid responsiveness, may ultimately inform the development of genotype-guided, individualized therapeutic strategies in SCA.

Conclusion

The present study provides evidence that the VEGF-A rs2010963 polymorphism, considered in isolation, does not serve as a reliable predictor of vaso-occlusive crisis frequency in adult patients with sickle cell anemia. Rather than diminishing the relevance of the VEGF pathway in SCA pathophysiology, this null finding redirects attention toward the broader genomic and environmental context within which individual variants operate. Vaso-occlusive crises are not driven by a single molecular switch; they emerge from the convergence of multiple interacting risk factors spanning inflammatory signaling, hemoglobin polymerization dynamics, endothelial dysfunction, and patient-specific environmental exposures. Single-variant association studies, while informative, are inherently limited in their capacity to capture this complexity. Progress in this field will therefore depend on shifting toward multilocus haplotype analyses, polygenic risk modeling, and integrative studies that incorporate serum biomarker data alongside genotypic information. Equally important is the inclusion of ethnically and geographically diverse cohorts to ensure that findings are broadly applicable rather than population-specific. Ultimately, a deeper and more nuanced understanding of the genetic contributors to VOC susceptibility holds real clinical promise, offering a pathway toward risk stratification tools and personalized therapeutic interventions that could meaningfully reduce the burden of this devastating complication in patients with sickle cell anemia.

Disclosures

Ethics Committee Approval: This study was approved by The Research Ethics Committee of Hatay Mustafa Kemal University (Approval No: 01, dated 03/09/2020) and conducted in accordance with the ethical standards of the 1964 Declaration of Helsinki.

Informed Consent: Written informed consents were obtained from patients who participated in this study.

Conflict of Interest: The author declare that there is no conflict of interest.

Use of AI for Writing Assistance: The authors declared that artificial intelligence was not used in the study.

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References

1. GBD 2021 Sickle Cell Disease Collaborators. Global, regional, and national prevalence and mortality burden of sickle cell disease, 2000-2021: a systematic analysis from the Global Burden of Disease Study 2021. Lancet Haematol 2023;10(8):e585–99.
2. Brousseau DC, Owens PL, Mosso AL, Panepinto JA, Steiner CA. Acute care utilization and rehospitalizations for sickle cell disease. JAMA 2010;303(13):1288–94. [CrossRef]
3. Sil S, Cohen LL, Dampier C. Psychosocial and functional outcomes in youth with chronic sickle cell pain. Clin J Pain 2016;32(6):527–33. [CrossRef]
4. Smith WR, Penberthy LT, Bovbjerg VE, McClish DK, Roberts JD, Dahman B, et al. Daily assessment of pain in adults with sickle cell disease. Ann Intern Med 2008;148(2):94-101. [CrossRef]
5. Kato GJ, Piel FB, Reid CD, Gaston MH, Ohene-Frempong K, Krishnamurti L, et al. Sickle cell disease. Nat Rev Dis Primers 2018;4:18010. [CrossRef]
6. Piel FB, Steinberg MH, Rees DC. Sickle cell disease. N Engl J Med 2017;376(16):1561–73. [CrossRef]
7. Rai P, Ataga KI. Drug therapies for the management of sickle cell disease. F1000Res 2020;9:F1000 Faculty Rev-592. [CrossRef]
8. Ware RE, de Montalembert M, Tshilolo L, Abboud MR. Sickle cell disease. Lancet 2017;390(10091):311–23. [CrossRef]
9. Darbari DS, Sheehan VA, Ballas SK. The vaso-occlusive pain crisis in sickle cell disease: Definition, pathophysiology, and management. Eur J Haematol 2020;105(3):237–46. [CrossRef]
10. Ballas SK, Lusardi M. Hospital readmission for adult acute sickle cell painful episodes: frequency, etiology, and prognostic significance. Am J Hematol 2005;79(1):17–25. [CrossRef]
11. Yusuf HR, Atrash HK, Grosse SD, Parker CS, Grant AM. Emergency department visits made by patients with sickle cell

disease: a descriptive study, 1999-2007. Am J Prev Med 2010;38(4 Suppl):S536–41. [CrossRef]
12. Evensen CT, Treadwell MJ, Keller S, Levine R, Hassell KL, Werner EM, et al. Quality of care in sickle cell disease: Cross-sectional study and development of a measure for adults reporting on ambulatory and emergency department care. Medicine (Baltimore) 2016;95(35):e4528. [CrossRef]
13. Udeze C, Evans KA, Yang Y, Lillehaugen T, Manjelievskaia J, Mujumdar U, et al. Economic and clinical burden of managing sickle cell disease with recurrent vaso-occlusive crises in the United States. Adv Ther 2023;40(8):3543–58. [CrossRef]
14. van Tuijn CF, van Beers EJ, Schnog JJ, Biemond BJ. Pain rate and social circumstances rather than cumulative organ damage determine the quality of life in adults with sickle cell disease. Am J Hematol 2010;85(7):532–5. [CrossRef]
15. Stannard AK, Khurana R, Evans IM, Sofra V, Holmes DI, Zachary I. Vascular endothelial growth factor synergistically enhances induction of E-selectin by tumor necrosis factor-alpha. Arterioscler Thromb Vasc Biol 2007;27(3):494–502. [CrossRef]
16. Ferrara N. Molecular and biological properties of vascular endothelial growth factor. J Mol Med (Berl) 1999;77(7):527–43. [CrossRef]
17. Connolly DT, Heuvelman DM, Nelson R, Olander JV, Eppley BL, Delfino JJ, et al. Tumor vascular permeability factor stimulates endothelial cell growth and angiogenesis. J Clin Invest 1989;84(5):1470–8. [CrossRef]
18. Senger DR, Ledbetter SR, Claffey KP, Papadopoulos-Sergiou A, Peruzzi CA, Detmar M. Stimulation of endothelial cell migration by vascular permeability factor/vascular endothelial growth factor through cooperative mechanisms involving the alphavbeta3 integrin, osteopontin, and thrombin. Am J Pathol 1996;149(1):293–305.
19. Connolly DT, Olander JV, Heuvelman D, Nelson R, Monsell R, Siegel N, et al. Human vascular permeability factor. Isolation from U937 cells. J Biol Chem 1989;264(33):20017–24. [CrossRef]
20. Kim YJ, Chung WC, Jun KH, Chin HM. Genetic polymorphisms of vascular endothelial growth factor (VEGF) associated with gastric cancer recurrence after curative resection with adjuvant chemotherapy. BMC Cancer 2019;19(1):483. [CrossRef]
21. Antwi-Boasiako C, Frimpong E, Gyan B, Kyei-Baafour E, Sey F, Dzudzor B, et al. Elevated proangiogenic markers are associated with vascular complications within ghanaian sickle cell disease patients. Med Sci (Basel) 2018;6(3):53. [CrossRef]
22. Amle DB, Patnayak RL, Verma V, Singh GK, Jain V, Khodiar PK, et al. VEGF promoter region 18-bp insertion-deletion polymorphism in sickle cell disease patients with microalbuminuria: a pilot study. Indian J Hematol Blood Transfus 2019;35(2):278–83. [CrossRef]
23. Farhan MS, Kamel MM, El-Desouky MA, Abdelhamid NA. Association of vascular endothelial growth factor gene polymorphism 2578C/A with vaso-occlusive crisis in sickle cell disease. Menoufia Med J 2023;36(1):16.
24. Al-Habboubi HH, Mahdi N, Abu-Hijleh TM, Abu-Hijleh FM, Sater MS, Almawi WY. The relation of vascular endothelial growth factor (VEGF) gene polymorphisms on VEGF levels and the risk of vasoocclusive crisis in sickle cell disease. Eur J Haematol 2012;89(5):403–9. [CrossRef]
25. Gürkan E, Tanriverdi K, Başlamişli F. Clinical relevance of vascular endothelial growth factor levels in sickle cell disease. Ann Hematol 2005;84(2):71–5. [CrossRef]
26. Redha NA, Mahdi N, Al-Habboubi HH, Almawi WY. Impact of VEGFA -583C>T polymorphism on serum VEGF levels and the susceptibility to acute chest syndrome in pediatric patients with sickle cell disease. Pediatr Blood Cancer 2014;61(12):2310–2. [CrossRef]
27. Rees DC, Williams TN, Gladwin MT. Sickle-cell disease. Lancet 2010;376(9757):2018–31. [CrossRef]
28. Gehling GM, Powell-Roach K, Wilkie DJ, Dungan JR. Single nucleotide polymorphisms and sickle cell disease-related pain: a systematic review. Front Pain Res (Lausanne) 2023;4:1223309. [CrossRef]
29. Rampersaud E, Kang G, Palmer LE, Rashkin SR, Wang S, Bi W, et al. A polygenic score for acute vaso-occlusive pain in pediatric sickle cell disease. Blood Adv 2021;5(14):2839–51. [CrossRef]

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Research Article

Recurrence of IgA Nephropathy in Protocol Kidney Biopsies After Renal Transplantation and Its Impact on Prognosis

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Abstract

Objectives: IgA nephropathy (IgAN) is a common primary glomerulonephritis with a wide clinical spectrum ranging from asymptomatic urinary abnormalities to rapidly progressive disease and is a major cause of chronic kidney failure. Although kidney transplantation is an effective treatment for primary IgAN, recurrence and graft loss remain significant concerns.

Methods: This study investigated predictors of post-transplant IgAN recurrence and graft loss. Seventy-six patients who underwent kidney transplantation due to end-stage kidney disease secondary to primary IgAN were retrospectively analyzed. First-year protocol biopsy results (6-month and 12-month) were evaluated using light microscopy and immunofluorescence. Baseline demographic, clinical, and laboratory data, along with post-transplant parameters, indication biopsies after the first year, graft loss, and mortality were analyzed.

Results: Binary logistic regression was used to identify predictors of recurrence, and Kaplan–Meier analysis assessed graft survival. Group comparisons were performed using appropriate statistical tests, with $p<0.05$ considered significant.

Conclusion: Recurrence rates were 9.3% at 6 months and 26.3% at 12 months. Isolated IgA deposition detected by immunofluorescence was common. Age at transplantation was independently associated with recurrence; each additional year reduced risk by 6%. No association was found between recurrence and 10-year graft survival. Younger age was strongly associated with recurrence.

Keywords: Biopsy, IgA nephropathy, kidney transplantation, prognosis, recurrence

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IgA nephropathy (IgAN) is one of the most common primary glomerulonephritides worldwide. The clinical course of IgAN may range from mild disease presenting with asymptomatic hematuria and proteinuria detected during routine screening to severe disease, such as acute rapidly progressive glomerulonephritis.

Previous studies have reported that a considerable proportion of patients with IgAN may progress to kidney failure during long-term follow-up.[1]

In patients who undergo kidney transplantation, close monitoring of transplant kidney function during follow-up is of great importance. Especially in the first year after transplantation, and subsequently at regular intervals, graft function should be closely monitored for possible allograft rejection,

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recurrence of the native kidney disease, or infections. Even when laboratory values are normal, rejection may be detected in follow-up biopsies (protocol biopsies), and this condition, referred to as subclinical rejection, has been reported to be associated with poorer kidney survival in subsequent years.

In our center, as in many centers worldwide, in addition to indication biopsies (performed for impaired kidney function, active urinary findings, etc.), protocol biopsies are performed in eligible patients to evaluate graft function and adjust treatments accordingly.

In this retrospective study, we aimed to determine the frequency of IgAN recurrence using sequential protocol biopsies in the population of patients who underwent kidney transplantation due to IgAN at a tertiary care center, to identify predictive factors, and to evaluate the clinical course of patients with IgA deposition without clinical evidence of disease.

Materials and Methods

This retrospective, single-center study included 76 patients who had follow-up records at a tertiary care kidney transplant outpatient clinic between June 2000 and June 2019. Pathological results of kidney biopsies were evaluated from retrospective follow-up files.

Sociodemographic characteristics and clinical and laboratory data at the time of transplantation and at the 6-month and 12-month post-transplant follow-up periods were recorded in a case report form, and a database was created. Six-month and 1-year protocol biopsy data were evaluated and entered into the database.

Patients aged ≥18 years with primary IgAN who underwent kidney transplantation due to end-stage kidney disease, had a post-transplant follow-up duration of at least 6 months, and had immunofluorescence staining performed in both the 6-month and 1-year protocol biopsies were included in the study. Exclusion criteria were the absence of immunofluorescence staining in protocol biopsies, missing clinical/laboratory data, or initiation of treatment at an external center. Clinical, laboratory, and treatment-related data were obtained from electronic medical records.

Independent variables included recipient age, sex, duration of kidney failure, transplantation type, number of transplantations, degree of tissue matching, donor age, donor sex, donor relationship, post-transplant medications, serum urea and creatinine levels, urinalysis findings, spot urine protein/creatinine ratio, presence of microscopic or macroscopic hematuria, and blood pressure values. Dependent variables were the presence of IgAN recurrence in follow-up kidney biopsies and graft survival.

Statistical Analysis

Binary logistic regression analysis was used to identify variables predicting post-transplant IgA recurrence. Kaplan–Meier analysis was used to determine the relationship between IgA recurrence and graft survival. SPSS version 22.0 (IBM Corp., Armonk, NY, USA) was used for descriptive statistics and group comparisons. Student's t-test and the Mann–Whitney U test were used for parametric and non-parametric variables, respectively, and the chi-square test was used for categorical variables. A p-value <0.05 was considered statistically significant.

Ethics Approval

Ethical approval was obtained on Ege University Medical Research Ethics Committee January 21, 2021 (decision number: 21-1.1T/57).

This study was conducted in accordance with the principles of the Declaration of Helsinki.

Results

Patient Characteristics

Data from 76 patients were retrospectively analyzed. The mean age at transplantation was 37.2±9.7 years. Seventy-nine percent of the patients were male. Preemptive transplantation was performed in 45% of cases. Before transplantation, 51% of patients were receiving chronic hemodialysis and 3% were on peritoneal dialysis (Table 1).

The mean duration of dialysis before transplantation was 14.6±29.5 months. Eleven percent of the patients underwent a second kidney transplantation.

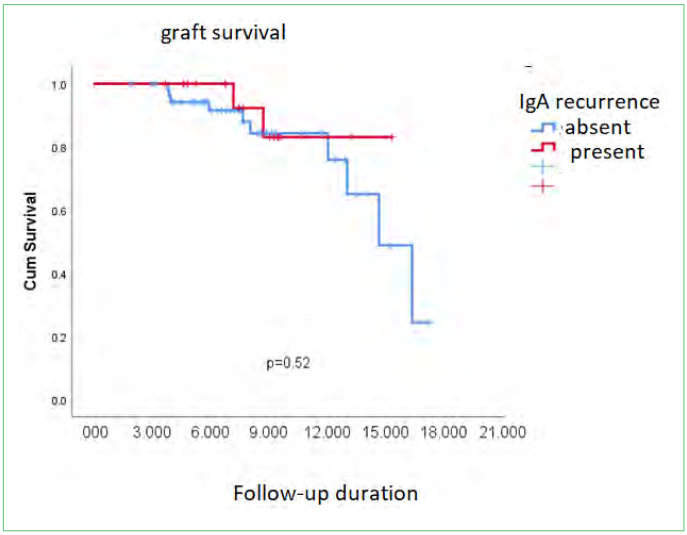

Figure 1. Shows graft survival in patients with and without IgA recurrence detected in the 1-year protocol biopsies.

Table 1. Characteristics of all patients and comparison between patients with and without IgA nephropathy recurrence in 1-year protocol biopsies after transplantation

	Overall group (n=76)	IgA recurrence negative (n=56)	IgA recurrence positive (n=20)	p
Follow-up duration (years)	7.4±2.2	7.2±2.3	7.7±2.1	0.53
Age at transplantation (years)	37.2±9.7	38.5±8.6	33.4±11.2	0.10
Gender (M, %)	79	78	85	0.15
Pre-transplant hemodialysis duration (months)	14.6±29.5	15.5±31.8	13.3±24.2	0.46
Pretransplant RRT modality				
Hemodialysis (%)	51	50	55.0	
Peritoneal dialysis (%)	3	1.9	5.0	0.77
Hemodialysis -Peritoneal dialysis (%)	1	1.9	-	
Preemptive Transplant (%)	45	46.3	40.0	0.61
Number of transplants (%)				
Transplant	89	85	100	0.02
Transplant	11	15	-	
Donor type (%)				
Living Related	63	59.3	80.0	0.05
Living-unrelated	21	20.4	15.0	0.12
Cadaveric	16	20.4	5	0.02
Age at donation (years)	50.1±11.8	51.8±12.0	45.6±11.0	0.46
Donor gender (M, %)	51	50	55	0.45
Haplotype match (%)				
1-haplotype match	88	90	85	0.84
Fullmatch	7	7	7	
immunosuppression)(%)				
Corticosteroids	100	100	100	-
Mycophenolate mofetil	97.3	100	90	0.07
Cyclosporine	52.7	53.7	50.0	0.77
Tacrolimus	45.9	44.4	50.0	0.79
Rapamycin	1.6	1.9	-	-
Induction therapy (%)				
ATG (%)	77.5	75.0	84.2	
Basiliximab (%)	11.3	13.5	5.3	0.56
None	11.3	11.5	10.5	

Regarding donor type, 63% received grafts from living related donors, 21% from living unrelated donors, and 16% from deceased donors. The mean donor age was 50.1±11.8 years, and 51% of donors were male. In terms of tissue matching, the full-match rate was 7%, and the 1-haplotype match rate was 88%.

The mean follow-up duration was 7.4±2.2 years. All patients received corticosteroid treatment after kidney transplantation; mycophenolate mofetil was used in 97%, cyclosporine in 45%, and tacrolimus in 45%. Induction therapy consisted of ATG in 77.5% and basiliximab in 11.3%, while 11.3% received no induction therapy.

The 3-month, 6-month, and 12-month post-transplant follow-up data are presented in Table 2. During the first 3 months after transplantation, the mean baseline creatinine level was 1.44±0.31 mg/dL, the mean proteinuria level was 0.21±0.19 g/day, and the frequency of microscopic hematuria was 13%.

At the 6-month protocol biopsy, the mean serum creatinine level was 1.44±0.34 mg/dL, proteinuria was 0.19±0.23 g/day, and the frequency of hematuria was 19%. Mean systolic and diastolic blood pressures were 131±17 mmHg and 83±10 mmHg, respectively. IgA recurrence was detected in 9.3% (n=7) of 6-month protocol biopsies (Table 3). Among these 7 patients, 5 (71%) had IgA deposition detected only by immunofluorescence staining, while 2 patients were diagnosed with IgA recurrence by both light microscopy and immunofluorescence. In the remaining 69 patients (90.7%), no IgA recurrence was detected in the 6-month biopsies. Among patients without recurrence at 6 months, focal tubulointerstitial nephritis was detected in 7, polyoma nephropathy in 2, and findings consistent with thrombotic

Table 2. First-year follow-up data of patients with and without IgA nephropathy recurrence

	All group	IgA recurrence negative	IgA recurrence positive	p
3 months				
Creatinine (mg/dL)	1.44±0.31	1.45±0.31	1.38±0.36	0.32
Proteinuria (g/day)	0.21±0.19	0.20±0.20	0.23±0.18	0.97
Hematuria (%)	13	10	23	0.23
6 months				
Systolic BP (mmHg)	131±17	130±17	133± 17	0.69
Diastolic BP (mmHg)	83±10	82±10	84 ±11	0.30
Creatinine (mg/dL)	1.44±0.34	1.47±0.36	1.39±0.27	0.17
Proteinuria (g/day)	0.19±0.23	0.18±0.23	0.21±0.25	0.33
Hematuria (%)	19	12	30	0.06
12 months				
Systolic BP (mmHg)	130±14	130±14	130±13	0.99
Diastolic BP (mmHg)	83±9	82±6	83±11	0.63
Creatinine (mg/dL)	1.43±0.36	1.46±0.37	1.34±0.32	0.20
Proteinuria (g/day)	0.18±0.21	0.19±0.25	0.16±0.14	0.58
Hematuria (%)	21	22	15	0.53

Table 3. Distribution of IgA nephropathy recurrence rates in 6-month and 12-month protocol biopsies

	IgA nephropathy recurrence positive			IgA nephropathy recurrence negative
	Total (n)	IM + IF	IF	Total (n)
6-month biopsy	7 (9.3%)	2 (29%)	5 (71%)	69 (90.7%)
12-month biopsy	20 (26.3%)	3 (15%)	17 (85%)	56 (73.7%)

microangiopathy in 1, while the remaining biopsies were considered nonspecific.

At the 12-month protocol biopsy, the mean serum creatinine level was 1.43±0.36 mg/dL, proteinuria was 0.18±0.21 g/day, and the frequency of hematuria was 21% (Table 2). Mean systolic and diastolic blood pressures were 130±14 mmHg and 83±9 mmHg, respectively. The recurrence rate of IgA nephropathy in the 1-year protocol biopsies was 26.3% (n=20), which was approximately three times higher than the rate detected at 6 months (Table 3). Among the 20 patients with recurrence, 17 (85%) had isolated IgA deposition detected only by immunofluorescence staining, while 3 patients were diagnosed by both light microscopy and immunofluorescence. In the remaining 56 patients (73.7%), no IgA recurrence was detected in the 12-month biopsies. Among those without recurrence, tubulointerstitial nephritis was reported in 9, polyoma nephropathy in 1, and glomerulitis with peritubular capillaritis in 1.

The baseline clinical and laboratory characteristics of patients with and without recurrence detected in the first year are presented in Table 1. When patients with IgA nephropathy recurrence in the 1-year follow-up biopsies were compared with those without recurrence, no differences were observed between the two groups in terms of pre-transplant dialysis duration, donor age, donor sex, haplotype matching, baseline immunosuppression, or induction therapies. Although patients with IgA nephropathy recurrence were younger and had a higher proportion of males, these differences were not statistically significant. Preemptive transplantation rates were similar between the two groups. The proportion of first-time transplantation was significantly higher among patients with IgA nephropathy recurrence, and the frequency of living-related donor transplantation was also significantly higher in this group. In addition, the use of mycophenolate mofetil as baseline immunosuppressive therapy tended to be less frequent in the recurrence group, approaching statistical significance.

First-year Data of Patients with and without IgA Nephropathy Recurrence

When parameters related to IgAN recurrence were examined, adjusted regression analysis including recipient age, recipient sex, number of transplantations, and donor type identified age at transplantation as an independent predictor of IgA recurrence on the 1-year biopsy (RR: 0.94, 95% CI: 0.88–0.99, p=0.04). Each additional year of age was associated with a 6% reduction in the risk of IgA recurrence.

Ten-year graft survival was 90% in the group with IgA recurrence and 87% in the group without IgA recurrence. During the study period, only one patient died, and at the end of the study, 87% of the patients continued follow-up with a functioning graft.

Discussion

IgAN is the most common primary glomerulonephritis and leads to end-stage kidney disease in 30–50% of patients within 20 to 30 years after diagnosis.[2] Because patients with IgAN are typically younger and have fewer comorbidities, they are among the most suitable candidates for kidney transplantation. However, recurrence of the original disease in the graft is a known complication of IgAN and often results in decreased graft function. Other glomerular or vascular lesions due to acute or chronic graft rejection may also negatively affect graft function.[2,3]

In previous studies, the male-to-female ratio has been reported to vary from less than 2:1 in Asian populations to as high as 6:1 in Europe and the United States. In our study, the vast majority of patients were male (79%), with an approximate male-to-female ratio of 4:1.[4-7]

While some studies have suggested that kidney disease progression occurs faster in men than in women, others have argued that sex differences are not determinants of the risk of renal progression.[4,8,9] In studies concluding that IgAN progresses more rapidly in men, possible mechanisms underlying the renal protective role of female sex are thought to be related to estrogen.[10] In our study, the frequency of male sex was moderately higher in the recurrence group (85% vs. 78%, p=0.15). Similarly, in the study by Maixnerova et al., 80% of patients with recurrence were male.[2] In some studies in the literature, male sex has been associated with a higher risk of recurrence, consistent with our findings.[11-13] In the study by Pippias et al. reporting 15-year post-transplant follow-up results, 71.2% of patients were male, and the median age was 47.5 years.[14] In our study, the mean age was 37.2±9.7 years. Overall, the sociodemographic characteristics of our cohort appear consistent with the literature. Additionally, younger age was identified as a significant risk factor for IgAN recurrence in 1-year protocol biopsies.

Among the 76 patients included, 89% underwent a first transplantation. The mean age at transplantation was 37.2±9.7 years. In the retrospective analysis of 62 patients who underwent transplantation due to IgAN by Avasare et al.[15], the mean age at transplantation was 38.1±12.2 years, and the duration of dialysis was approximately 10 months. In our study, the mean dialysis duration in 42 patients receiving hemodialysis or peritoneal dialysis before transplantation was 14.6±29.5 months. In Pippias's study[16], although the mean age at transplantation (47.5 years) was slightly higher than ours, the mean pre-transplant dialysis duration was similar at approximately 13 months. In the study by Maixnerova et al.[2], including 313 patients, the mean age at transplantation was 46 years, similar to Pippias's findings.

In our study, the living-donor transplantation rate was 84%, the mean donor age was 50.1±11.8 years, and the most common donor type was living-related donors (63%). In the study by Boğa[16], the living-donor rate was 63.2%, 54.3% of living donors were first-degree relatives, and the mean donor age was 32.2±11.7 years. In Ünsal's[17] study, the mean donor age was 44±14.5 years, and 59% were living donors. According to the Turkish Nephrology, Dialysis and Transplantation Registry 2015 report, the predominance of male recipients and the highest transplantation frequency in the 20–45 age group are consistent with our findings.[16,18] Similarly, Avasare et al.[15] reported that 74% of transplants were from living donors. In Ünsal's[17] study, unlike ours, 53% of donors were female.

In the article by Şantaş evaluating the current status of organ donation in Türkiye, it was stated that organs used for transplantation are largely obtained through donation from relatives, whereas in developed countries, most organ needs are met through deceased donors. A major issue in developing countries, including Türkiye, is the insufficient number of deceased donor transplants.[19]

In our cohort, the most frequently used medications after transplantation were corticosteroids, mycophenolate mofetil, cyclosporine, and tacrolimus. Most patients received ATG as induction therapy. In the literature, early initiation of immunosuppressive regimens in patients with IgAN at risk of progression has been reported to slow progression to end-stage kidney disease.[2] In the study by Ordin et al.[20], patients used an average of 6.72±1.92 (min–max: 2–13) medications daily; 76% received tacrolimus, 48% received cyclosporine, and 8% received everolimus-containing immunosuppressive therapy, and 36.7% used triple therapy consisting of corticosteroids, tacrolimus, and mycophenolate mofetil.

Although immunosuppression protocols vary between centers, the goal is to achieve the lowest acute rejection rate and the highest patient and graft survival. Therefore, except for transplants between identical twins, continuous immunosuppressive therapy is essential for long-term graft function, either as induction or maintenance therapy.[21] In our study, no significant difference was found between patients with and without IgAN recurrence in 1-year protocol biopsies in terms of immunosuppressive treatment regimens.

In our study, the graft loss rate was 10.8% during a mean follow-up period of 7.4±2.2 years, and no significant association was found between IgAN recurrence in protocol biopsies and 10-year graft survival. Graft survival was similar between patients with and without recurrence. Only one patient death occurred. In the literature, graft loss due to IgAN recurrence has been reported to be approximately 3–4%.[15-22] Moroni et al.[12] reported graft loss due to IgAN recurrence at a rate of 8.4%, which is similar to our findings. Clayton et al.[23] also reported that recurrent IgAN was the cause of graft loss in 12.6% of patients transplanted for primary IgAN, which is close to our result.

In our study, the characteristics of patients with and without recurrence in 1-year protocol biopsies were compared. Although there were numerical differences between the two groups in variables such as follow-up duration, age at transplantation, recipient sex, pre-transplant hemodialysis, RRT type, preemptive transplantation, donor sex, haplotype matching, immunosuppressive treatment, and induction therapy, these differences were not statistically significant. Chailimpamontree et al.[24] reported that age at transplantation was not associated with recurrence risk. In contrast to our findings, Avasare et al.[15] reported that age at transplantation was higher in patients with recurrence, and this difference was statistically significant.

In our results, a statistically significant association was found between recurrence and the number of transplantations and donor type. Similar to our findings, Han et al.[13] reported a significant relationship between living-related donation and recurrence, and Choy et al.[25] stated that living-related donation increased the likelihood of IgAN recurrence 2.5-fold. Conversely, in Han's[13] study, the presence of anti-HLA antibodies was not associated with recurrent IgAN, and chronic allograft nephropathy was more frequent in deceased-donor transplantation. In our study, similarly, no significant association was found between haplotype matching and recurrence.

In adjusted analyses, each additional year of recipient age was associated with a 6% reduction in the risk of IgA recurrence. In the study by Moroni et al.[12], transplant year, younger age, and immunosuppressive use were identified as predictive variables for recurrence. Han et al.[13] also reported that younger age at transplantation was associated with a higher risk of recurrence.

Since Berger's first report,[102] many studies have evaluated recurrent IgAN.[13,15,24-26] In our study, the IgAN recurrence rate was 26.3% in 1-year protocol biopsies and 65% in indication biopsies performed beyond the first year. Kim et al.[27] reported a 44% risk of recurrent IgAN after 10 years, while Han et al.[13] reported 30.8%. We believe these differences may be due to the ethnic characteristics of the studied populations and the frequent use of indication biopsies in our center when graft dysfunction or active urinary findings are detected.

Conclusion

In our study, 10-year graft loss was found to be 10%. This rate was similar in patients with and without IgAN recurrence in 1-year protocol biopsies. The rate of living-donor transplantation was higher among patients with IgAN recurrence. Younger age at the time of transplantation was a risk factor for IgAN recurrence (RR: 0.94, 95% CI: 0.88–0.99, p=0.04). Each additional year of age was associated with a 6% reduction in the risk of IgA recurrence.

In our study, the recurrence rate of IgAN was 26.3% in 1-year protocol biopsies among all patients and 65% in indication biopsies during the study period. The wide range of recurrence rates reported in the literature may be explained by differences in the baseline characteristics of study populations and biopsy policies. In addition, the inclusion of small sample sizes and/or patients from multiple ethnic backgrounds, as well as varying biopsy strategies, may lead to differences in reported findings. Although our study includes data from a single center, our findings regarding the risk of recurrent IgAN are considered reliable due to multivariable analyses, long-term follow-up results, and the relatively large sample size compared with the existing literature. Our data also showed that immunosuppressive use and induction therapy were not associated with the prevention of recurrent IgAN. Moreover, no significant association was found between haplotype matching and recurrent IgAN.

Although our results contribute to the literature, our study has several limitations. The study design was retrospective, and all patients in our dataset were from a single center.

Although further prospective, well-designed studies are needed, our data may help evaluate risk factors for IgAN recurrence in protocol biopsies and determine prognosis in recurrent IgAN among kidney transplant recipients.

Disclosures

Ethics Committee Approval: This study was approved by The Ege University Medical Research Ethics Committee (January 21,2021 / 21-1.1T/57).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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References

1. Akın S. İmmünglobulin A nefropatisinin klinik ve patolojik özelliklerinin retrospektif olarak incelenmesi [Dissertation]. İzmir: T.C. Ege Üniversitesi, Tıp Fakültesi, İç Hastalıkları Anabilim Dalı; 2012. [in Turkish]
2. Maixnerova D, Hruba P, Neprasova M, Bednarova K, Slatinska J, Suchanek M, et al. Outcome of 313 Czech patients with IgA nephropathy after renal transplantation. Front Immunol 2021;12:726215. [CrossRef]
3. Working Group of the International IgA Nephropathy Network and the Renal Pathology Society; Cattran DC, Coppo R, Cook HT, Feehally J, Roberts IS, et al. The Oxford classification of IgA nephropathy: rationale, clinicopathological correlations, and classification. Kidney Int 2009;76:534–45.
4. Deng W, Tan X, Zhou Q, Ai Z, Liu W, Chen W, et al. Gender-related differences in clinicopathological characteristics and renal outcomes of Chinese patients with IgA nephropathy. BMC Nephrol 2018;19(1):31. [CrossRef]
5. Geddes CC, Rauta V, Gronhagen-Riska C, Bartosik LP, Jardine AG, Ibels LS, et al. A tricontinental view of IgA nephropathy. Nephrol Dial Transplant 2003;18(8):1541–8. [CrossRef]
6. Donadio JV, Grande JP. IgA nephropathy. N Engl J Med 2002;347(10):738–48. [CrossRef]
7. Duan ZY, Cai GY, Chen YZ, Liang S, Liu SW, Wu J, et al. Aging promotes progression of IgA nephropathy: a systematic review and meta-analysis. Am J Nephrol 2013;38(3):241–52. [CrossRef]
8. Riispere Ž, Laurinavičius A, Kuudeberg A, Seppet E, Sepp K, Ilmoja M, et al. IgA nephropathy clinicopathologic study following the Oxford classification: Progression peculiarities and gender-related differences. Medicina (Kaunas) 2016;52(6):340–8. [CrossRef]
9. Moriyama T, Tanaka K, Iwasaki C, Oshima Y, Ochi A, Kataoka H, et al. Prognosis in IgA nephropathy: 30-year analysis of 1,012 patients at a single center in Japan. PLoS One 2014;9(3):e91756. [CrossRef]
10. Garovic VD, August P. Sex Differences and Renal Protection: Keeping in Touch with Your Feminine Side. J Am Soc Nephrol 2016;27(10):2921–4. [CrossRef]
11. Ponticelli C, Glassock RJ. Posttransplant recurrence of primary glomerulonephritis. Clin J Am Soc Nephrol 2010;5(12):2363–72. [CrossRef]
12. Moroni G, Longhi S, Quaglini S, Gallelli B, Banfi G, Montagnino G, et al. The long-term outcome of renal transplantation of IgA nephropathy and the impact of recurrence on graft survival. Nephrol Dial Transplant 2013;28(5):1305–14. [CrossRef]
13. Han SS, Huh W, Park SK, Ahn C, Han JS, Kim S, et al. Impact of recurrent disease and chronic allograft nephropathy on the long-term allograft outcome in patients with IgA nephropathy. Transpl Int 2010;23(2):169–75. [CrossRef]
14. Pippias M, Stel VS, Areste-Fosalba N, Couchoud C, Fernandez-Fresnedo G, Finne P, et al. Long-term kidney transplant outcomes in primary glomerulonephritis: analysis from the ERA-EDTA registry. Transplantation 2016;100(9):1955–62. [CrossRef]
15. Avasare RS, Rosenstiel PE, Zaky ZS, Tsapepas DS, Appel GB, Markowitz GS, et al. Predicting post-transplant recurrence of iga nephropathy: the importance of crescents. Am J Nephrol 2017;45(2):99–106. [CrossRef]
16. Boğa İ. *Böbrek nakli yapılan hastalarda böbrek biyopsi sonuçlarının prognoza etkisi* [dissertation]. Ankara: Hacettepe Üniversitesi, Tıp Fakültesi, İç Hastalıkları Anabilim Dalı; 2017. [in Tukish]
17. Ünsal A, Tanrısev M, Oluç C. Böbrek nakli yapılan hastalarda proteinüri sıklığı ve nedenleri. İstanbul Bilim Üniversitesi Florence Nightingale Tıp Dergisi 2016;2:4–11. [Article in Turkish] [CrossRef]
18. Süleymanlar G, Ateş K, Seyahi N. *Türkiye'de nefroloji, diyaliz ve transplantasyon: Registry 2015*. Ankara: Türk Nefroloji Derneği Yayınları; 2016. [in Tukish]
19. Şantaş G, Şantaş F. Türkiye'de organ bağışının mevcut durumu ve organ bağışında stratejik iletişimin önemi. Süleyman Demirel Üniversitesi Sağlık Bilimleri Dergisi 2018;9:163–8. [Article in Turkish] [CrossRef]
20. Ordin YS, Karayurt Ö, Ertan N, Yıldız S. Böbrek Nakli Alıcılarında İmmünosupresif Tedaviye Uyumun Farklı Yöntemler ile Değerlendirilmesi. Turkish Journal of Nephrology 2018;27(3):254–61
21. Suárez Fernández ML, G-Cosío F. Causes and consequences of proteinuria following kidney transplantation. Nefrologia 2011;31(4):404–14. [Article in Spanish]
22. Sevillano AM, Gutiérrez E, Yuste C, Cavero T, Mérida E, Rodríguez P, et al. Remission of hematuria improves renal survival in IgA nephropathy. J Am Soc Nephrol 2017;28(10):3089–99. [CrossRef]

23. Clayton P, McDonald S, Chadban S. Steroids and recurrent IgA nephropathy after kidney transplantation. Am J Transplant 2011;11(8):1645–9. [CrossRef]
24. Chailimpamontree W, Dmitrienko S, Li G, Balshaw R, Magil A, Shapiro RJ, et al. Probability, predictors, and prognosis of posttransplantation glomerulonephritis. J Am Soc Nephrol 2009;20(4):843–51. [CrossRef]
25. Choy BY, Chan TM, Lo SK, Lo WK, Lai KN. Renal transplantation in patients with primary immunoglobulin A nephropathy. Nephrol Dial Transplant 2003;18(11):2399–404. [CrossRef]
26. Berthoux F, Mohey H, Laurent B, Mariat C, Afiani A, Thibaudin L. Predicting the risk for dialysis or death in IgA nephropathy. J Am Soc Nephrol 2011;22(4):752–61. [CrossRef]
27. Kim YS, Moon JI, Jeong HJ, Kim MS, Kim SI, Choi KH, et al. Live donor renal allograft in end-stage renal failure patients from immunoglobulin A nephropathy. Transplantation 2001;71(2):233–8. [CrossRef]

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Research Article

Carotid Intima-media Thickness and Thrombosis Risk in Polycythemia Vera and Essential Thrombocythemia

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Abstract

Objectives: Thrombotic complications are the most important cause of morbidity and mortality in patients with polycythemia vera (PV) and essential thrombocythemia (ET). Carotid intima-media thickness (CIMT) is a surrogate marker used in the noninvasive assessment of subclinical atherosclerosis and cardiovascular event risk. This study aimed to evaluate CIMT in patients with PV and ET and to investigate its usefulness in risk stratification.

Methods: This study was conducted at the Hematology Clinic of Istanbul Training and Research Hospital, Health Sciences University, with patients diagnosed with PV and ET and a control group. CIMT measurement by ultrasonography was performed at the Radiology Clinic of Istanbul Training and Research Hospital, Health Sciences University. The study included 84 patients diagnosed between July 2006 and December 2016, including 42 with PV and 42 with ET, and 46 individuals in the control group.

Results: The median age of the patients was 57 years (min-max, 24-82); 38 were male (45%), and 46 were female (55%). Sixty individuals (71%) had a positive JAK2 mutation. There was no statistically significant difference in CIMT between the patient and control groups (0.83 vs. 0.85 mm; p=0.470). When the relationship between risk factors associated with increased CIMT was examined, a significant increase in CIMT was found in patients with JAK2 mutation, hypertension, and diabetes mellitus (DM) (p<0.05). In the correlation analysis between CIMT increase and age, CIMT was found to be correlated with age (p<0.001, r=0.647). In the univariate analysis, age (B=0.584, p<0.001), JAK2 mutation (B=0.319, p=0.003), hypertension (B=0.395, p<0.001), and DM (B=0.311, p=0.004) were significantly associated with increased CIMT. However, in the multivariate analysis, only age was found to be an independent predictor of increased CIMT (B=0.584, p<0.001).

Conclusion: The lack of an independent association between thrombotic tendency and CIMT in patients with PV and ET suggests that thrombotic risk may not be explained solely by subclinical atherosclerosis.

Keywords: Carotid intima-media thickness (CIMT), essential thrombocythemia (ET), polycythemia vera (PV)

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The majority of patients with BCR-ABL-negative classical chronic myeloproliferative neoplasms (MPNs) are those with polycythemia vera (PV) and essential thrombocythemia (ET); in these patients, the most important causes of morbidity and mortality are thrombotic complications, such as acute coronary syndrome and ischemic stroke.[1-6] Although the pathogenesis of clonal proliferation in PV and ET has not been fully elucidated, the JAK-STAT signaling pathway is known to be an important mechanism. The annual incidence of cumulative thrombosis is approximately 2.5-5% in patients with PV and around 1.9-4% in patients with ET.[1,7-9] Advanced age (>60 years) and a history of thrombosis are the most important risk factors for thrombosis development in both PV and ET.[1,4,7,8,10,11]

Atherosclerosis-related diseases, primarily coronary heart disease and ischemic stroke, are among the most important causes of morbidity and mortality, especially in developed countries.[12-14] The evaluation of carotid arteries by ultrasound and measurement of carotid intima-media thickness (CIMT) for the detection of subclinical atherosclerosis are widely used because of their noninvasive nature, use of nonionizing radiation, low cost, easy accessibility, and lack of disruption to bodily integrity.[13,15-19]

Considering that thrombotic events are a serious cause of morbidity and mortality in PV and ET, CIMT may be important in risk assessment in this patient group. This study aimed to evaluate carotid intima-media thickness in patients with PV and ET and to investigate the usefulness of CIMT in risk assessment.

Methods

This study was conducted at the Hematology Clinic of Istanbul Training and Research Hospital, Health Sciences University, with patients diagnosed with PV and ET and a control group, in accordance with the Declaration of Helsinki. The study protocol was approved by the Istanbul Training and Research Hospital Clinical Research Ethics Committee (Date: 11.11.2016, Approval Number: 2011-KAEK-50).

Ultrasonography-based CIMT measurement was performed at the Radiology Clinic of Istanbul Training and Research Hospital, Health Sciences University.

Patients

A total of 84 patients, including 42 with PV and 42 with ET, who were diagnosed between July 2006 and December 2016 and presented for follow-up to the Hematology Outpatient Clinic of Istanbul Training and Research Hospital, Health Sciences University, were included in the study. The 2016 WHO criteria were used for the diagnosis of polycythemia vera and ET, and patients who did not meet these criteria were excluded from the study. The 46 individuals included in the control group were selected from those who applied to the Internal Medicine Outpatient Clinic of Istanbul Training and Research Hospital, Health Sciences University, and from healthcare professionals. Individuals who did not use any medication, had no additional diseases, and had no risk factors for increased CIMT were selected for the control group. Informed consent forms were signed by the volunteers. The demographic characteristics of the patients; JAK2 V617F and BCR-ABL gene mutation status; complete blood count; blood lipid profile; fasting glucose; medical history and medications used; history of thrombosis and its location, if any; and smoking history were recorded from patient files and the hospital's automation system.

Carotid Intima-media Thickness Measurement

Carotid intima-media thickness measurement was performed at the Radiology Clinic of Istanbul Training and Research Hospital, Health Sciences University, by the same specialist radiologist. As defined by the Mannheim consensus, it was measured from the distal wall of both common carotid arteries 1 cm proximal to the carotid artery bifurcation.[20] (Fig. 1)

Statistical Analysis

Data were analyzed using IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, NY, USA, 2013). Descriptive statistics were performed for the characteristics of the patient groups. Median (min-max) values and proportions (percentages) were used for the descriptive statistics of the data. Independent samples t-tests, Kruskal-Wallis tests, and

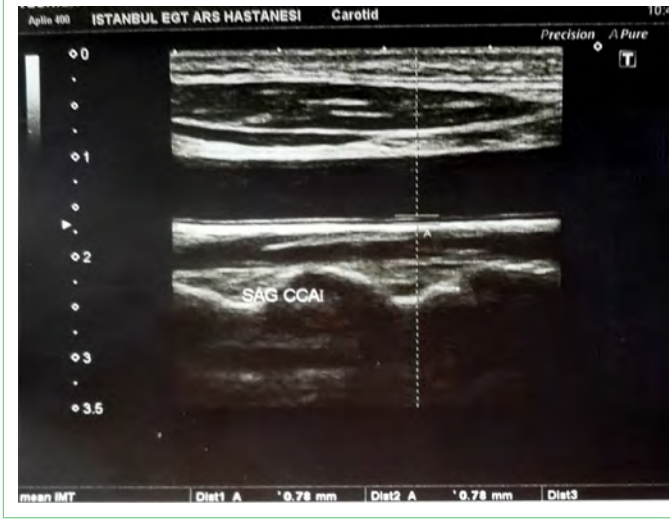

Figure 1. Carotid intima-media thickness measurement using ultrasonography.

Mann-Whitney U tests were used in the analysis of quantitative independent data, while chi-square tests were used in the analysis of qualitative independent data. The relationship between carotid intima-media thickness and certain risk factors was investigated using correlation analysis. Linear regression analysis was performed to examine the effects of classical and potential risk factors on CIMT. A p-value of 0.05 was used as the significance level. Foreign language support and writing assistance in this study were provided with the aid of Claude Sonnet 4.6 (Anthropic), a large language model.

Results

A total of 84 patients, including 42 with PV and 42 with ET, and 46 healthy individuals were included in the study. The median age of the patients was 57 years (range, 24-82), with 38 males (45%) and 46 females (55%). Sixty patients (71%) had a positive JAK2 mutation, while 11 patients (13.1%) had a history of thrombosis. The number of patients with a history of HT was 39 (46.4%), 20 patients (23.8%) had a history of DM, 29 patients (35%) had a history of hyperlipidemia, 26 patients (31%) were obese according to body mass index, and 19 patients (22.6%) were smokers (Table 1).

Hematological and biochemical parameters at the time of measurement did not show a significant correlation with CIMT.

Information comparing age, sex, and CIMT values of the patient subgroups and the control group is summarized in Table 2. No statistically significant difference was found in CIMT between the PV, ET, and control groups.

When the relationship between risk factors associated with increased carotid intima-media thickness was examined, a significant increase in CIMT was found in patients with JAK2 mutation, HT, and DM ($p<0.05$) (Table 3).

In the correlation analysis performed with age, disease duration, lipid levels, Hb, Hct, WBC, and platelet counts during USG, which may be related to increased carotid intima-media thickness, age was found to be correlated with CIMT ($p<0.001$, $r=0.647$).

When the relationship between risk factors and CIMT was examined in the patient group, univariate and multivariate analyses were performed for the factors found to be significant. In the univariate analysis, age, sex, JAK2 mutation, HT, and diabetes were evaluated. For age, B=0.584 and $p<0.001$.

Table 1. General characteristics of patients

Number of patients	**n= 84**
Median age, years, (min-max)	57 (24-82)
Gender, n (%)	
Male	38 (45%)
Female	46 (55%)
MPN subtype, n (%)	
PV	42 (50%)
ET	42 (50%)
JAK-2 mutation, n (%)	
Yes	60 (71%)
No	24 (29%)
History of thrombosis, n (%)	
Yes	11 (13.1%)
No	73 (86.9%)
HT, n (%)	
Yes	39 (46.4%)
No	45 (53.6%)
DM, n (%)	
Yes	20 (23.8%)
No	64 (76.2%)
Hyperlipidemia, n (%)	
Yes	29 (35%)
No	55 (65%)
Body mass index, n (%)	
<30 kg/m^2	58 (69%)
> 30 kg/m^2	26 (31%)
Smoking, n (%)	
Yes	19 (22.6%)
No	65 (77.4%)

MPN: Myeloproliferative neoplasm; PV: Polycythemia vera; ET: Essential thrombocythemia; JAK-2: Janus Kinase 2 gene; HT: Hypertension; DM: Diabetes mellitus.

Table 2. Comparison of demographic characteristics and CIMT between patient subgroups and the control group

	PV n=42	**ET n=42**	**Control group n=46**	**p**
Median age, years (min-max)	57 (27-74)	58 (24-82)	55 (26-77)	0.672
Gender, n (%)				
Male	21 (%50)	25 (%59.5)	24 (%52.2)	0.655
Female	21 (%50)	17 (%40.5)	22 (%47.8)	
CIMT, median (min-max)	0.78 (0.5-2)	0.85 (0.42-1.13)	0.85 (0.33-1.21)	0.782

PV: Polycythemia vera; ET: Essential thrombocythemia; CIMT: CIMT: Carotid intima-media thickness.

Table 3. Relationship between CIMT and risk factors

n=84	CIMT Median, mm	CIMT Min-max, mm	p
Gender			
Female	0.83	0.42-2	0.828
Male	0.8	0.33-1.13	
Body mass index			
<30 kg/m^2	0.82	0.42-2	0.289
>30 kg/m^2	0.83	0.6-1.13	
JAK-2 mutation			
Yes	0.85	0.54-2	**0.001**
No	0.72	0.42-1.13	
Thrombosis			
Yes	0.85	0.5-1.06	0.790
No	0.81	0.42-2	
HT			
Yes	0.92	0.6-2	**0.000**
No	0.73	0.42-1.13	
DM			
Yes	0..89	0.7-2	**0.009**
No	0.78	0.42-1.13	
Hyperlipidemia			
Yes	0.85	0.42-2	0.083
No	0.78	0.5-1.13	
Smoking			
Yes	0.80	0.42-1.13	0.295
No	0.85	0.5-2	

CIMT: Carotid intima-media thickness; JAK-2: Janus Kinase 2 gene; HT: Hypertension; DM: Diabetes mellitus.

Discussion

This study found that carotid intima-media thickness values in patients with PV and ET were similar to those in the control group. Considering that PV and ET are high-risk diseases for thrombosis, the failure to demonstrate an increase in CIMT suggests that the tendency for thrombosis may not be directly related to structural atherosclerotic changes.

In subgroup analyses, higher CIMT values were found in patients with hypertension, diabetes, and JAK2 mutation. However, in the multivariate analysis, only age was found to be an independent predictor of increased CIMT. The literature has demonstrated a strong and linear relationship between age and CIMT.[12,14,21] This finding supports that the increase in CIMT in our study primarily reflects age-related vascular changes.

The association between hypertension and increased CIMT has been well established in the literature.[13,22-25] The association between diabetes and increased CIMT has also been well established.[14,16,19,26-28] In our study, these variables were also found to be significant in the univariate analysis but failed to maintain their independence. Similarly, while the higher CIMT values observed in JAK2-positive patients are noteworthy, the fact that this relationship was not independent in the multivariate analysis suggests that the observed increase may be related to accompanying classical cardiovascular risk factors.

The relationship between the JAK2 mutation and thrombosis has been demonstrated in previous studies.[29-34] However, the finding in our study that JAK2 positivity did not constitute an independent structural atherosclerotic burden via CIMT raises the possibility that the thrombosis-enhancing effect of this mutation may occur through inflammatory, endothelial dysfunction, and procoagulant mechanisms rather than structural atherosclerosis.

No statistically significant association was found between obesity, hyperlipidemia, male sex, smoking, and CIMT in our study.

One of the strengths of this study is that CIMT was evaluated in patients with PV and ET in comparison with a control group, and the effects of potential confounding factors were examined using multivariate analysis. Furthermore, the inclusion of a disease-specific molecular variable, such as the JAK2 mutation, in the analysis contributes to the biological interpretation of the findings. However, the cross-sectional and single-center design of the study limits the establishment of causal relationships. The limited sample size and the fact that CIMT reflects only the structural atherosclerotic burden are among the other limitations of the study.

Conclusion

In conclusion, the fact that the presence of PV and ET alone is not associated with an increase in CIMT suggests that the development of thrombosis in these diseases may not be fully explained by classical atherosclerotic burden. Larger prospective studies evaluating the role of disease-specific biological processes in the pathogenesis of thrombosis are needed.

Disclosures

Ethics Committee Approval: The study protocol was approved by the Istanbul Training and Research Hospital Clinical Research Ethics Committee (Date: 11.11.2016, Approval Number: 2011-KAEK-50).

Informed Consent: Informed consent forms were signed by the volunteers.

Conflict of Interest: The author declare that there is no conflict of interest.

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ing assistance during the preparation of this manuscript. The tool was used to translate and edit text from Turkish to English. No AI tool was used for data analysis, interpretation, or generation of scientific content.

Authorship Contributions: Concept: MB, ES; Design: MB,ES; Supervision: ES; Fundings: MB; Materials: MB; Data Collection and/or Processing: MB, OK; Analysis and/or Interpretation: MB, ES; Literature Search: MB, ES; Writing: MB; Critical Reviews: MB, OK, ES.

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References

1. Casini A, Fontana P, Lecompte TP. Thrombotic complications of myeloproliferative neoplasms: risk assessment and risk-guided management. J Thromb Haemost 2013;11(7):1215–27. [CrossRef]
2. Vianello F, Battisti A, Cella G, Marchetti M, Falanga A. Defining the thrombotic risk in patients with myeloproliferative neoplasms. ScientificWorldJournal 2011;11:1131–7. [CrossRef]
3. Martinelli I, De Stefano V, Carobbio A, Randi ML, Santarossa C, Rambaldi A, et al. Cerebral vein thrombosis in patients with Philadelphia-negative myeloproliferative neoplasms. An European Leukemia Net study. Am J Hematol 2014;89(11):E200–5.
4. Kessler CM. Propensity for hemorrhage and thrombosis in chronic myeloproliferative disorders. Semin Hematol 2004;41(Suppl 3):10–4.
5. Kaifie A, Kirschner M, Wolf D, Maintz C, Hänel M, Gattermann N, et al. Bleeding, thrombosis, and anticoagulation in myeloproliferative neoplasms (MPN): analysis from the German SAL-MPN-registry. J Hematol Oncol 2016;9:18. [CrossRef]
6. De Stefano V, Fiorini A, Rossi E, Za T, Farina G, Chiusolo P, et al. Incidence of the JAK2 V617F mutation among patients with splanchnic or cerebral venous thrombosis and without overt chronic myeloproliferative disorders. J Thromb Haemost 2007;5(4):708–14. [CrossRef]
7. Barbui T, Finazzi G, Falanga A. Myeloproliferative neoplasms and thrombosis. Blood 2013;122(13):2176–84. [CrossRef]
8. Kreher S, Ochsenreither S, Trappe RU, Pabinger I, Bergmann F, Petrides PE, et al. Prophylaxis and management of venous thromboembolism in patients with myeloproliferative neoplasms: consensus statement of the Haemostasis Working Party of the German Society of Hematology and Oncology (DGHO), the Austrian Society of Hematology and Oncology (ÖGHO) and Society of Thrombosis and Haemostasis Research (GTH e.V.). Ann Hematol 2014;93(12):1953–63. [CrossRef]
9. Barbui T, De Stefano V. Management of venous thromboembolism in myeloproliferative neoplasms. Curr Opin Hematol 2017;24(2):108–14. [CrossRef]
10. Montanaro M, Latagliata R, Cedrone M, Spadea A, Rago A, Di Giandomenico J, et al. Thrombosis and survival in essential thrombocythemia: a regional study of 1,144 patients. Am J Hematol 2014;89(5):542–6. [CrossRef]
11. Harrison CN. Platelets and thrombosis in myeloproliferative diseases. Hematology Am Soc Hematol Educ Program 2005;2005(1):409–15. [CrossRef]
12. Zaid M, Fujiyoshi A, Kadota A, Abbott RD, Miura K. Coronary artery calcium and carotid artery intima media thickness and plaque: clinical use in need of clarification. J Atheroscler Thromb 2017;24(3):227–39. [CrossRef]
13. Ho SS. Current status of carotid ultrasound in atherosclerosis. Quant Imaging Med Surg 2016;6(3):285–96. [CrossRef]
14. Qu B, Qu T. Causes of changes in carotid intima-media thickness: a literature review. Cardiovasc Ultrasound 2015;13:46. [CrossRef]
15. Lee W. General principles of carotid Doppler ultrasonography. Ultrasonography 2014;33(1):11–7. [CrossRef]
16. Katakami N, Kaneto H, Shimomura I. Carotid ultrasonography: A potent tool for better clinical practice in diagnosis of atherosclerosis in diabetic patients. J Diabetes Investig 2014;5(1):3–13. [CrossRef]
17. Naik V, Gamad RS, Bansod PP. Carotid artery segmentation in ultrasound images and measurement of intima-media thickness. Biomed Res Int 2013;2013:801962. [CrossRef]
18. Bots ML, Sutton-Tyrrell K. Lessons from the past and promises for the future for carotid intima-media thickness. J Am Coll Cardiol 2012;60(17):1599–604. [CrossRef]
19. Sibal L, Agarwal SC, Home PD. Carotid intima-media thickness as a surrogate marker of cardiovascular disease in diabetes. Diabetes Metab Syndr Obes 2011;4:23–34. [CrossRef]
20. Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Bornstein N, et al. Mannheim carotid intima-media thickness and plaque consensus (2004-2006-2011). An update on behalf of the advisory board of the 3rd, 4th and 5th watching the risk symposia, at the 13th, 15th and 20th European Stroke Conferences, Mannheim, Germany, 2004, Brussels, Belgium, 2006, and Hamburg, Germany, 2011. Cerebrovasc Dis 2012;34(4):290–6. [CrossRef]
21. Herder M, Johnsen SH, Arntzen KA, Mathiesen EB. Risk factors for progression of carotid intima-media thickness and total plaque area: a 13-year follow-up study: the Tromsø Study. Stroke 2012;43(7):1818–23. [CrossRef]
22. Acevedo M, Tagle R, Kramer V, Arnaíz P, Marín A, Pino F, et al. Risk factors for a high carotid intima media thickness among healthy adults. Rev Med Chil 2011;139(3):290–7. [Article in Spanish] [CrossRef]
23. Magnussen CG. Carotid artery intima-media thickness and hypertensive heart disease: a short review. Clin Hypertens 2017;23:7. [CrossRef]
24. Bots ML, Hofman A, de Bruyn AM, de Jong PT, Grobbee DE. Isolated systolic hypertension and vessel wall thickness of

the carotid artery. The Rotterdam Elderly Study. Arterioscler Thromb 1993;13(1):64–9. [CrossRef]
25. Cuspidi C, Sala C, Tadic M, Rescaldani M, De Giorgi GA, Grassi G, et al. Untreated masked hypertension and carotid atherosclerosis: a meta-analysis. Blood Press 2015;24(2):65–71. [CrossRef]
26. Yapei Y, Xiaoyan R, Sha Z, Li P, Xiao M, Shuangfeng C, et al. Clinical significance of arterial stiffness and thickness biomarkers in Type 2 diabetes mellitus: an up-to-date meta-analysis. Med Sci Monit 2015;21:2467–75. [CrossRef]
27. Wagenknecht LE, D'Agostino RB Jr, Haffner SM, Savage PJ, Rewers M. Impaired glucose tolerance, type 2 diabetes, and carotid wall thickness: the Insulin Resistance Atherosclerosis Study. Diabetes Care 1998;21(11):1812–8. [CrossRef]
28. Brohall G, Odén A, Fagerberg B. Carotid artery intima-media thickness in patients with Type 2 diabetes mellitus and impaired glucose tolerance: a systematic review. Diabet Med 2006;23(6):609–16. [CrossRef]
29. Finazzi G, Rambaldi A, Guerini V, Carobbo A, Barbui T. Risk of thrombosis in patients with essential thrombocythemia and polycythemia vera according to JAK2 V617F mutation status. Haematologica 2007;92(1):135–6. [CrossRef]
30. De Stefano V, Za T, Rossi E, Fiorini A, Ciminello A, Luzzi C, et al. Influence of the JAK2 V617F mutation and inherited thrombophilia on the thrombotic risk among patients with essential thrombocythemia. Haematologica 2009;94(5):733–7. [CrossRef]
31. De Stefano V, Rossi E, Za T, Ciminello A, Betti S, Luzzi C, Leone G, et al. JAK2 V617F mutational frequency in essential thrombocythemia associated with splanchnic or cerebral vein thrombosis. Am J Hematol 2011;86(6):526–8. [CrossRef]
32. Dahabreh IJ, Zoi K, Giannouli S, Zoi C, Loukopoulos D, Voulgarelis M. Is JAK2 V617F mutation more than a diagnostic index? A meta-analysis of clinical outcomes in essential thrombocythemia. Leuk Res 2009;33(1):67–73. [CrossRef]
33. Lussana F, Caberlon S, Pagani C, Kamphuisen PW, Büller HR, Cattaneo M. Association of V617F Jak2 mutation with the risk of thrombosis among patients with essential thrombocythaemia or idiopathic myelofibrosis: a systematic review. Thromb Res 2009;124(4):409–17. [CrossRef]
34. Borowczyk M, Wojtaszewska M, Lewandowski K, Gil L, Lewandowska M, Lehmann-Kopydłowska A, et al. The JAK2 V617F mutational status and allele burden may be related with the risk of venous thromboembolic events in patients with Philadelphia-negative myeloproliferative neoplasms. Thromb Res 2015;135(2):272–80. [CrossRef]

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Research Article

Cytokine and Chemokine Profiles in COVID-19: Why Did Conventional Inflammatory Markers Outperform Cytokines?

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Abstract

Objectives: Dysregulated inflammation drives severe COVID-19. Cytokine profiling has been proposed for risk stratification. Its value over conventional markers is unclear. We compared admission cytokine and chemokine levels in mild and severe COVID-19. We also examined why routine markers may outperform cytokines.

Methods: This single-center cross-sectional study enrolled 78 adults with confirmed COVID-19. Patients were classified as mild or severe using WHO criteria at emergency admission. Serum IL-13, TNF-alpha, IFN-gamma, macrophage inflammatory protein-1 beta, and monocyte chemoattractant protein-1 were measured. Demographics, routine laboratory markers, and cytokines were compared between groups.

Results: Thirty-two patients had mild disease and 46 had severe disease. Cytokine and chemokine levels were numerically higher in severe disease. None of these differences were statistically significant.

Conclusion: Conventional inflammatory and tissue-injury markers tracked severity better than single-time-point cytokine measurements. Standalone cytokine profiling at admission may offer limited value. Larger studies with serial sampling, broader panels, and adjustment for confounders are needed.

Keywords: COVID-19, cytokines, chemokines, disease severity, MCP-1, MIP-1β

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Coronavirus disease 2019 (COVID-19) is a heterogeneous infectious disease ranging from asymptomatic or mild upper respiratory tract involvement to severe viral pneumonia, acute respiratory distress syndrome, thromboembolic complications, multiorgan dysfunction, and death.[1,2] Although direct viral cytopathic injury contributes to early disease pathogenesis, the clinical deterioration observed in severe COVID-19 is largely shaped by dysregulated host inflammation, endothelial injury, coagulation activation, and tissue damage.[3,4] This inflammatory phenotype has led to intense interest in cytokines and chemokines as potential biomarkers of disease severity and therapeutic targets.

Early reports of severe COVID-19 described increased circulating concentrations of several inflammatory mediators, including interleukin-6, interleukin-8, interleukin-10, tumor necrosis factor-alpha, interferon-gamma-related pathways, monocyte chemoattractant protein-1, and macrophage inflammatory proteins.[5,6] These findings supported the concept that a cytokine-driven hyperinflammatory response may contribute to respiratory failure and systemic complications in a subgroup of patients. Subsequent studies and reviews, however, have shown that cytokine profiles in COVID-19 are not uniform and may vary according to disease phase, timing of sampling, assay platform,

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viral variant, age, comorbidities, and prior or ongoing anti-inflammatory treatment.[7,8]

In routine clinical practice, conventional inflammatory and tissue injury markers, such as C-reactive protein, ferritin, lactate dehydrogenase, D-dimer, leukocyte and neutrophil counts, lymphocyte count, and platelet count, have remained more accessible and more consistently associated with severe disease than specialized cytokine measurements. Several studies have reported that elevated CRP, ferritin, LDH, D-dimer, and neutrophil-to-lymphocyte ratio are associated with worse clinical outcomes, while lymphopenia and thrombocytopenia reflect immune dysregulation and disease severity.[9-12]

Despite the biological plausibility of cytokine profiling, its incremental value over conventional laboratory markers remains uncertain, particularly when measured at a single time point at hospital admission. In this context, negative or nonsignificant cytokine findings are clinically informative, as they may indicate that isolated baseline cytokine measurements do not sufficiently capture the dynamic inflammatory trajectory of COVID-19. This is especially relevant for cytokines and chemokines such as interleukin-13, tumor necrosis factor-alpha, interferon-gamma, monocyte chemoattractant protein-1, and macrophage inflammatory protein-1 beta, which may show biological elevation without providing robust discrimination between severity groups.

Therefore, this study aimed to compare admission serum cytokine and chemokine levels between patients with mild and severe COVID-19 and to evaluate their relationship with conventional inflammatory and tissue injury markers. We specifically examined whether selected serum cytokines and chemokines measured at emergency department admission could distinguish severe disease and why conventional laboratory markers may have outperformed cytokine measurements in reflecting COVID-19 severity. The study included 78 patients with COVID-19, classified as 32 mild and 46 severe cases according to World Health Organization disease severity criteria.[13] Cytokine and chemokine measurements were performed using the Bio-Plex Pro Human Cytokine Screening Panel.

Materials and Methods

Study Design and Setting

This was a single-center, cross-sectional observational study conducted at İstanbul University, Faculty of Medicine, between January 2022 and March 2022. The study included adult patients who presented to the emergency department with suspected COVID-19 and had confirmed SARS-CoV-2 infection by real-time reverse transcription polymerase chain reaction from combined nasopharyngeal and oropharyngeal swab samples. Patients were enrolled at hospital admission, and blood samples for cytokine and chemokine analyses were obtained before the initiation of COVID-19-specific treatment.

The study was designed to evaluate whether pretreatment serum cytokine and chemokine levels measured at admission were associated with COVID-19 disease severity and to compare their performance with conventional inflammatory and tissue injury markers.

Study Population

Patients aged 18 years or older with confirmed COVID-19 were eligible for inclusion. A total of 78 patients were included in the final analysis. At the time of emergency department admission, patients were evaluated according to clinical findings, laboratory parameters, and radiological features. Based on the World Health Organization COVID-19 disease severity classification, patients were categorized into two groups: mild disease and severe disease. Accordingly, 32 patients were classified as having mild COVID-19 and 46 patients as having severe COVID-19.

For all included patients, routine laboratory tests were obtained as part of clinical care, and an additional 10 mL blood sample was collected for cytokine and chemokine measurement.

Disease Severity Classification

COVID-19 disease severity was determined according to the World Health Organization classification. Patients without evidence of viral pneumonia or hypoxia were classified as having mild disease. Patients with clinical, radiological, or laboratory findings consistent with severe COVID-19 were classified as having severe disease. Classification was based on the overall assessment at emergency department admission, including clinical status, oxygenation, laboratory findings, and radiological involvement.

Data Collection

Demographic, clinical, laboratory, and radiological data were collected at the time of admission. The following baseline variables were recorded:

- age
- sex
- clinical presentation
- disease severity category
- routine hematological parameters
- biochemical markers
- inflammatory markers
- coagulation-related markers

Laboratory parameters included leukocyte count, neutrophil count, lymphocyte count, platelet count, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, urea, ferritin, D-dimer, and C-reactive protein. These parameters were compared between the mild and severe disease groups.

Cytokine and Chemokine Measurement

Blood samples for cytokine and chemokine analysis were obtained at emergency department admission before the initiation of COVID-19-specific treatment. This timing was chosen to minimize the potential confounding effects of corticosteroids, antiviral agents, anticoagulants, or other anti-inflammatory treatments on circulating cytokine and chemokine concentrations.

Samples were centrifuged at 1000×g for 15 minutes at 4°C. The plasma fraction was then transferred into polypropylene tubes. To remove residual platelets and precipitates, plasma samples were centrifuged again at 4000×g for 15 minutes at 4°C. The samples were diluted at a 1:4 ratio and stored at –80°C until analysis.

Serum cytokine and chemokine levels were measured using the Bio-Plex Pro Human Cytokine Screening Panel (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. The measured mediators included tumor necrosis factor-alpha, interferon-gamma, interleukin-13, monocyte chemoattractant protein-1, and macrophage inflammatory protein-1 beta. Measurements were performed using the Bio-Plex 200 multiplex system platform.

Outcomes

The primary outcome was the association between pretreatment admission serum cytokine and chemokine levels and COVID-19 disease severity, defined according to World Health Organization severity criteria.

The secondary outcome was the association between conventional laboratory markers and COVID-19 disease severity. Conventional markers included leukocyte count, neutrophil count, lymphocyte count, platelet count, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, urea, ferritin, D-dimer, and C-reactive protein.

Data on intensive care unit admission, mortality, mechanical ventilation, or detailed oxygen requirement were not available; therefore, these endpoints were not analyzed.

Statistical Analysis

All statistical analyses were performed using SPSS version 28.0 (IBM Corp., Armonk, NY, USA). Continuous and categorical variables were presented as median (IQR) and n (%), respectively. The Shapiro-Wilk and Kolmogorov-Smirnov tests were used to assess the assumption of normality. Independent samples t-tests and Mann-Whitney U tests were used to compare normally distributed and nonnormally distributed independent variables, respectively. Categorical variables were compared using the chi-square test. A p-value of <0.05 was considered statistically significant.

Ethical Considerations

The study was approved by the Istanbul Faculty of Medicine Clinical Research Ethics Committee, Istanbul University, Türkiye, with approval dated December 31, 2021, and approval number 671545. Written informed consent was obtained from all participants. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Results

Study Population and Demographic Characteristics

A total of 78 patients with confirmed COVID-19 were included in the study. Of these, 54 patients were male and 24 were female. According to the World Health Organization COVID-19 disease severity classification, 46 patients were classified as having severe disease and 32 patients as having mild disease.

The overall mean age was 51.7±16.1 years. Patients with severe COVID-19 were significantly older than those with mild disease, with mean ages of 58.2±15.0 years and 43.1±13.3 years, respectively ($p<0.001$). Male sex was more frequent in the severe disease group than in the mild disease group (36/46 patients [78.3%] vs. 18/32 patients [56.2%], $p=0.048$).

Comorbidities were more common among patients with severe disease. Diabetes mellitus was present in 10 patients with severe disease and in 1 patient with mild disease (21.7% vs. 3.1%, $p=0.021$). Hypertension was also significantly more frequent in severe disease (24 patients [52.2%] vs. 2 patients [6.2%], $p<0.001$). Cardiovascular disease and chronic obstructive pulmonary disease were observed only in the severe disease group (23.9% and 15.2%, respectively), and both were significantly associated with severe disease. The demographic characteristics of patients with mild and severe COVID-19 are presented in Table 1.

Clinical Characteristics

The most common symptoms in the overall cohort were myalgia or fatigue (53 patients [67.9%]), cough (36 patients [46.3%]), dyspnea (28 patients [35.9%]), and fever (27 patients [34.6%]).

Fever was observed only in the severe disease group (27 patients [58.7%] vs. 0 patients in the mild disease group, $p<0.001$).

Table 1. Demographic characteristics of patients with mild and severe COVID-19

	All (n=78)	Severe (n=46)	Mild (n=32)	p
Age	51.7±16.1 (18-92)	58±15 (35-92)	43.1±13.3 (18-65)	**<0.001**
Sex				
Male	54 (69.2%)	36 (78.3%)	18 (56.2%)	0.048
Female	24 (30.8%)	10 (21.7%)	14 (43.8%)	
Comorbidity				
DM	11 (14.1%)	10 (21.7%)	1 (3.1%)	0.021
HT	26 (33.3%)	24 (52.2%)	2 (6.2%)	**<0.001**
CardiovascularyDisease	11 (14.1%)	11 (23.9%)	-	0.002
COPH	7 (9%)	7 (15.2%)	-	0.037
Malignancy	1 (1.3%)	1 (2.2%)	-	1
Chronic Hepatic Disease	1 (1.3%)	1 (2.2%)	-	1

Cough was also significantly more frequent in severe disease (31 patients [67.4%] vs. 5 patients [15.6%], p<0.001). Dyspnea showed one of the clearest differences between groups, being present in 27 patients with severe disease (58.7%) and in only 1 patient with mild disease (3.1%) (p<0.001).

Myalgia or fatigue was more common in the severe disease group than in the mild disease group (36 patients [78.3%] vs. 17 patients [53.1%], p=0.027). Headache was more frequent in the mild disease group (11 patients [34.4%] vs. 3 patients [6.5%], p=0.003). Nausea and vomiting were observed only in the severe disease group (6 patients [13.0%], p=0.038). Diarrhea and sore throat did not differ significantly between groups. The clinical characteristics of patients according to COVID-19 disease severity are summarized in Table 2.

Conventional Laboratory Markers According to Disease Severity

Several conventional laboratory markers differed significantly between the mild and severe disease groups. Patients with severe COVID-19 had significantly higher leukocyte counts than patients with mild disease [7.8±3.7 vs. 5.4±2.2×10^9/L, p=0.002]. Neutrophil counts were also significantly higher in severe disease [6.1±3.7 vs. 3.7±1.9×10^9/L, p=0.001], whereas lymphocyte counts were significantly lower [1.0±0.6 vs. 1.5±0.6×10^9/L, p<0.001].

Platelet counts were lower in severe disease than in mild disease [216.2±52.6 vs. 257.4±100.6×10^9/L, p=0.045]. INR was also significantly different between groups [1.0±0.1 vs. 1.2±0.2, p=0.002]. D-dimer levels were higher in severe disease [1.8±3.4 vs. 0.5±0.7 ng/L, p=0.042].

Markers of liver injury and tissue damage were also more elevated in severe disease. Alanine aminotransferase was higher in severe disease [57.5±55.3 vs. 32.4±29.9 U/L, p=0.030], as was aspartate aminotransferase [57.3±50.9 vs. 26.6±11.5 U/L, p=0.002]. Lactate dehydrogenase showed a marked increase in severe disease [373.3±165.3 vs. 203.3±40.6 U/L, p<0.001].

Inflammatory markers showed the strongest differences between groups. Ferritin levels were substantially higher in pa-

Table 2. Clinical characteristics of patients according to COVID-19 disease severity

Symptoms and Signs	All (n=78)	Severe (n=46)	Mild (n=32)	p
Fewer	27 (34.6%)	27 (58.7%)	-	**<0.001**
Cough	36 (46.3%)	31 (39.7%)	5 (15.6%)	**<0.001**
Myalgia or fatigue	53 (67.9%)	36 (78.3%)	17 (53.1%)	**0.027**
Headache	14 (17.9%)	3 (6.5%)	11 (34.4%)	**0.003**
Diarrhea	4 (5.1%)	3 (6.5%)	1 (3.1%)	0.64
Dispnea	28 (35.9%)	27 (58.7%)	1 (3.1%)	**<0.001**
Neausea and vomitting	6 (7.7%)	6 (13%)	-	**0.038**
Sore Throat	12 (15.4%)	4 (8.7%)	8 (25%)	0.062

tients with severe COVID-19 [1303.3±860.4 vs. 171.4±147.3 ng/mL, p<0.001]. C-reactive protein levels were also markedly higher in the severe disease group [139.2±84.1 vs. 1.0±2.6 mg/dL, p<0.001]. Urea levels were higher in severe disease [46.7±24.7 vs. 33.0±12.6 mg/dL, p=0.013].

In contrast, prothrombin time, activated partial thromboplastin time, creatine kinase, total bilirubin, potassium, sodium, and creatinine levels did not differ significantly between the two groups. Laboratory findings of the patients according to COVID-19 disease severity are shown in Table 3.

Cytokine and Chemokine Levels According to Disease Severity

Serum cytokine and chemokine levels were compared between patients with mild and severe COVID-19. Overall, cytokine and chemokine concentrations tended to be numerically higher in patients with severe disease; however, none of the measured mediators showed a statistically significant difference between the groups.

Tumor necrosis factor-alpha levels were higher in the severe disease group than in the mild disease group [48.0±53.9 vs. 30.7±11.6 pg/mL], but the difference was not statistically significant (p=0.644). Interferon-gamma levels were also higher in severe disease [14.3±26.3 vs. 6.5±7.1 pg/mL], without statistical significance (p=0.271).

Interleukin-13 levels were slightly higher in severe disease [1.6±0.7 vs. 1.3±0.3 pg/mL], but again, the difference did not reach statistical significance (p=0.119). Monocyte chemoattractant protein-1 levels were numerically higher in severe disease [159.5±262.8 vs. 86.4±51.7 pg/mL], but this difference was not significant (p=0.533). Macrophage inflammatory protein-1 beta levels were also higher in severe disease [54.1±89.2 vs. 38.6±15.6 pg/mL], with no statistically significant difference between the groups (p=0.854).

These findings indicate that, despite a numerical upward trend in selected cytokine and chemokine levels among patients with severe COVID-19, pretreatment single-timepoint measurements of TNF-α, IFN-γ, IL-13, MCP-1, and MIP-1β did not significantly discriminate between mild and severe disease. Admission serum cytokine and chemokine levels according to COVID-19 disease severity are presented in Table 4.

Discussion

In this single-center, cross-sectional study, we evaluated pretreatment admission serum cytokine and chemokine levels in patients with mild and severe COVID-19 and

Table 3. Laboratory findings according to COVID-19 disease severity

Laboratory findings	All (78) Mean±SD	Severe (46) Mean±SD	Mild (32) Mean±SD	p
Whice cell count (x 10^9/ L, 3.7-10.4)	6.8±3.4	7.8±3.7	5.4±2.2	0.002
Neutrophil count (x 10^9/ L, 1.8-7.8)	5.1±3.2	6.1±3.7	3.7±1.9	0.001
Lymphocyte count (x 10^9/ L, 0.9-3.7)	1.2±0.6	1±0.6	1.5±0.6	**<0.001**
Platelet count (x 10^9/ L, 149-371)	240±85.7	216.2±52.6	257.4±100.6	0.045
Protrombin time (s, 9.7-13.9)	13.3±3.7	13.1±3.9	13.8±2.9	0.549
Active partial thromboplastin time (s, 22-45)	33.8±11	33.4±9.5	35.1±14.6	0.616
INR (0.8-1.24)	1,1±0.2	1±0.1	1.2±0.2	0.002
D-dimer (ng/L, 0-500)	1.3±2.8	1.8±3.4	0.5±0.7	0.042
ALT (U/L, 0-41)	47.1±47.9	57.5±55.3	32.4±29.9	0.03
AST (U/L, 0-40)	44.7±42.5	57.3±50.9	26.6±11.5	0.002
CK (IU/L, 0-190)	142.1±125.7	154.7±137.3	108.5±82.1	0.228
LDH (U/L, 135-225)	305.8±154.9	373.3±165.3	203.3±40.6	**<0.001**
Total bilirubin (mg/dL, 0-1.2)	0.6±0.3	0.6±0.3	0.5±0.2	0.3
Potassium level (mmol/L,.5-5.1)	6.1±15.9	7.5±20.8	4.1±0.4	0.384
Sodium level (mmol/L, 136-145)	135,4±16.7	132.6±20.9	139.7±3.1	0.089
Urea (mg/dL, 10-50)	41.5±21.7	46.7±24.7	33.3±12.6	0.013
Creatinine (mg/dL, 0.7-1.2)	1.1±0.6	1.1±0.7	1±0.3	0.323
Ferritin (ng/mL, 30-400)	844±870.3	1303.3±860.4	171.4±147.3	**<0.001**
CRP (mg/dL, 0-0.5)	52.1±84.1	139.2±84.1	1±2.6	**<0.001**

Table 4. Admission serum cytokine and chemokine levels according to COVID-19 disease severity

	Severe (n=46)		Mild (n=32)		
Immune Mediatory	**Mean±SD**	**[Q1:Q3] Median**	**Mean±SD**	**[Q1:Q3] Median**	**p***
TNF-α, pg/mL	48±53.9	[19.28:66.00] 35.94	30.7±11.6	[29.34:45.85] 35.98	0.644
IFN-γ, pg/mL	14.3±26.3	[3.71:11.49] 5.82	6.5±7.1	[3.01:5.47] 3.01	0.271
IL-13, pg/mL	1.6±0.7	[1.14:1.63] 1.63	1.3±0.3	[1.14:1.14] 1.14	0.119
MCP-1, pg/mL	159.5±262.8	[91.92:286.97] 107.35	86.4±51.7	[65.32:126.36] 99.03	0.533
MIP-1β, pg/mL	54.1±89.2	[29,51:75,93] 53	38.6±15.6	[41,07:53,33] 43.96	0.854

compared their association with disease severity against conventional inflammatory and tissue injury markers. The main finding was that routinely available laboratory parameters, including C-reactive protein, ferritin, lactate dehydrogenase, D-dimer, leukocyte and neutrophil counts, lymphocyte count, platelet count, alanine aminotransferase, aspartate aminotransferase, and urea, were significantly associated with severe disease. In contrast, selected cytokines and chemokines, including TNF-α, IFN-γ, IL-13, MCP-1, and MIP-1β, were numerically higher in severe COVID-19 but did not reach statistical significance. This pattern suggests that conventional inflammatory and tissue injury markers may better reflect the integrated systemic burden of COVID-19 at admission than isolated single-time-point cytokine measurements.

Patients with severe disease in our cohort were older, more frequently male, and had a higher burden of comorbidities, particularly hypertension, diabetes mellitus, cardiovascular disease, and chronic obstructive pulmonary disease. These findings are consistent with the recognized clinical phenotype of severe COVID-19, in which host vulnerability substantially modifies the inflammatory response to SARS-CoV-2 infection. Age, cardiometabolic disease, and chronic pulmonary disease may amplify endothelial dysfunction, basal inflammatory tone, coagulation activation, and susceptibility to organ injury. Therefore, the stronger association of conventional markers with severe disease may partly reflect their ability to capture both acute infection-related inflammation and the underlying systemic vulnerability of the host.[14,15]

The clearest laboratory differences between mild and severe disease were observed in CRP, ferritin, LDH, D-dimer, neutrophil count, lymphocyte count, and platelet count. This is biologically plausible. CRP is a downstream marker of hepatic acute-phase activation, largely driven by upstream inflammatory signaling, such as the IL-6 pathway. Ferritin reflects macrophage activation, cellular stress, and systemic hyperinflammation. LDH indicates tissue injury and cellular turnover. D-dimer reflects coagulation activation, endothelial injury, and thromboinflammation. Neutrophilia and lymphopenia reflect the combined effects of innate immune activation, stress response, and impaired adaptive immune regulation. Thrombocytopenia may reflect platelet consumption, endothelial activation, inflammation-associated coagulopathy, or severe systemic illness. Systematic reviews of COVID-19 biomarkers have similarly identified D-dimer, ferritin, neutrophil-to-lymphocyte ratio, and related hematologic or inflammatory markers as clinically relevant indicators of disease severity and mortality.[9,10]

Importantly, our panel did not include IL-6 or IL-8, two of the most consistently reported cytokines associated with severe COVID-19. Therefore, our findings should not be interpreted as evidence against cytokine involvement in COVID-19, but rather as evidence that the selected cytokines and chemokines measured at a single admission time point did not outperform conventional markers.

Although all measured cytokines and chemokines were numerically higher in the severe group, large standard deviations suggest substantial interindividual variability. In a modest-sized cohort, such variability may reduce statistical power and obscure biologically meaningful trends. Our analysis reflects disease severity at admission, not subsequent clinical outcomes.

TNF-α may be associated with endothelial activation, inflammation, and tissue damage in severe COVID-19. Del Valle and colleagues demonstrated that elevated levels of IL-6, IL-8, and TNF-α at the time of hospital admission were strong and independent predictors of survival.[7] In our study, TNF-α was also elevated, but not significantly; this may be explained by the sample size and variability. IFN-γ is an indicator of the antiviral Th1 response, but timing is critical in COVID-19. While an early antiviral response may be protective, a late and dysregulated response may increase inflammation. Therefore, severity discrimination may be difficult with a single measurement. MCP-1 is associated with monocyte chemotaxis and is important in terms of pulmonary inflammation and monocyte-macrophage acti-

vation.[16,17] In some studies, MCP-1 has been reported to be elevated in COVID-19. In the study by Pinarlik et al., IFN-α2, TNF-α, MCP-1/CCL2, IL-6, IL-8, IL-18, and IL-33 levels were reported to be higher in patients with COVID-19 than in healthy controls, while increased CRP levels and decreased lymphocyte counts were observed in critical illness.[18] MIP-1β is involved in the macrophage- and T-cell-related chemotactic response. Its numerical elevation in the severe group may be biologically expected, but due to wide variation and the small sample size, it may not have reached statistical significance.

The absence of statistically significant differences in TNF-α, IFN-γ, IL-13, MCP-1, and MIP-1β should not be interpreted as evidence against cytokine involvement in COVID-19 pathogenesis. Rather, our findings indicate that the selected cytokines and chemokines measured at a single pretreatment admission time point did not discriminate mild from severe disease as effectively as conventional laboratory markers. Cytokines are dynamic rather than static biomarkers. Their circulating concentrations may vary according to disease phase, timing from symptom onset, viral replication kinetics, compartmentalized pulmonary inflammation, host immune status, assay platform, and prior immune priming. Longitudinal cytokine studies have emphasized that early and late cytokine profiles may differ across disease severity groups, supporting the idea that inflammatory trajectories may be more informative than isolated baseline values.[8]

A second explanation is that cytokines may peak earlier or later than the downstream laboratory markers used in routine practice. CRP, ferritin, LDH, and D-dimer are more stable integrated readouts of systemic inflammation, tissue injury, and thromboinflammatory burden. By contrast, cytokines can be transient, pulsatile, locally compartmentalized, and technically more variable. A patient sampled early in the course of the disease may not yet have developed a measurable systemic cytokine increase, whereas another sampled later may already show downstream acute-phase activation after the cytokine peak has passed. Therefore, conventional markers may outperform cytokines at admission because they summarize the biological consequences of inflammation rather than only a narrow upstream signaling snapshot.

Another important point is that our cytokine panel did not include IL-6 or IL-8, two of the most consistently reported cytokines associated with severe COVID-19. Del Valle et al. showed that high serum IL-6, IL-8, and TNF-α levels at hospitalization were strong predictors of survival and that IL-6 and TNF-α remained independently associated with disease severity and death after adjustment for clinical and laboratory variables.[7,19,20] IL-6 is also central to the acute-phase response and has been repeatedly discussed as a key mediator in COVID-19-associated cytokine storm. Therefore, the absence of IL-6 and IL-8 measurements is a relevant limitation of our panel. In our cohort, the marked CRP elevation in severe disease may indirectly reflect upstream IL-6-related acute-phase activation, but this cannot be confirmed without direct IL-6 measurement.

The measured cytokines and chemokines still have biological relevance. TNF-α contributes to endothelial activation, inflammation, vascular permeability, and tissue injury. IFN-γ reflects antiviral Th1 immune activation, but its clinical meaning depends strongly on timing; early interferon signaling may support viral control, whereas delayed or dysregulated responses may contribute to immunopathology. MCP-1, also known as CCL2, promotes monocyte recruitment and may participate in monocyte-macrophage accumulation within inflamed tissues. MIP-1β, also known as CCL4, is involved in the chemotactic recruitment of immune cells and macrophage-associated inflammation. IL-13 is more closely related to type 2 immune responses, mucus production, tissue remodeling, and fibrotic pathways, and it may not be expected to behave as a dominant acute severity marker in the same way as IL-6 or IL-8.[21-23] Thus, the lack of significant group differences in our study may reflect not only sample size and timing but also the fact that not all cytokines are equally suited for acute severity discrimination.

The numerical elevation of all measured cytokines and chemokines in the severe group is nevertheless noteworthy. TNF-α, IFN-γ, IL-13, MCP-1, and MIP-1β were all higher in patients with severe disease, but standard deviations were wide, especially for TNF-α, IFN-γ, MCP-1, and MIP-1β. This suggests substantial interindividual variability. In a modest-sized cohort, such variability can reduce statistical power and obscure biologically meaningful trends. Therefore, our results should be interpreted as showing the limited discriminatory performance of these pretreatment single-time-point measurements in this cohort, rather than the complete absence of inflammatory differences between mild and severe disease.

From a clinical perspective, these findings are relevant because emergency department risk stratification requires rapid, reproducible, inexpensive, and easily interpretable biomarkers. Conventional markers, such as CRP, ferritin, LDH, D-dimer, complete blood count parameters, and routine biochemical tests, are widely available and reflect multiple downstream processes, including acute-phase activation, macrophage response, endothelial injury, coagulation activation, immune cell redistribution, and tissue damage. Cytokine profiling, although biologically attractive, is more

expensive, technically demanding, less standardized across platforms, and more sensitive to sampling-time effects. Accordingly, our findings support the view that selected single-time-point cytokine measurements should not be used as standalone admission severity markers but may be more informative when combined with serial sampling, broader cytokine panels, and clinical variables.[24-26]

This study has several strengths. First, cytokine and chemokine measurements were performed using pretreatment blood samples obtained at emergency department admission, reducing the potential confounding effects of corticosteroids, antiviral agents, anticoagulants, or other COVID-19-specific therapies on inflammatory mediator levels. Second, patients were classified according to World Health Organization disease severity criteria, providing a clinically recognizable severity framework. Third, the study directly compared specialized cytokine and chemokine measurements with routinely available laboratory markers, which is relevant for real-world clinical decision-making.

The study also has limitations. It was a single-center study with a relatively small sample size. Cytokines and chemokines were measured at only one time point, preventing the assessment of inflammatory trajectories. The panel did not include IL-6 or IL-8, two cytokines frequently linked to COVID-19 severity. Detailed data on intensive care unit admission, mortality, mechanical ventilation, and oxygen requirement were not available; therefore, the analysis was limited to disease severity at admission rather than subsequent clinical outcomes. In addition, age, sex, and comorbidity differences between severity groups may have influenced both conventional laboratory markers and cytokine levels. Larger studies with serial sampling, broader cytokine panels, and multivariable adjustment are needed to clarify whether cytokine profiling adds independent value beyond conventional markers.

Conclusion

In conclusion, conventional inflammatory, hematological, coagulation-related, and tissue injury markers were more strongly associated with COVID-19 severity at admission than selected pretreatment serum cytokine and chemokine levels. Although TNF-α, IFN-γ, IL-13, MCP-1, and MIP-1β were numerically higher in severe disease, none significantly distinguished severe from mild COVID-19. These findings suggest that the apparent superiority of conventional markers may derive from their greater biological stability, clinical accessibility, and ability to reflect integrated systemic inflammation and tissue injury. Cytokine profiling may still be valuable in COVID-19 research, but its clinical utility likely depends on broader inflammatory panels, longitudinal sampling, and integration with routine laboratory and clinical parameters.

Disclosures

Ethics Committee Approval: This study was approved by The Istanbul Faculty of Medicine Clinical Research Ethic Committee (Approval Number: 671545 - 31.12.2021).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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References

1. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395(10223):497–506. [CrossRef]
2. Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, transmission, diagnosis, and treatment of Coronavirus Disease 2019 (COVID-19): A review. JAMA 2020;324(8):782–93. [CrossRef]
3. Berlin DA, Gulick RM, Martinez FJ. Severe Covid-19. N Engl J Med 2020;383(25):2451–60. [CrossRef]
4. Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, Mehra MR, et al. Endothelial cell infection and endotheliitis in COVID-19. Lancet 2020;395(10234):1417–8. [CrossRef]
5. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ, et al. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 2020 Mar 28;395(10229):1033–4. [CrossRef]
6. Hadjadj J, Yatim N, Barnabei L, Corneau A, Boussier J, Smith N, et al. Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. Science 2020;369(6504):718–24. [CrossRef]
7. Del Valle DM, Kim-Schulze S, Huang HH, Beckmann ND, Nirenberg S, Wang B, et al. An inflammatory cytokine signature predicts COVID-19 severity and survival. Nat Med 2020;26(10):1636–43. [CrossRef]
8. Lucas C, Wong P, Klein J, Castro TBR, Silva J, Sundaram M, et al. Longitudinal analyses reveal immunological misfiring in severe COVID-19. Nature 2020;584(7821):463–9. [CrossRef]

9. Huang I, Pranata R, Lim MA, Oehadian A, Alisjahbana B. C-reactive protein, procalcitonin, D-dimer, and ferritin in severe coronavirus disease-2019: a meta-analysis. Ther Adv Respir Dis 2020;14:1753466620937175. [CrossRef]
10. Simadibrata DM, Calvin J, Wijaya AD, Ibrahim NAA. Neutrophil-to-lymphocyte ratio on admission to predict the severity and mortality of COVID-19 patients: A meta-analysis. Am J Emerg Med 2021;42:60–9. [CrossRef]
11. Henry BM, de Oliveira MHS, Benoit S, Plebani M, Lippi G. Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. Clin Chem Lab Med 2020;58(7):1021–8. [CrossRef]
12. Tan L, Wang Q, Zhang D, Ding J, Huang Q, Tang YQ, et al. Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study. Signal Transduct Target Ther 2020;5(1):33. [CrossRef]
13. World Health Organization. Clinical management of COVID-19: living guideline. Geneva: World Health Organization; 2023. Available at: https://www.who.int/publications/i/item/WHO-2019-nCoV-clinical-2023.2. Accessed July 01, 2026.
14. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020;395(10229):1054–62. [CrossRef]
15. Williamson EJ, Walker AJ, Bhaskaran K, Bacon S, Bates C, Morton CE, et al. Factors associated with COVID-19-related death using OpenSAFELY. Nature 2020;584(7821):430–6. [CrossRef]
16. Chi Y, Ge Y, Wu B, Zhang W, Wu T, Wen T, et al. Serum cytokine and chemokine profile in relation to the severity of Coronavirus Disease 2019 in China. J Infect Dis 2020;222(5):746–54. [CrossRef]
17. Liao M, Liu Y, Yuan J, Wen Y, Xu G, Zhao J, et al. Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. Nat Med 2020;26(6):842–4. [CrossRef]
18. Pınarlık F, Babuççu G, Albayrak Ö, Doğan Ö, Keske Ş, Beşli Y, et al. Predictive role and clinical value of serum cytokines in COVID-19. Infect Dis Clin Microbiol 2024;6(4):258–67. [CrossRef]
19. McElvaney OJ, McEvoy NL, McElvaney OF, Carroll TP, Murphy MP, Dunlea DM, et al. Characterization of the Inflammatory response to severe COVID-19 illness. Am J Respir Crit Care Med 2020;202(6):812–21. [CrossRef]
20. RECOVERY Collaborative Group. Tocilizumab in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. Lancet 2021;397(10285):1637–45. [CrossRef]
21. Donlan AN, Sutherland TE, Marie C, Preissner S, Bradley BT, Carpenter RM, et al. IL-13 is a driver of COVID-19 severity. JCI Insight 2021;6(15):e150107. [CrossRef]
22. Gómez-Escobar LG, Hoffman KL, Choi JJ, Borczuk A, Salvatore S, Alvarez-Mulett SL, et al. Cytokine signatures of end organ injury in COVID-19. Sci Rep. 2021;11(1):12606. [CrossRef]
23. Schulte-Schrepping J, Reusch N, Paclik D, Baßler K, Schlickeiser S, Zhang B, et al. Severe COVID-19 is marked by a dysregulated myeloid cell compartment. Cell 2020;182(6):1419–40.e23.
24. Mathew D, Giles JR, Baxter AE, Oldridge DA, Greenplate AR, Wu JE, et al. Deep immune profiling of COVID-19 patients reveals distinct immunotypes with therapeutic implications. Science 2020;369(6508):eabc8511. [CrossRef]
25. Liang W, Liang H, Ou L, Chen B, Chen A, Li C, et al. Development and validation of a clinical risk score to predict the occurrence of critical illness in hospitalized patients with COVID-19. JAMA Intern Med 2020;180(8):1081–9. [CrossRef]
26. Yang Y, Shen C, Li J, Yuan J, Wei J, Huang F, et al. Plasma IP-10 and MCP-3 levels are highly associated with disease severity and predict the progression of COVID-19. J Allergy Clin Immunol 2020;146(1):119–27.e4. [CrossRef]

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Research Article

Endocrine Complications and Their Relationship with Iron Overload in Adult Patients with Sickle Cell Anemia

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Abstract

Objectives: The objective of this study was to investigate the prevalence of endocrine complications and to determine their potential relationship with transfusional iron accumulation in adult patients diagnosed with sickle cell anemia (SCA) residing in the Mersin region.

Methods: A retrospective multiparametric study was conducted involving 55 adult patients (aged 18–60 years) followed at the Mersin University Faculty of Medicine, Adult Hematology Clinic, between July 2018 and July 2019. Comprehensive clinical and laboratory data were extracted, including hemoglobin variants (HbS, HbF), serum ferritin, and specific endocrine markers such as calcium, phosphorus, 25-OH Vitamin D3, parathormone (PTH), thyroid functions (TSH, sT4, sT3), and gonadotropins (FSH, LH). Glucose metabolism was evaluated through fasting blood glucose, insulin, and HOMA-IR scores. Statistical analysis was performed using descriptive statistics and Chi-square tests to evaluate associations between ferritin levels and biochemical markers, with the threshold for significance set at $p<0.05$.

Results: The study cohort was comprised of 55 patients with a mean age of 32.02±11.97 years, with the female gender representing 41.8% of the population. High rates of metabolic and endocrine abnormalities were identified: Vitamin D deficiency was found in 61.8% of patients, while borderline levels were noted in an additional 23.6%. Furthermore, low corrected calcium was observed in 16.4% and elevated PTH was identified in 12.7% of the cohort. Regarding thyroid function, one patient (1.8%) exhibited low TSH levels, although this individual had a known history of primary hypothyroidism. In gonadal assessments, FSH and LH levels were elevated in 16.4% and 23.6% of patients, respectively, while no cases of hypogonadotropic hypogonadism were recorded. Glucose metabolism analysis revealed impaired fasting glucose in 18.2% and a HOMA-IR score >3 in 9.1%. No statistically significant association was found between serum ferritin levels and endocrine parameters such as ALP ($p=0.744$), Vitamin D ($p=0.505$), or TSH ($p=0.503$).

Conclusion: Although various endocrine complications—particularly those involving bone metabolism and Vitamin D—have been frequently identified in adult SCA patients, these dysfunctions have not been found to correlate with serum ferritin levels in this cohort. This lack of association suggests that factors such as chronic ischemia, micro-infarctions, and malperfusion may contribute to endocrine damage in SCA. However, the potential role of iron overload cannot be excluded, particularly given the limitations of ferritin as a surrogate marker. Early and regular screening remains essential for the management of these patients.

Keywords: Endocrine Complications, Ferritin, Iron Overload, Sickle Cell Anemia, Vitamin D

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Sickle cell anemia (SCA) has been characterized as a multisystemic, inherited disorder resulting from a specific genetic mutation in the beta-globin chain. This mutation, involving the substitution of valine for glutamic acid at the sixth position (GAG-GTG mutation), has been identified as the cause of Hemoglobin S (HbS) production. Under conditions of reduced oxygen tension, HbS has been known to polymerize, leading to the formation of rigid, sickle-shaped erythrocytes. This process has been recognized as the fundamental driver of chronic hemolysis and vaso-occlusion.[1–3]

The clinical manifestations of SCA have been described as extensive, encompassing multisystemic effects such as acute painful vaso-occlusive crises, acute chest syndrome, and progressive organ damage. Chronic damage to the microvasculature has been frequently observed, resulting in complications such as leg ulcers, pulmonary hypertension, and renal dysfunction.[4]

Endocrine organs have been identified as particularly vulnerable in the context of SCA. The pathophysiology of endocrine dysfunction has been regarded as multifaceted, potentially arising from micro-infarctions caused by vaso-occlusive events or from secondary iron overload resulting from chronic blood transfusions.[5] While growth retardation and pubertal delay have been extensively studied in pediatric populations, a significant need has been identified to evaluate the prevalence of endocrine complications in adult patients.[5] Consequently, this study has been designed to evaluate the endocrine profile of adult SCA patients in the Mersin region and to determine the extent to which these complications have been linked to iron accumulation as measured by serum ferritin.

Methods

Study Design

A retrospective multiparametric study was conducted at the Mersin University Hospital, Department of Hematology. The study protocol was reviewed and approved by the Mersin University Clinical Research Ethics Committee (Decision No: 2018/317, Date: 01/08/2018).

Patient Population

A total of 55 patients diagnosed with SCA were included in the study. All subjects presented to the adult hematology clinic between July 14, 2018, and July 14, 2019. Inclusion criteria were restricted to patients aged between 18 and 60 years. Exclusion criteria were defined as pregnancy, lactation, and a history of chemotherapy.

Parameters Evaluated

Laboratory and clinical data were collected from institutional medical records. The parameters analyzed included:

- **Hematological Profiles:** HbS, HbF, and total hemoglobin.
- **Bone and Mineral Metabolism:** Corrected calcium, 25-OH Vitamin D3, phosphorus, alkaline phosphatase (ALP), and parathormone (PTH).
- **Thyroid Function:** TSH, sT4, and sT3.
- **Gonadal Function:** FSH, LH, testosterone, and estradiol.
- **Glucose Metabolism:** Fasting blood glucose, insulin, and HOMA-IR scores.
- **Iron Status:** Serum ferritin levels.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 21.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (SD) or median (minimum–maximum) according to data distribution, while categorical variables were presented as frequencies and percentages.

The normality of continuous variables was assessed using the Kolmogorov–Smirnov test. Since ferritin levels were categorized into predefined groups (normal, high, and very high), comparisons between ferritin categories and categorical endocrine parameters were performed using the Chi-square test or Fisher's exact test when appropriate.

A p-value of <0.05 was considered statistically significant.

Results

Demographic Profile

The study cohort was constituted by 55 patients with a mean age of 32.02±11.97 years (range: 18–60). The female gender was represented by 41.8% (n=23) of the group, while males accounted for 58.2% (n=32).

Biochemical Findings

Metabolic and endocrine abnormalities were identified across several physiological systems. Regarding bone metabolism, Vitamin D deficiency (25-OH D3 <20 µg/L) was found in 61.8% (n=34) of the patients, while 23.6% (n=13) were recorded in the borderline range (20–30 µg/L). Low corrected calcium levels were observed in 16.4% (n=9) of the patients, and elevated PTH was identified in 12.7% (n=7).

In the evaluation of thyroid and gonadal functions, low TSH levels were identified in 1.8% (n=1) of the patients; however, this individual was noted to have a pre-existing

diagnosis of primary hypothyroidism. No cases of central hypothyroidism or central hyperthyroidism were detected. In the gonadal assessment, FSH levels above the reference range were identified in 16.4% (n=9) of patients, and elevated LH levels were found in 23.6% (n=13). No instances of hypogonadotropic hypogonadism were identified, and no history of hormone replacement therapy was reported by any patient.

Glucose Metabolism

Normal fasting blood glucose levels were maintained by 78.2% (n=43) of the patients. Impaired fasting glucose (100–126 mg/dL) was observed in 18.2% (n=10) of the subjects, and levels exceeding 126 mg/dL were recorded in 1.8% (n=1). A pre-existing diagnosis of Type 2 Diabetes Mellitus was identified in 3.6% (n=2) of the cohort. Elevated HOMA-IR scores (>3) were identified in 9.1% (n=5) of the patients.

Association Analysis

Statistical tests were performed to determine if a relationship existed between serum ferritin levels and endocrine markers. Patients were categorized by ferritin levels: normal, high (336–1000 ng/mL), and very high (>1000 ng/mL). No significant relationships were identified (Table 1).

Discussion

The results of this study offer a detailed overview of the endocrine status of adult SCA patients in the Mersin region. A significant finding was the relatively low prevalence of diabetes mellitus (3.6%) in the cohort compared to the 13.7% prevalence reported for the general Turkish population in the TURDEP-2 study.[6] This observation is consistent with previous studies suggesting a relatively lower prevalence of diabetes in patients with SCA, which may be attributed to a lower body mass index (BMI) and increased metabolic demand in this population.[1,7]

Bone metabolism represents a critical area of clinical concern. The high prevalence of Vitamin D deficiency (61.8%) and associated calcium imbalances are consistent with established literature regarding the impact of chronic hemolysis and vaso-occlusion on skeletal health.[8,9] These findings are consistent with previous studies demonstrating that metabolic deficiencies in SCA are associated with

Table 1. Association between ferritin levels and endocrine markers

		Ferritin			
		Normal n (%)	High n (%)	Very High n (%)	p
ALP	Normal	20 (87)	14 (82.4)	10 (76.9)	0.744
	High	3 (13)	3 (17.6)	3 (23.1)	
Creatinin	Normal	24 (100)	16 (94.1)	12 (92.3)	0.294
	High	0 (0)	1(5.9)	1 (7.7)	
Calcium	Low	3 (12.5)	4 (23.5)	2 (15.4)	0.650
	Normal	21 (87.5)	13 (76.5)	11(84.6)	
25(OH)D	Deficiency	13 (59.1)	11 (68.8)	10 (76.9)	0.505
	Incuffiency	6 (27.3)	4 (25)	3 (23.1)	
	Normal	3 (13.6)	1 (6.3)	0 (0)	
Parathormon	Normal	17 (94.4)	14 (82.4)	10 (76.9)	0.322
	High	1 (5.6)	3 (17.6)	3 (23.1)	
TSH	Low	1 (4.2)	0 (0)	0 (0)	0.503
	Normal	22 (91.7)	17 (100)	13 (100)	
	High	1 (4.2)	0 (0)	0 (0)	
Hyphotyroidism	None	23 (95.8)	17 (100)	12 (92.3)	0.418
	Primary	1 (4.2)	0 (0)	1 (7.7)	
FBG	Normal	20 (83.3)	12 (70.6)	11 (84.6)	0.274
	IFG	4 (16.7)	5 (29.4)	1 (7.7)	
	DM	0 (0)	0 (0)	1 (7.7)	

ALP: Alkaline phosphatase; TSH: Thyroid functions; IFG: Impaired fasting glucose; FBG: Fasting blood glucose; DM: Diabetes mellitus.

chronic bone pain and reduced bone mineral density (BMD), which may manifest earlier in the SCA population compared to the general population.[9,10]

A central observation of this study was the lack of a significant association between ferritin levels and endocrine dysfunction, representing a "ferritin paradox" in comparison to other hemoglobinopathies. In thalassemia major, iron overload is a well-established primary driver of endocrinopathy. However, in this SCA cohort, thyroid, gonadal, and glucose metabolism parameters did not differ significantly according to iron status. These findings suggest that the pathophysiology of organ damage in SCA is likely multifactorial, with vaso-occlusive micro-infarctions and chronic malperfusion playing a significant role alongside potential contributions from iron overload.[11] Furthermore, chronic malnutrition and zinc deficiency, which have been associated with growth failure and hypogonadism, may also contribute to endocrine dysfunction in this population.[12]

One important limitation of this study is the use of serum ferritin as a surrogate marker for iron overload. Ferritin is known to be influenced by inflammatory status and may not accurately reflect total body iron stores in patients with sickle cell anemia. Previous studies have demonstrated that more reliable assessments of iron overload, such as MRI T2 or liver iron concentration measurements, may be required to accurately evaluate iron burden.[13,14] Therefore, the absence of an observed association between ferritin levels and endocrine dysfunction should be interpreted with caution.

Other limitations of this study include its retrospective design and relatively small sample size (n=55), which may have limited the ability to detect more subtle biochemical associations.

Conclusion

In this retrospective cohort of adult patients with sickle cell anemia, endocrine and metabolic abnormalities were frequently observed, particularly Vitamin D deficiency and disturbances in bone metabolism. However, serum ferritin levels were not significantly associated with the evaluated endocrine parameters. These findings suggest that endocrine dysfunction in adult sickle cell anemia may not be explained solely by iron overload and may also reflect chronic vaso-occlusion, microvascular injury, and malperfusion. Regular endocrine screening should be considered in adult patients with sickle cell anemia, regardless of ferritin status.

Disclosures

Ethics Committee Approval: The study protocol was reviewed and approved by the Mersin University Clinical Research Ethics Committee (Decision No: 2018/317, Date: 01/08/2018).

Informed Consent: Informed consent was waived due to the retrospective nature of the study.

Conflict of Interest: The authors declare no conflicts of interest.

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References

1. Kato GJ, Piel FB, Reid CD, Gaston MH, Ohene-Frempong K, Krishnamurti L, et al. Sickle cell disease. Nat Rev Dis Primers 2018;4:18010. [CrossRef]
2. Bunn HF. Pathogenesis and treatment of sickle cell disease. N Engl J Med 1997;337(11):762–9. [CrossRef]
3. Rees DC, Williams TN, Gladwin MT. Sickle-cell disease. Lancet 2010;376(9757):2018–31. [CrossRef]
4. Ballas SK. Sickle cell anaemia: progress in pathogenesis and treatment. Drugs 2002;62(8):1143-72. [CrossRef]
5. Mandese V, Bigi E, Bruzzi P, Palazzi G, Predieri B, Lucaccioni L, et al. Endocrine and metabolic complications in children and adolescents with Sickle Cell Disease: an Italian cohort study. BMC Pediatr 2019;19(1):56. [CrossRef]
6. Satman I, Omer B, Tutuncu Y, Kalaca S, Gedik S, Dinccag N, et al; TURDEP-II Study Group. Twelve-year trends in the prevalence and risk factors of diabetes and prediabetes in Turkish adults. Eur J Epidemiol 2013;28(2):169–80.
7. Özen S, Ünal S, Erçetin N, Taşdelen B. Frequency and risk factors of endocrine complications in Turkish children and adolescents with sickle cell anemia. Turk J Haematol 2013;30(1):25–31. [CrossRef]
8. Buison AM, Kawchak DA, Schall J, Ohene-Frempong K, Stallings VA, Zemel BS. Low vitamin D status in children with sickle cell disease. J Pediatr 2004;145(5):622–7. [CrossRef]
9. Osunkwo I, Hodgman EI, Cherry K, Dampier C, Eckman J, Ziegler TR, et al. Vitamin D deficiency and chronic pain in sickle cell disease. Br J Haematol 2011;153(4):538–40. [CrossRef]
10. Sahu A, Narayan Padhi U, Bhaskar LVKS. Defining vitamin D deficiency in patients with sickle cell disease: a meta-analysis. J Parathyroid Dis 2022;10:11154. [CrossRef]
11. Fung EB, Harmatz PR, Lee PD, Milet M, Bellevue R, Jeng MR, et al; Multi-Centre Study of Iron Overload Research Group. Increased prevalence of iron-overload associated endocrinop-

athy in thalassaemia versus sickle-cell disease. Br J Haematol 2006;135(4):574–82. [CrossRef]

12. Zemel BS, Kawchak DA, Fung EB, Ohene-Frempong K, Stallings VA. Effect of zinc supplementation on growth and body composition in children with sickle cell disease. Am J Clin Nutr 2002;75(2):300–7. [CrossRef]
13. Wood JC. Use of magnetic resonance imaging to monitor iron overload. Hematol Oncol Clin North Am 2014;28(4):747–64, vii. [CrossRef]
14. Coates TD, Wood JC. How we manage iron overload in sickle cell patients. Br J Haematol 2017;177(5):703 [CrossRef]

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Research Article

Etiological Investigation of Cryptogenic Cirrhosis Patients Undergoing Liver Transplantation

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Abstract

Objectives: This study aimed to investigate hidden etiologies in patients with cryptogenic cirrhosis by evaluating post-liver transplantation explant pathology findings.

Methods: Between 2010 and 2018, 324 patients with cryptogenic cirrhosis from a total cohort of 2,400 were analyzed retrospectively. Pretransplant clinical scores, including FIB-4, APRI, Child, and MELD scores, and posttransplant pathology results were recorded. Patients diagnosed with NASH were further evaluated for body mass index (BMI) and diabetes mellitus (DM). Ascites and hepatocellular carcinoma (HCC) conversion rates were also analyzed.

Results: The mean age of the patients was 47.5±14.6 years. Posttransplant pathology identified specific etiologies in 58.3% of cases: NASH/NAFLD (24.7%), HCC (7.7%), veno-occlusive disease (6.8%), biliary pathology (5.2%), autoimmune hepatitis (4.3%), and Wilson's disease (3%). Etiology remained undetermined in 135 patients (41.7%). Ascites was detected in 71.5% (n=232) of patients, and HCC was found in 17.3% (n=57). Among patients with NASH, 66.15% had DM/insulin resistance, and 72.5% had a BMI >25. The pretransplant mean Child and MELD scores were 12.1±1.4 and 28.5±5.3, respectively.

Conclusion: Explant pathology reveals that various diseases, particularly NASH/NAFLD, often underlie cryptogenic cirrhosis. NASH should be investigated thoroughly in cases of metabolic syndrome. Additionally, occult HBV and veno-occlusive disease should be considered in unexplained cirrhosis, even when standard serology and imaging are negative.

Keywords: Cryptogenic cirrhosis, liver transplantation, non-alcoholic fatty liver disease

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Liver cirrhosis is a chronic liver disease characterized by progressive fibrosis and regenerative nodules, which can develop due to various causes, such as viral hepatitis, chronic alcohol use, autoimmune diseases, vascular causes, and metabolic and toxic conditions.[1] As the disease progresses, serious complications, such as ascites, portal hypertension, esophageal varices, hepatorenal syndrome, and hepatocellular carcinoma (HCC), may develop.[2]

In developed countries, the most common causes of cirrhosis are alcohol and non-alcoholic fatty liver disease (NAFLD), whereas in developing countries, viral hepatitis plays a more frequent role in the etiology. However, cases in which the etiology cannot be determined despite detailed clinical, laboratory, and imaging evaluations are referred to as "cryptogenic cirrhosis."[3] While up to 30% of cirrhosis cases were classified as cryptogenic in the past, this rate has decreased to around 10% today due to advancements in diagnostic methods.[4]

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Recent studies suggest that in a significant proportion of patients diagnosed with cryptogenic cirrhosis, the underlying cause may be silently progressing non-alcoholic steatohepatitis (NASH), and that latent viral infections or autoimmune hepatitis may also play a role in the etiology.[5] In particular, obesity, type 2 diabetes mellitus, insulin resistance, hyperlipidemia, and the presence of metabolic syndrome are considered significant risk factors for the development of cryptogenic cirrhosis. In addition, occult HBV infection that cannot be detected serologically, vascular diseases, autoimmune hepatitis, and rare metabolic disorders may also play a role in the etiology of cryptogenic cirrhosis.[6]

Liver tissue obtained from explants following liver transplantation offers a significant opportunity to identify etiological causes that could not be detected before transplantation. In particular, the ability to reevaluate histopathological findings that are lost in advanced-stage cirrhosis through detailed posttransplant pathological examination may contribute to determining the true etiology in patients classified as having cryptogenic cirrhosis.

This study aimed to evaluate the explant pathology results of patients with cryptogenic cirrhosis who underwent liver transplantation and to investigate underlying occult etiologies. Furthermore, degrees of fibrosis were assessed using pretransplant APRI and FIB-4 scores, and MELD and Child-Pugh scores were calculated. Metabolic risk factors, such as obesity, diabetes mellitus, and insulin resistance, were examined in patients diagnosed with NASH. Additionally, the presence of HCC, the development of ascites, and the distribution of blood groups were evaluated to reveal the clinical characteristics of cryptogenic cirrhosis in more detail.

Methods

Study Design and Patient Selection

In our study, the medical records of patients who presented to the Hepatology Outpatient Clinic at İnönü University Turgut Özal Medical Center between 2010 and 2018 and underwent liver transplantation were retrospectively reviewed. Despite comprehensive pretransplant clinical, biochemical, serological, microbiological, radiological (ultrasonography, portal vein Doppler, elastography, etc.), and histopathological (liver biopsy) examinations, the etiology remained unclear; a total of 324 patients diagnosed with "cryptogenic cirrhosis" who underwent liver transplantation were included in the study.

Inclusion and Exclusion Criteria

The inclusion and exclusion criteria for the study were determined as follows:

Inclusion Criteria: (1) Being 18 years of age or older, (2) having received a diagnosis of "cryptogenic cirrhosis" despite detailed pretransplant investigations that failed to identify the etiology, and (3) having undergone liver transplantation at İnönü University Turgut Özal Medical Center and having access to posttransplant explant liver pathology results.

Exclusion Criteria: (1) Patients whose cirrhosis etiology was determined before transplantation, including viral hepatitis such as HBV, HCV, HDV, HAV, or HEV; autoimmune or biliary pathologies confirmed by ASMA, LKM-1, ANA, or AMA positivity; ischemic hepatitis; known history of alcoholism; Wilson's disease; hemochromatosis; or drug-induced toxic hepatitis, etc.; (2) patients who had not undergone liver transplantation or for whom explant pathology data were unavailable; and (3) patients with missing pretransplant laboratory or clinical data required for the scoring systems to be used in the study.

Data Collection and Analysis

The post-liver-transplant explant liver pathology results of the 324 patients included in the study were retrospectively analyzed to determine the actual etiologies. Using the patients' pretransplant laboratory data, APRI and FIB-4 scores were calculated to predict the degree of liver fibrosis; Child and MELD scores were calculated to determine the severity of liver failure and the urgency of transplantation.

For patients whose explant pathology was consistent with non-alcoholic steatohepatitis (NASH), body mass index (BMI) and the presence of diabetes mellitus (DM) were examined to calculate NAFLD scores. Additionally, the radiological, endoscopic, and pathological data of all patients were reviewed to document the presence of major complications of cirrhosis, including hepatocellular carcinoma (HCC), ascites, and esophageal varices. Pretransplant tumor marker levels, primarily alpha-fetoprotein (AFP), were also included in the analysis.

Statistical Analysis

Statistical analysis was performed using SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were expressed as mean±standard deviation for continuous variables and as number (n) and percentage (%) for categorical variables. Only descriptive statistics were used in the study.

AI-Assisted Technology Statement: In the production of this work, the authors utilized Gemini 3 Flash (Google, May 2026 version) as an AI-assisted tool. The technology was employed to restructure the manuscript into a blinded format by separating the title page and main text, ensure that the

statistical reporting met the journal's specific requirements, such as adding location data for the software, and improve English language flow. The prompts used primarily focused on "reformatting existing clinical data and text according to specific submission guidelines." All AI-generated structural and linguistic suggestions were critically appraised by the authors to ensure scientific accuracy and integrity.

Ethical Approval

Approval for this study was obtained from the İnönü University Non-Interventional Clinical Research Ethics Committee (Date: 30.07.2019 and Decision/Protocol Number: 2019/310), and the study was conducted in accordance with the principles of the Declaration of Helsinki.

Results

The study included a total of 324 patients (126 female [38.9%] and 198 male [61.1%]) who were followed up with a preliminary diagnosis of cryptogenic cirrhosis and underwent liver transplantation. The mean age of the patients was 47.5±14.6 years. When pretransplant clinical scores were examined, the mean Child score was 12.1±1.4, and the mean MELD score was 28.5±5.3. Based on imaging methods, ascites was observed in 71.5% of patients (n=232), while hepatocellular carcinoma (HCC) was detected in 17.3% of patients (n=57) based on clinical, radiological, and pathological data (Table 1).

In the etiological analysis based on posttransplant explanted liver pathology results, the etiology of 135 patients (41.7%) could not be determined despite all investigations and was classified as "cryptogenic cirrhosis." The most common etiological factor identified was hepatic steatosis (NASH/NAFLD) in 80 patients (24.7%). The etiological distribution of the remaining patients was as follows: primary HCC (n=25, 7.7%), veno-occlusive disease (n=22, 6.8%), biliary pathologies (PSC, PBC; n=17, 5.2%), autoimmune hepatitis (n=14, 4.3%), and Wilson's disease (n=10, 3%). In the remaining 21 patients (6.4%), rarer causes, such as HBV, HCV, hemochromatosis, ischemic hepatitis, hemophagocytic syndrome, glycogen storage disease, and amyloidosis, were identified (Table 2).

In our study, subgroup analysis of 80 patients with findings consistent with NASH revealed that 66.15% of these patients (n=53) had diabetes mellitus (DM) or insulin resistance. When the body mass index (BMI) of the NASH group was evaluated, 40% (n=32) were found to be obese (BMI >30), while 32.5% (n=26) were overweight (BMI 25–30).

While the APRI and FIB-4 scores of the patients were consistent with advanced fibrosis in all cases, the NAFLD score supported advanced fibrosis in 91% of the NASH group.

Table 1. Clinical characteristics of the patients

Variables	n=324
Mean Age	47.5 (±14.6)
Sex	
Male	198 (%61.1)
Female	126 (%38.9)
MELD	28.5 (±5.3)
CHILD	12.1(±1.4)
Ascites	
Yes	232 (71.5%)
No	92 (28.5%)
HCC	
Yes	57 (17.3%)
No	267 (82.7%)

MELD: Model for end-stage liver disease; CHILD: Child–pugh classification/score; HCC: Hepatocellular carcinoma

Discussion

In our study, examination of the pathology of explanted livers from patients with cryptogenic cirrhosis who underwent liver transplantation revealed that the underlying etiology could be reclassified in a significant proportion of

Table 2. Etiological distribution according to explant liver pathology

Variables	n=324 (%)
Veno-occlusive disease	22 (6.8%)
NASH/NAFLD	80 (24.7%)
HCC	25 (7.7%)
Cryptogenic	135 (41.7%)
Alpha-1 antitrypsin deficiency	1 (0.3%)
Hemochromatosis	4 (1.2%)
Hepatitis B virus related hepatitis	7 (2.1%)
Biliary pathology-related cirrhosis	17 (5.2%)
Ischemic hepatitis	2 (0.6%)
Autoimmune hepatitis	14 (4.3%)
Wilson disease	10 (3.0%)
Glycogen storage disease	1 (0.3%)
Amyloidosis	2 (0.6%)
Hepatitis C virus related hepatitis	2 (0.6%)
Hemophagocytic syndrome	2 (0.6%)

NASH: Non-alcoholic steatohepatitis; NAFLD: Non-alcoholic fatty liver disease; HCC: Hepatocellular carcinoma

cases. The fact that NASH/NAFLD was the most frequently identified cause supports the role of metabolic liver diseases in the development of cryptogenic cirrhosis.

In a study conducted by Charlton and colleagues, the authors reported a high prevalence of obesity, diabetes mellitus, and dyslipidemia among patients with cryptogenic cirrhosis who underwent liver transplantation. They suggested that a significant proportion of these patients may have NASH. The study evaluated patients with cryptogenic cirrhosis who underwent liver transplantation and reported that, despite advanced investigations, the exact etiology could not be determined in some cases, and that this situation might be related to the blurring of histopathological features, particularly in advanced-stage liver disease.[4] In our study as well, the finding that the NASH/NAFLD rate in explant pathology was 24.7% supports this view. Furthermore, the fact that 66.15% of patients diagnosed with NASH had diabetes mellitus or insulin resistance and 72.5% were overweight or obese clearly demonstrates the decisive role of metabolic syndrome components in the etiology of cryptogenic cirrhosis. These results highlight the need for careful evaluation of metabolic risk factors, even if steatosis findings have resolved before transplantation.

Despite explant pathology, the fact that the etiology could not be determined in 41.7% of patients is noteworthy, as it suggests that current diagnostic approaches may be insufficient in a significant proportion of cryptogenic cirrhosis cases. Therefore, our study indicates that routine serological tests, imaging methods, and classical histopathological examinations are not always sufficient for etiological differentiation in advanced cirrhosis. In particular, genetic analyses, advanced immunophenotyping, viral nucleic acid testing, and metagenomic sequencing are recognized as important methods for identifying underlying hidden etiologies in cases of cryptogenic cirrhosis.

In a study by Wong and colleagues, it was noted that a significant proportion of patients awaiting liver transplantation have NASH.[5] Similarly, a study by Huang and colleagues indicated that metabolic dysfunction-associated fatty liver disease is one of the fastest-growing causes of cirrhosis.[6] In our study, the fact that NASH/NAFLD was the most frequently identified cause in the posttransplant pathological examination of patients with unknown pretransplant etiology suggests that this global trend is also evident in our country. In particular, the increase in the prevalence of obesity and type 2 diabetes mellitus necessitates a more systematic investigation of metabolic-origin diseases in patients diagnosed with cryptogenic cirrhosis.

DeLeve and colleagues noted that sinusoidal obstruction syndrome is characterized by perisinusoidal fibrosis and lobular congestion and that the primary mechanism underlying its pathogenesis is damage to sinusoidal endothelial cells. The study demonstrated that vascular liver diseases are often diagnosed late due to nonspecific laboratory and imaging findings and that some cases can only be diagnosed through liver biopsy or posttransplant pathology.[7] In our study as well, the fact that the diagnosis was established posttransplant via pathology in 6.8% of patients initially classified as cryptogenic is consistent with these literature findings. In this context, the findings of our study support the necessity of considering vascular causes in patients with unexplained cirrhosis through Doppler ultrasound, cross-sectional imaging, and histopathological evaluation when indicated.

The presence of cases of autoimmune hepatitis in which autoantibodies, such as ANA, ASMA, and LKM-1, may be negative can lead to delayed diagnosis and result in patients being managed as having cryptogenic cirrhosis. Czaja reported that seronegative autoimmune hepatitis poses diagnostic challenges, particularly in the advanced stages of fibrosis or cirrhosis, due to the blurring of typical histological and immunological findings. It is emphasized that the absence of autoantibodies does not rule out the diagnosis of autoimmune hepatitis, and this diagnosis must always be considered in cases of unexplained chronic hepatitis or cirrhosis.[8] Similarly, in cholestatic diseases, such as primary biliary cholangitis and primary sclerosing cholangitis, serological, biochemical, and imaging findings may be absent, making diagnosis challenging. The American Association for the Study of Liver Diseases guidelines recommend systematically investigating autoimmune and cholestatic causes in cases of unexplained cirrhosis.[9] In our study, autoimmune hepatitis was observed in 4.3% of cases, while biliary pathologies, including primary biliary cholangitis and primary sclerosing cholangitis, were detected in 5.2% of cases. These findings indicate that autoimmune and cholestatic liver diseases represent significant yet frequently overlooked causes among cases classified as cryptogenic cirrhosis.

Ferenci et al.[10] reported that Wilson's disease can present with highly variable clinical manifestations and that some patients may present directly with decompensated cirrhosis; however, when diagnosed early, the progression of liver damage can be halted with chelation therapy. Pietrangelo demonstrated that hereditary hemochromatosis can remain asymptomatic for many years and that untreated patients may progress to cirrhosis; however, if detected early through serum ferritin and transferrin saturation levels, the development of cirrhosis and hepatocellular carcinoma can be largely prevented with regular phlebotomy.[11]

Eriksson and colleagues, on the other hand, reported that alpha-1 antitrypsin deficiency, particularly in the PiZZ genotype, increases the risk of progressive fibrosis, cirrhosis, and primary liver cancer.[12]

In our study, when the transplant pathology of patients with cryptogenic cirrhosis was examined, Wilson's disease was detected in 3%, hereditary hemochromatosis in 1.2%, and alpha-1 antitrypsin deficiency in 0.3% of cases. Although the overall prevalence of these diseases appears low, our findings indicate that hereditary and metabolic liver diseases constitute a clinically significant proportion of cases classified as cryptogenic cirrhosis. It is important to investigate these conditions, particularly in cases of cirrhosis that develop at a young age, have a family history, or cannot be explained by common etiological factors, such as viral hepatitis, alcohol use, and metabolic syndrome. Our study demonstrates that these conditions may easily be overlooked in subsequent pathology, as clinical and laboratory findings lose their specificity in advanced-stage cirrhosis. However, the availability of effective disease-modifying treatment options for all these conditions strongly supports the necessity of evaluating Wilson's disease, hemochromatosis, and alpha-1 antitrypsin deficiency in the differential diagnosis of unexplained cirrhosis cases.

In our study, HBV-associated hepatitis was detected in 2.1% of cases and HCV-associated hepatitis in 0.6%. In these patients, who had negative serological test results before transplantation and were diagnosed with cryptogenic cirrhosis, the identification of viral etiology through explant pathology supports the importance of occult viral hepatitis in the etiology of cryptogenic cirrhosis. Similarly, Raimondo et al.[13] noted that in cases of occult HBV infection, particularly where HBV DNA persists at low levels, viral persistence may continue in the liver despite HBsAg negativity, and this could lead to the development of cirrhosis and hepatocellular carcinoma. Studies by Im et al.[14] also emphasized that while these serological markers may become negative during the low-replicative phase of HBV infection, HBV DNA can still be detected, particularly in liver tissue, and therefore serology alone is insufficient. Regarding HCV, Pawlotsky et al.[15] reported that HCV RNA is the only reliable diagnostic method in cases with negative or low-titer anti-HCV and that molecular tests are of critical importance, particularly in patients with advanced fibrosis. The findings of our study support the need to avoid overlooking occult viral hepatitis, particularly in seronegative or atypically presenting patients, and underscore the significant role of molecular tests in the diagnostic algorithm.

The incidence of hepatocellular carcinoma (HCC) was found to be 17.3% in our study. This rate supports the notion that cryptogenic cirrhosis carries a significant risk of malignancy, particularly in patients with advanced-stage disease. Previous studies have also shown that a significant proportion of cryptogenic cirrhosis is associated with underlying NASH/NAFLD and that the development of HCC is not uncommon in this patient group. A study by Bugianesi and colleagues emphasized that, given the global increase in the prevalence of NAFLD, NASH-related cirrhosis constitutes a significant risk group for the development of hepatocellular carcinoma. Furthermore, it has been shown that many cases classified as cryptogenic cirrhosis are actually of NASH origin and that inflammation, oxidative stress, and insulin resistance are the key mechanisms triggering carcinogenesis in these patients.[16]

These mechanisms accelerate HCC development, particularly through the effects of chronic low-grade inflammation and metabolic dysfunction on DNA damage and hepatic cell proliferation. The high HCC rate identified in our study underscores the need for close monitoring of patients with cryptogenic cirrhosis not only for liver failure but also for the development of malignancy. In this context, ensuring early diagnosis through regular ultrasound and alpha-fetoprotein (AFP) monitoring is of critical clinical importance.

The fact that the patients' mean MELD score was 28.5 and their Child-Pugh score was 12.1 indicates that the cases included in the study belonged to the advanced-stage decompensated cirrhosis group. At this stage of liver failure, initial etiological clues diminish because advanced fibrosis and nodular regeneration become predominant in the hepatic parenchyma. In evaluations of the MELD score by Kamath and colleagues, it was demonstrated that as the MELD score increases, decompensation worsens during the pretransplant period, and the diagnostic power of clinical and biochemical parameters for etiological differentiation decreases. The Child-Pugh classification, on the other hand, has revealed that in advanced cirrhosis, liver functional reserve is significantly reduced, and at this stage, complications take precedence over the etiology of the disease.[17] In this context, the literature also reports that histopathological specificity decreases in advanced cirrhosis and that many cases classified as cryptogenic can only be reclassified through posttransplant explant examination.

In our study, the finding that APRI and FIB-4 scores were consistent with advanced fibrosis in all cases was an expected result and supports the role of these indices in reflecting the severity of fibrosis in patients with advanced cirrhosis. In particular, the fact that the NAFLD score confirmed advanced fibrosis in 91% of the NASH group demonstrates that noninvasive scoring systems are reliable tools for assessing fibrosis in metabolic-related liver diseases. In vali-

dation studies of the FIB-4 index developed by Sterling and Lai,[18] it was demonstrated that this score, based on age, AST, ALT, and platelet counts, has a high negative predictive value for detecting advanced fibrosis. Similarly, Wai et al.[19] reported that the APRI score can be used to predict the stage of fibrosis in patients with chronic hepatitis B and C but that it reflects the degree of fibrosis rather than establishing an etiological diagnosis. In studies conducted by McPherson et al.[20] in a NAFLD population, it was demonstrated that FIB-4 and similar noninvasive scores are particularly valuable in ruling out advanced fibrosis and cirrhosis but may have limited sensitivity in early-stage disease. In this context, the findings of our study indicate that scores such as APRI and FIB-4 reflect the severity of fibrosis, particularly in advanced-stage patients, but are not sufficient on their own to determine the underlying etiology in cryptogenic cirrhosis.

Our study has certain limitations, such as its single-center and retrospective design. Since the study included only advanced-stage patients who underwent liver transplantation, it may not be representative of patients with earlier-stage cryptogenic cirrhosis. Additionally, the inability to perform advanced molecular tests that could identify occult viral infections or genetic causes in some patients may have contributed to the high proportion of cases with unexplained etiology.

The most significant strengths of our study include the large patient sample size, the standardized transplantation and pathology evaluations conducted at a single center, and the detailed examination of explanted liver tissue. Furthermore, the systematic assessment of metabolic risk factors contributes to a better understanding of NASH-associated cryptogenic cirrhosis.

Conclusion

This study, which examined the pathology of explanted livers in patients followed up with a preliminary diagnosis of cryptogenic cirrhosis who underwent transplantation, demonstrated that non-alcoholic fatty liver disease (NASH/NAFLD) underlies a significant proportion of cases in which the etiology was previously considered unclear. In cirrhotic patients with metabolic syndrome components, such as obesity, diabetes, and hypertension, which have become major problems of the modern era, NASH/NAFLD should be considered the primary etiology. The use of noninvasive tests, such as imaging methods and the NAFLD score in the early stages, diagnosis through liver biopsy when necessary, and a focus on lifestyle changes are critical for preventing disease progression. On the other hand, the ability to detect pathologically latent hepatitis B in patients with negative serological tests and the presence of veno-occlusive diseases that may be missed in routine imaging highlight the need for advanced vascular imaging methods in etiological investigations. Our study highlights the importance of accessible, low-cost, and clinically feasible tests, such as Child and MELD. The fact that the proportion of true cryptogenic cirrhosis cases with an unexplained etiology, despite thorough investigations, was found to be higher in our study compared with the literature suggests a need for further genetic and molecular studies to elucidate the underlying mechanisms of this disease.

Disclosures

Ethics Committee Approval: This study was approved by The İnönü University Scientific Research and Publication Ethics Committee (Approval No: 2019/310 -30.07.2019)

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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Authorship Contributions:

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References

1. Tsochatzis EA, Bosch J, Burroughs AK. Liver cirrhosis. Lancet 2014;383(9930):1749–61. [CrossRef]
2. Cattau EL Jr, Benjamin SB, Knuff TE, Castell DO. The accuracy of the physical examination in the diagnosis of suspected ascites. JAMA 1982;247(8):1164–6. [CrossRef]
3. Heidelbaugh JJ, Bruderly M. Cirrhosis and chronic liver failure: part I. Diagnosis and evaluation. Am Fam Physician 2006;74(5):756–62.
4. Charlton MR, Kondo M, Roberts SK, Steers JL, Krom RA, Wiesner RH. Liver transplantation for cryptogenic cirrhosis. Liver Transpl Surg 1997;3(4):359–64. [CrossRef]
5. Wong RJ, Aguilar M, Cheung R, Perumpail RB, Harrison SA, Younossi ZM, et al. Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. Gastroenterology 2015;148(3):547–55. [CrossRef]
6. Huang DQ, Terrault NA, Tacke F, Gluud LL, Arrese M, Bugianesi E, et al. Global epidemiology of cirrhosis - aetiology,

trends and predictions. Nat Rev Gastroenterol Hepatol 2023;20(6):388–98. [CrossRef]
7. DeLeve LD, Valla DC, Garcia-Tsao G; American Association for the Study Liver Diseases. Vascular disorders of the liver. Hepatology 2009;49(5):1729–64. [CrossRef]
8. Czaja AJ. Autoimmune hepatitis in special patient populations. Best practice & research. Clinical gastroenterology 2011;*25*(6):689–700. [CrossRef]
9. Lindor KD, Bowlus CL, Boyer J, Levy C, Mayo M. Primary biliary cholangitis: 2018 practice guidance from the American Association for the study of liver diseases. Hepatology 2019;69(1):394–419. [CrossRef]
10. Ferenci P, Caca K, Loudianos G, Mieli-Vergani G, Tanner S, Sternlieb I, et al. Diagnosis and phenotypic classification of Wilson disease. Liver Int 2003;23(3):139–42. [CrossRef]
11. Pietrangelo A. Hereditary hemochromatosis--a new look at an old disease. N Engl J Med 2004;350(23):2383–97. [CrossRef]
12. Eriksson S, Carlson J, Velez R. Risk of cirrhosis and primary liver cancer in alpha 1-antitrypsin deficiency. N Engl J Med 1986;314(12):736–9. [CrossRef]
13. Raimondo G, Allain JP, Brunetto MR, Buendia MA, Chen DS, Colombo M, et al. Statements from the Taormina expert meeting on occult hepatitis B virus infection. J Hepatol 2008;49(4):652–7. [CrossRef]
14. Im YR, Jagdish R, Leith D, Kim JU, Yoshida K, Majid A, et al. Prevalence of occult hepatitis B virus infection in adults: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol 2022;7(10):932–42. [CrossRef]
15. Pawlotsky JM. Use and interpretation of virological tests for hepatitis C. Hepatology 2002;36(Suppl 1):S65–73. [CrossRef]
16. Bugianesi E, Leone N, Vanni E, Marchesini G, Brunello F, Carucci P, et al. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. Gastroenterology 2002;123(1):134–40. [CrossRef]
17. Kamath PS, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Kosberg CL, et al. A model to predict survival in patients with end-stage liver disease. Hepatology 2001;33(2):464–70. [CrossRef]
18. Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology 2006;43(6):1317–25. [CrossRef]
19. Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology 2003;38(2):518–26. [CrossRef]
20. McPherson S, Stewart SF, Henderson E, Burt AD, Day CP. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. Gut 2010;59(9):1265–9. [CrossRef]

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Research Article

Frequency of Thyroid Nodules and Autoimmune Thyroid Disease in Patients with Adrenal Incidentaloma

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Abstract

Objectives: To evaluate the clinical, laboratory, and radiological characteristics of patients with adrenal incidentaloma and to investigate the relationship between adrenal incidentaloma and autoimmune thyroid disease.

Methods: This retrospective study included 66 patients diagnosed with adrenal incidentaloma between December 2018 and February 2019. Demographic characteristics, hormonal status, radiological findings, metabolic comorbidities, and thyroid ultrasonography findings were evaluated. Autoimmune thyroid disease was assessed according to thyroid autoantibody positivity and thyroid parenchymal heterogeneity.

Results: The mean age was 56.2±7.9 years, and 77.3% of the patients were female. Overall, 83.3% of the patients were overweight or obese. Hypertension (29.5%), diabetes mellitus (21.4%), and hyperlipidemia (13.4%) were the most common comorbidities. Nonfunctioning adrenal adenoma was detected in 83.3% of patients. Thyroid nodules were significantly more frequent in patients with functioning adrenal adenoma than in those with nonfunctioning adenoma (90.9% vs. 56.4%, p=0.04). No significant association was found between hormonal activity and thyroid autoantibody positivity or thyroid parenchymal heterogeneity.

Conclusion: Functioning adrenal adenoma may be associated with increased thyroid nodule frequency; however, no relationship was demonstrated between adrenal hormonal activity and autoimmune thyroid disease.

Keywords: Adrenal incidentaloma, autoimmune thyroid disease, thyroid nodule

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Adrenal incidentaloma (AI) is defined as an adrenal mass incidentally detected during imaging studies performed without suspicion of adrenal disease. In recent years, the frequency of adrenal incidentaloma has increased significantly due to the widespread use of computed tomography (CT) and magnetic resonance imaging (MRI).[1] The prevalence of adrenal incidentaloma in autopsy series has been reported to range between 1.4% and 8.7%.[2] Adrenal incidentalomas are more frequently observed in the elderly population, and most cases are diagnosed between the sixth and eighth decades of life.[3]

The majority of adrenal incidentalomas are benign and nonfunctioning adrenal adenomas. However, clinically significant pathologies such as pheochromocytoma, primary hyperaldosteronism, subclinical hypercortisolism, adrenocortical carcinoma, and metastatic lesions may also be included in the etiology of adrenal incidentaloma.[4] Therefore,

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evaluation of the hormonal activity and malignant potential of the adrenal mass is of great importance.

A detailed clinical evaluation, hormonal investigations, and radiological imaging methods are used together in the assessment of patients with adrenal incidentaloma. Current guidelines recommend the 1-mg dexamethasone suppression test in all patients to exclude autonomous cortisol secretion.[5] In addition, plasma or urinary metanephrine levels are evaluated for pheochromocytoma,[6] and primary hyperaldosteronism is investigated in the presence of hypertension or hypokalemia. In radiological evaluation, the size, density, and imaging characteristics of the adrenal mass provide important clues regarding malignancy.

Autoimmune thyroid diseases (AITD) are chronic autoimmune disorders that develop as a result of the interaction between genetic susceptibility and environmental factors. Hashimoto's thyroiditis and Graves' disease are the most common clinical forms of autoimmune thyroid diseases, and the prevalence in the general population is reported to be approximately 5%.[7] HLA-associated genetic factors, immune regulatory genes, female sex, age, iodine intake, and environmental triggers are known to play a role in the development of autoimmune thyroid diseases.

In recent years, there has been increasing interest in the relationship between adrenal incidentaloma and various endocrine and autoimmune diseases. However, there are limited data in the literature regarding the frequency of autoimmune thyroid disease and associated clinical characteristics in patients with adrenal incidentaloma. Therefore, the present study aimed to investigate the frequency of autoimmune thyroid disease in patients with adrenal incidentaloma and to evaluate the accompanying clinical, hormonal, and radiological characteristics.

Methods

In this retrospective study, the clinical, laboratory, and radiological data of patients diagnosed with adrenal incidentaloma (AI) were evaluated.

A total of 66 patients with radiologically detected "adrenal adenoma," "adrenal lesion," or "adrenal mass" identified during imaging studies performed for any reason between December 2018 and February 2019 were included in the study. Patient data were retrospectively obtained from hospital automation records and patient files.

Demographic characteristics, presenting complaints, accompanying diseases, medications, smoking status, and alcohol consumption history of the patients were recorded. Clinical findings suggestive of pheochromocytoma, Cushing syndrome, hyperaldosteronism, and malignancy were also evaluated. Anthropometric measurements including height, body weight, and body mass index (BMI) were calculated. Systolic and diastolic blood pressure values were recorded. Physical examination findings including central obesity, moon face, dorsocervical fat accumulation, supraclavicular fullness, purple striae, and proximal muscle weakness were assessed.

The clinical indications leading to the diagnosis of adrenal incidentaloma and the imaging modalities used [ultrasonography (USG), computed tomography (CT), and/or magnetic resonance imaging (MRI)] were evaluated. Radiological assessment included the localization, size, density, and other imaging characteristics of the adrenal masses.

Hormonal evaluation included serum sodium and potassium levels, plasma aldosterone, plasma renin activity, basal ACTH, serum cortisol, dehydroepiandrosterone sulfate (DHEA-S), 24-hour urinary free cortisol levels, results of the 1-mg or 2-mg dexamethasone suppression tests, and 24-hour urinary catecholamine and metabolite levels. In addition, fasting blood glucose, HbA1c, liver and kidney function tests, and lipid parameters were recorded.

According to the hormonal evaluation results, patients were classified as having nonfunctioning adrenal adenoma, subclinical Cushing syndrome, Cushing syndrome, pheochromocytoma, or hyperaldosteronism. Patients with incomplete hormonal evaluations were assessed separately. Whether biopsy was performed, biopsy indications, and histopathological results were also recorded.

The relationship between adrenal adenoma and autoimmune thyroid disease was evaluated using thyroid ultrasonographic parenchymal heterogeneity and thyroid autoantibody levels.

Statistical analyses were performed using IBM SPSS Statistics for Windows Version 22.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean±standard deviation or median (minimum–maximum), while categorical variables were expressed as numbers and percentages. Pearson's chi-square test was used for comparisons of categorical variables. Fisher's exact test was used when the expected cell count was less than 5. A p-value of <0.05 was considered statistically significant.

Ethics committee approval for the study was obtained from the local ethics committee (Approval No: 2019/373, Date: 25.12.2019). The study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Results

A total of 66 patients diagnosed with adrenal incidentaloma were included in the study. The mean age of the patients was 56.2±7.9 years (range: 40–71 years). Of the patients, 51

(77.3%) were female and 15 (22.7%) were male. According to body mass index (BMI) classification, 11 patients (16.7%) had normal weight, 26 (39.4%) were overweight, and 29 (43.9%) were obese.

Regarding the localization of adrenal masses, 26 patients (39.4%) had right adrenal involvement, 30 patients (45.5%) had left adrenal involvement, and 10 patients (15.1%) had bilateral adrenal masses.

The relationship between BMI and sex was evaluated using Pearson's chi-square test, and BMI was found to be significantly higher in female patients compared to male patients (p=0.012) (Table 1).

Pearson's chi-square test, p=0.012

When accompanying comorbidities were evaluated, the most common conditions were hypertension (29.5%), diabetes mellitus (21.4%), hyperlipidemia (13.4%), and hypothyroidism (8.9%). A history of malignancy was present in 11 patients (Table 2).

Regarding thyroid diseases, 5 patients (7.6%) had a history of thyroidectomy, and 14 patients (21.2%) had a history of levothyroxine use. Thyroid nodules were detected in 41 patients (62.1%), while thyroid parenchymal heterogeneity was observed in 35 patients (53%). Thyroid autoantibody positivity was present in 10 patients (15.2%).

Table 1. Comparison of body mass index distribution between female and male patients

Body mass index	Female n (%)	Male n (%)	Total n (%)
Normal (18.5–24.9 kg/m^2)	5 (45.5)	6 (54.5)	11 (16.7)
Overweight (25–29.9 kg/m^2)	20 (76.9)	6 (23.1)	26 (39.4)
Obese (≥30 kg/m^2)	26 (89.7)	3 (10.3)	29 (43.9)

Table 2. Distribution of accompanying comorbid diseases in patients

Comorbid disease	n (%)	Comorbid disease	n (%)
Hypertension	33 (29.5)	Rheumatoid arthritis	2 (1.8)
Diabetes mellitus	24 (21.4)	Multinodular goiter	2 (1.8)
Hyperlipidemia	15 (13.4)	Renal cell carcinoma	2 (1.8)
Hypothyroidism	10 (8.9)	Hyperparathyroidism	1 (0.9)
Asthma	7 (6.3)	Atrial fibrillation	1 (0.9)
Chronic kidney disease	3 (2.7)	Colon cancer	1 (0.9)
Thyroid cancer	3 (2.7)	Cervical cancer	1 (0.9)
Breast cancer	3 (2.7)	Endometrial cancer	1 (0.9)
Coronary artery disease	2 (1.8)	Ankylosing spondylitis	1 (0.9)

Table 3. Comparison of thyroid nodule frequency between patients with functioning and nonfunctioning adrenal adenoma

Thyroid nodule	Nonfunctioning adenoma n (%)	Functioning adenoma n (%)	Total n (%)
Present	31 (56.4)	10 (90.9)	41 (62.1)
Absent	24 (43.6)	1 (9.1)	25 (37.9)
Total	55 (100.0)	11 (100.0)	66 (100.0)

Pearson's chi-square test, p=0.04

Among the study population, 55 patients were classified as having nonfunctioning adrenal adenoma, whereas 11 patients had functioning adrenal adenoma [subclinical Cushing syndrome, Cushing syndrome, primary hyperaldosteronism, and pheochromocytoma]. Thyroid nodules were detected in 31 patients (56.4%) with nonfunctioning adrenal adenoma and in 10 patients (90.9%) with functioning adrenal adenoma. The relationship between hormonal activity and the presence of thyroid nodules was found to be statistically significant according to Pearson's chi-square test (p=0.04) (Table 3).

The relationship between functioning and nonfunctioning adrenal adenomas and autoimmune thyroid disease was evaluated based on thyroid autoantibody positivity and thyroid parenchymal heterogeneity. No significant association was found between hormonal activity and thyroid autoantibody positivity (p=0.66) (Table 4). Similarly, no statistically significant relationship was observed between hormonal activity and thyroid parenchymal heterogeneity (p=0.46) (Table 5).

Discussion

The frequency of adrenal incidentalomas has increased significantly in recent years, mainly due to the widespread use of imaging modalities.[1] In the present study, the mean age of patients diagnosed with adrenal incidentaloma was 56.2±7.9 years, which is consistent with previously reported data in the literatüre.[3] The higher prevalence of adrenal incidentalomas in elderly populations may be explained by the increased use of imaging techniques with advancing age and the age-related increase in nodular changes within the adrenal cortex.[8]

Previous studies have reported that adrenal incidentalomas are more common in women. In a large series conducted by the Italian Association of Clinical Endocrinologists Study Group on Adrenal Tumors, the female-to-male ratio was reported as 1.39.[9] Similarly, the majority of patients in our study were female (77.3%). This finding may be associated

Table 4. Distribution of thyroid autoantibody positivity among adrenal adenoma subgroups

Hormonal activity	Thyroid Autoantibody positive n (%)	Thyroid Autoantibody negative n (%)	Total n (%)
Nonfunctioning adenoma	8 (14.5)	47 (85.5)	55 (83.3)
Subclinical cushing syndrome	0 (0.0)	3 (100.0)	3 (4.5)
Cushing syndrome	0 (0.0)	2 (100.0)	2 (3.0)
Primary hyperaldosteronism	1 (25.0)	3 (75.0)	4 (6.1)
Pheochromocytoma	1 (50.0)	1 (50.0)	2 (3.0)
Total	10 (15.2)	56 (84.8)	66 (100.0)

Pearson's chi-square test, p=0.66.

Table 5. Distribution of thyroid parenchymal characteristics among adrenal adenoma subgroups

Hormonal activity	Homogeneous parenchyma n (%)	Heterogeneous parenchyma n (%)	Total n (%)
Nonfunctioning adenoma	25 (45.5)	30 (54.5)	55 (83.3)
Subclinical Cushing syndrome	1 (33.3)	2 (66.7)	3 (4.5)
Cushing syndrome	1 (50.0)	1 (50.0)	2 (3.0)
Primary hyperaldosteronism	3 (75.0)	1 (25.0)	4 (6.1)
Pheochromocytoma	1 (50.0)	1 (50.0)	2 (3.0)
Total	31 (47.0)	35 (53.0)	66 (100.0)

Pearson's chi-square test, p=0.46.

with the more frequent use of imaging studies in female patients.

Metabolic disorders are known to occur more frequently in patients with adrenal incidentaloma.[10] Previous studies have demonstrated associations between adrenal incidentaloma and obesity, hypertension, diabetes mellitus, and hyperlipidemia.[11] In our study, 83.3% of the patients were overweight or obese. In addition, hypertension, diabetes mellitus, and hyperlipidemia were the most common accompanying comorbidities. These findings support the association between adrenal incidentaloma and metabolic disorders.

In the present study, adrenal masses were most frequently localized in the left adrenal gland (45.5%). In contrast, previous reports have indicated that adrenal masses are more commonly detected in the right adrenal gland, which has been attributed to the limited ability of ultrasonography (USG) to visualize the left adrenal gland.[12] The difference observed in our study may be related to the predominant use of CT and MRI as imaging modalities.

Functional evaluation demonstrated that the majority of patients had nonfunctioning adrenal adenoma (83.3%), which is consistent with the rates reported in the literature.[13,14] Among the functional lesions, primary hyperaldosteronism was the most common (6.1%), whereas the frequency of pheochromocytoma was 3%.

One of the most remarkable findings of our study was the significantly higher frequency of thyroid nodules in patients with functioning adrenal adenoma. Thyroid nodules were detected in 90.9% of patients with functioning adrenal adenoma compared to 56.4% of patients with nonfunctioning adrenal adenoma (p=0.04). Similarly, a previous study conducted in Türkiye reported a higher prevalence of thyroid nodules in patients with adrenal incidentaloma compared to the control group.[15] This finding suggests that cortisol and other adrenal hormones may contribute to thyroid proliferation through growth factors and insulin resistance mechanisms.

However, no significant association was found between hormonal activity and autoimmune thyroid disease in our study. Evaluations based on thyroid autoantibody positivity and thyroid parenchymal heterogeneity demonstrated no significant differences between functioning and nonfunctioning adrenal adenoma groups. These findings suggest that adrenal hormonal activity may be associated with thyroid nodule formation but may not be directly related to autoimmune mechanisms.

Limitations of the Study

The retrospective and single-center design of the study, as well as the limited sample size, are important limitations. In addition, the absence of a control group prevented a

clear evaluation of whether the frequency of thyroid nodules was increased compared to the general population. In addition, the retrospective design may have introduced selection bias.

Conclusion

Adrenal incidentaloma has become an increasingly common condition in clinical practice due to the widespread use of imaging modalities. Comprehensive clinical, laboratory, and radiological evaluation of these patients is essential for the assessment of hormonal activity and malignancy risk.

In our study, obesity, hypertension, diabetes mellitus, and hyperlipidemia were frequently observed in patients with adrenal incidentaloma. In addition, the frequency of thyroid nodules was found to be significantly higher in patients with functioning adrenal adenoma. However, no significant association was demonstrated between autoimmune thyroid disease and adrenal hormonal activity.

These findings suggest that adrenal hormonal activity may be associated with the development of thyroid nodules. Therefore, more careful evaluation for the presence of thyroid nodules may be beneficial in patients with adrenal incidentaloma. Nevertheless, further prospective studies with larger patient populations are needed to better clarify the relationship between adrenal incidentaloma and autoimmune thyroid diseases.

Disclosures

Ethics Committee Approval: The study was conducted in accordance with the principles of the Declaration of Helsinki. Ethics committee approval was obtained from the Local Ethics Committee of Selcuk University Faculty of Medicine before the study (Approval No: 2019/373, Date: 25.12.2019).

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References

1. Sippel RS, Chen H. Subclinical Cushing's syndrome in adrenal incidentalomas. Surg Clin North Am 2004;84(3):875–85. [CrossRef]
2. Abecassis M, McLoughlin MJ, Langer B, Kudlow JE. Serendipitous adrenal masses: prevalence, significance, and management. Am J Surg 1985;149(6):783–8. [CrossRef]
3. Mansmann G, Lau J, Balk E, Rothberg M, Miyachi Y, Bornstein SR. The clinically inapparent adrenal mass: update in diagnosis and management. Endocr Rev 2004;25(2):309–40. [CrossRef]
4. Jain SM. Adrenal incidentaloma: a puzzle for clinician. Indian J Endocrinol Metab 2013;17(Suppl 1):S59–63. [CrossRef]
5. Aron D, Terzolo M, Cawood TJ. Adrenal incidentalomas. Best Pract Res Clin Endocrinol Metab 2012;26(1):69–82. [CrossRef]
6. Young WF Jr. The incidentally discovered adrenal mass. N Engl J Med 2007;356(6):601–10. [CrossRef]
7. Antonelli A, Ferrari SM, Corrado A, Di Domenicantonio A, Fallahi P. Autoimmune thyroid disorders. Autoimmun Rev 2015;14(2):174–80. [CrossRef]
8. Dobbie JW. Adrenocortical nodular hyperplasia: the ageing adrenal. J Pathol 1969;99(1):1–18. [CrossRef]
9. Mantero F, Terzolo M, Arnaldi G, Osella G, Masini AM, Alì A, et al. A survey on adrenal incidentaloma in Italy. study group on adrenal tumors of the ıtalian society of endocrinology. J Clin Endocrinol Metab 2000;85(2):637–44. [CrossRef]
10. Terzolo M, Pia A, Alì A, Osella G, Reimondo G, Bovio S, et al. Adrenal incidentaloma: a new cause of the metabolic syndrome? J Clin Endocrinol Metab 2002;87(3):998–1003. [CrossRef]
11. Terzolo M, Stigliano A, Chiodini I, Loli P, Furlani L, Arnaldi G, et al; Italian Association of Clinical Endocrinologists. AME position statement on adrenal incidentaloma. Eur J Endocrinol 2011;164(6):851–70. [CrossRef]
12. Kloos RT, Gross MD, Francis IR, Korobkin M, Shapiro B. Incidentally discovered adrenal masses. Endocr Rev 1995;16(4):460–84. [CrossRef]
13. Androulakis II, Kaltsas G, Piaditis G, Grossman Advanced. The clinical significance of adrenal incidentalomas. Eur J Clin Invest 2011;41(5):552–60. [CrossRef]
14. Comlekci A, Yener S, Ertilav S, Secil M, Akinci B, Demir T, et al. Adrenal incidentaloma: clinical, metabolic, and follow-up aspects. Endocrine 2010;37(1):40–6. [CrossRef]
15. Arduc A, Isik S, Ozuguz U, Tutuncu YA, Kucukler FK, Ozcan HN, Berker D, Guler S. Relationship between thyroid nodules and non-functioning adrenal incidentalomas and their association with insulin resistance. Endocr Res 2014;39(3):99–104. [CrossRef]