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ORIGINAL ARTICLE

Lumbar level distribution of acute abdomen with no history of abdominal surgery or trauma: Is there an aggregation?*

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ABSTRACT

Objective: In the majority of the population, the anatomic location of intra-abdominal structures varies slightly but is at certain vertebral levels, excluding postoperative and traumatic positional changes. Our aim was to investigate the distribution of pathologic findings at each lumbar level in acute abdomen patients.

Materials and Methods: This retrospective study included patients admitted to the emergency department between May 2017 and 2019 without abdominal trauma or surgery. CT images by vertebral length were assessed by two radiologists. Primary and secondary findings for each condition were examined for each lumbar level.

Results: In 553/1008 patients (54.8%), CT had findings explaining the cause of pain. However, in 48/553 (8.67%), no primary or secondary findings were found in any lumbar level, and most (n=42) were gynecologic, while three had appendicitis and three had sigmoid diverticulitis. The distribution of primary and secondary findings is as follows: 19.16% (n=106) and 19.34% (n=107) for L1, 28.57% (n=158) and 21.33% (n=118) for L2, 16.09% (n=89) and 27.84% (n=154) for L3, 22.78% (n=126) and 27.48% (n=152) for L4, and 31.64% (n=175) and 18.26% (n=101) for L5, respectively. There were no patients with primary or secondary findings at any lumbar level, who did not also have findings at L1, L2, and L5. The CT assessment of L1, L2, and L5 yielded 91.32% (88.66-93.53%CI) sensitivity, 90.46% NPV (87.85-92.55%CI), and 95.24% (93.74-96.47%CI) accuracy.

Conclusion: Due to the specific anatomic location of the organs, acute abdomen findings in patients without trauma or surgical changes tend to cluster at certain lumbar levels.

Keywords: Acute Abdomen (D000006), Emergency Medicine (D004635), Lumbar Vertebrae (D008159), Tomography (D014057).

INTRODUCTION

Computed tomography (CT) has been widely accepted and indispensable for patients presenting with acute abdominal pain, supplanting traditional physical exams and history in many instances [1, 2]. The increased demand for CT assessment by emergency physicians seems understandable if one disregards the increase in both radiation exposure and workload in emergency radiology (ER) units [3-5].

In the majority of the population, the anatomical positions of the intra-abdominal structures vary slightly but are located at certain vertebral levels [6]. However, this verdict may not be appropriate for both posttraumatic and postoperative patients, as the distortion of the normal anatomic configuration may occur [7]. It should also be noted that certain pathological conditions (such as acute appendicitis, acute cholecystitis, urinary stones, diverticulitis) account for a large proportion of acute abdominal pain [8].

The axial CT findings of a particular population with acute abdomen who are admitted to the emergency department may accumulate in certain lumbar levels, over a wide period of a year.

With this study, we aimed to obtain two important results. First, to investigate the detection rate of major and minor findings at each lumbar vertebral level in a selected group of patients with acute abdominal pain. Second, to determine if there is a particular combination of spinal levels with optimal outcomes and the highest negative predictive value.

MATERIALS AND METHODS

Approval for this study was granted by the Institutional Ethics Board (GO 19/800). Informed consent was not obtained due to the retrospective nature of the study.

Data Collection

Between January 2017 and May 2019, patients who presented to the emergency department (ED) and underwent a whole abdominal CT (n: 3352) were documented. Subsequently, patients with posttraumatic injury (n: 1583) and a history of any abdominal surgery (n: 761) were excluded (Fig.1). A total of 1008 patients, as the final sample size, were enrolled in this study.

All documents (patient history, US findings, and other follow-up information besides final clinical admissions) of each patient including reports from CT were evaluated. Also, no follow-up patients with acute abdominal pain were included in the study to prevent sampling repetition.

CT Technique

All CT examinations included in the study were contrast-enhanced (iodinated water-soluble intravenous contrast medium -Iohexol 5mg/kg- was administered at a rate of 4ml/sn) and were performed in the emergency radiology station, via Somatom Perspective 64-slice Siemens® CT (Erlangen/Germany) device. Oral contrast agents were administered in 75.9% (n=766) of patients. The CT acquisition parameters were as follows: Tube voltage: 120 kV, tube current determined with optimized automatic exposure control (the ref mAs value of CT was 140 mAs), collimation thickness: 0.6-2 mm, tube rotation time: 0.6-1 seconds and collimated section thickness: 2-5 mm. Iterative reconstruction (ADMIRE, strength 2) with a 30s soft-tissue kernel was used as a reconstruction algorithm to reduce radiation dose.

CT Image Analysis

CT images limited to specific lumbar levels were assessed by two reviewers (E.A. and İ.İ.) prior to evaluation of the entire CT examination to avoid both bias and false positivity in the analysis of the images. İ.İ. and E.A. created the sample with the final diagnosis, and all patients in this sample were selected by consensus of them. The determination of the main pathological condition found in the patients was made by examining the entire clinical course and consultations of the patients. In addition, surgical reports, interventions and pathology reports, if any, were also reviewed.

All CT images were reevaluated by consensus of two radiologists with 5 years (A.G.E.) and 18 years (M.R.O.) of experience in abdominal radiology to define the lumbar level(s) in which primary and/or secondary findings were observed. The longitudinal

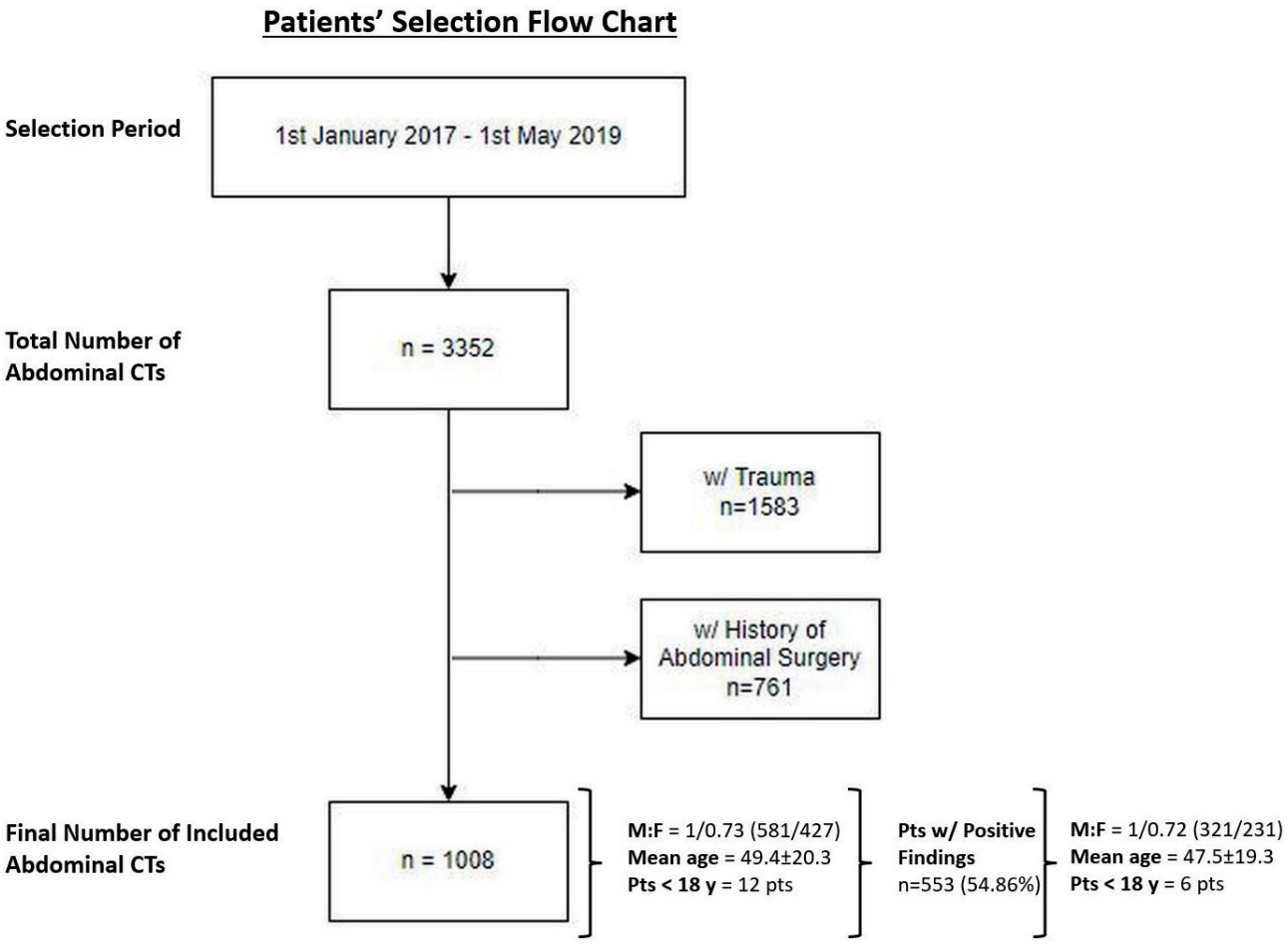

Figure 1. Flow Chart of Patients' Selection.

axis height of the assessment for each lumbar level was designated as shown in Fig. 2.

Fat stranding, pneumoperitoneum, fluid collection, urinary dilatation, biliary dilatation, and dilated bowel were considered as secondary findings in acute abdomen. Henceforth, a dilated appendix, a hydropic gallbladder with thickened wall, obstructive urinary stones, a perforation focus of the intestine, a tumor causing intestinal obstruction, etc., were considered as primary findings. The primary and secondary findings are listed in Table 1. In cases where the primary finding was accompanied by a secondary finding at the same vertebral level, the secondary finding was ignored and only the primary finding was recorded.

Considering the frequency of occurrence in the ED, patients who had findings explaining acute abdomen were divided into 10 groups and analyzed separately with respect to the distribution of primary and secondary findings for each lumbar level. These 10 groups consisted of appendicitis, hepatobiliary-pancreatic, inflammatory-infectious gastrointestinal (GI) diseases, diverticulitis - epiploic appendagitis, intestinal obstruction, gynecological, peritoneal - retroperitoneal and mesenteric diseases, urinary tract diseases, incidental malignancies, and miscellaneous causes.

Radiaiton Dose Calculation

Radiation exposure dose values for the CT examinations were recorded as CT Dose Index Volume (CTDIvol) and Dose Length Product (DLP) values within the PACS software (Syngo.Via, Siemens®). DLP values for each lumbar vertebra were calculated by determining the length of the vertebra in the z-axis direction using the topogram image (Fig.2). The DLP values of the segments with minor and major findings were summed and related to the total DLP values to determine the ratio of the actual radiation dose required and to decide which segments to scan to complete the examination.

Statistical Analysis

Data were summarized as "mean ± SD" or "median (with minimum-maximum)" for continuous variables, depending on the distributional

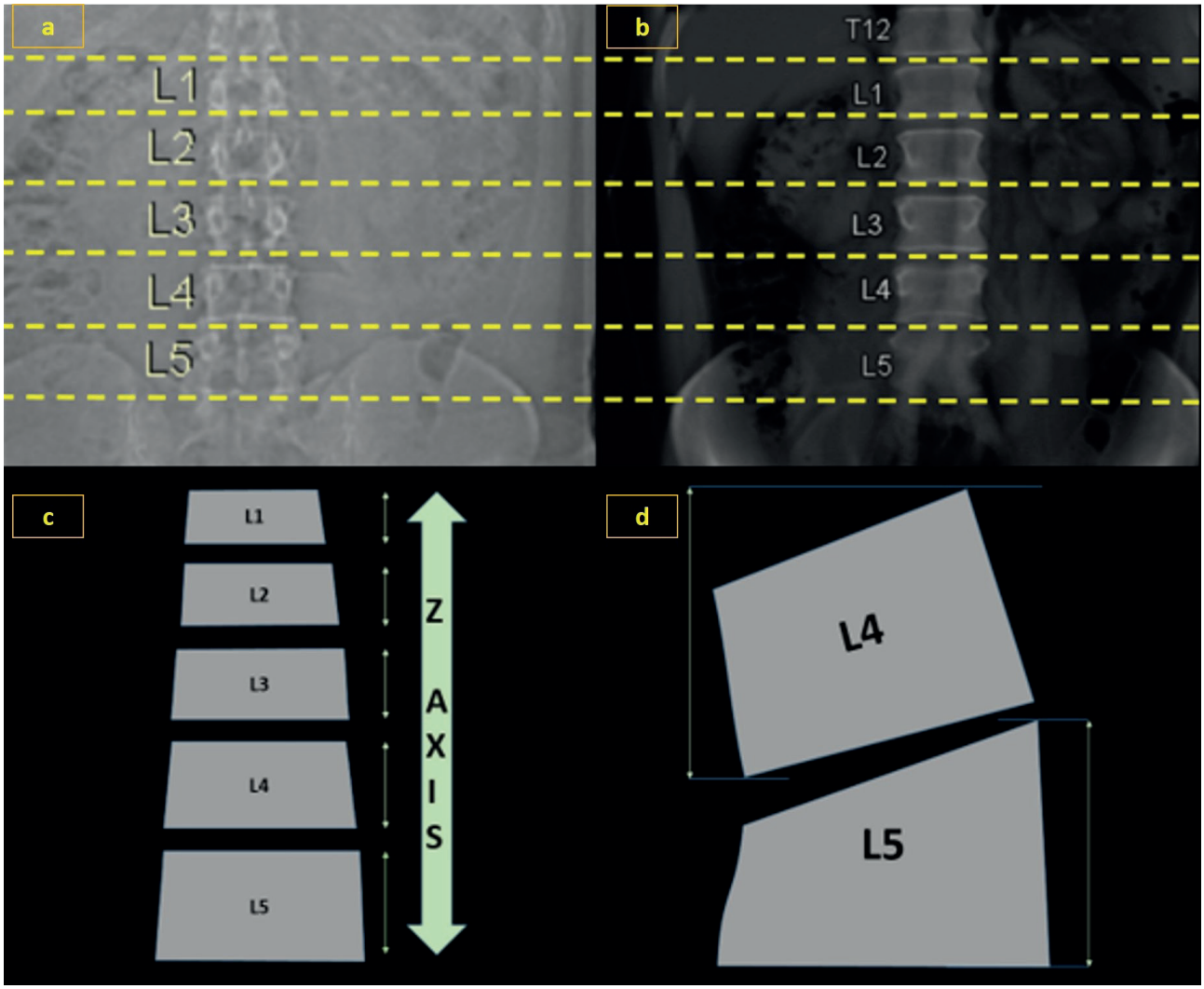

Figure 2. Determining the limits of scanning levels.

Topogram (a) and Maximal Intensity Projection (b) images alongside with simple illustrations used to determine the length of z-axis (c,d). Length for vertebrae were determined with topogram guidance (a), excluding intervertebral spaces (c). Though, in patients with abnormal vertebral order, the longer lateral margin of vertebral body were considered as the length of z axis, whether it overlaps which each other or not (d).

Table 1. The list of primary and secondary findings

Primary	Secondary
• Dilated appendix • Hydropic gall-bladder with thickened wall • Obstructive biliary or urinary stone • Biliary dilatation with thickened wall and fluid level • Pancreatic thickening with fat stranding, fluid collection or unenhancing area • Intestinal obstruction transition site • Perforation focus • Thickened intestinal wall • Inflammed diverticule or epiploic appendix • Ruptured or hemorrhagic cyst • Torsion site • Lymphadenopathy or tumor • Abscess or hematoma • Thrombosed vessel, dissection or ruptured aneurysm	• Mesenteric, omental, pelvic, retroperitoneal or perirenal fat stranding • Fluid collection • Peritoneal thickening[§] • Pneumoperitoneum • Intestinal dilatatiom • Urinary dilatation • Biliary dilatation • Ill-defined visceral enhancement

[§] Secondary peritonitis is considered as the secondary finding of the primary etiologies.

properties of the data. Normality of variables was tested using the Kolmogorov-Smirnov. Percentiles were given for categorical data. Kruskal-Wallis analysis was performed for comparison of DLP at different lumbar levels. For all tests, a two-tailed p-value of less than 0.05 was considered statistically significant.

RESULTS

Out of 1008 patients, the male/female ratio was 1/0.73 (581/427), while the mean age was 49.4 ± 20.3 (varied between 10-96), with 12 pediatric (<18) patients. Out of 1008 patients, CT assessments revealed normal findings in 455 patients (45.13%). In the remaining 553 patients (54.86%), CT assessments have positive findings for acute abdomen. Out of 553 patients, the male/female ratio was 1/0.72 (321/232), while the mean age was 47.5 ± 19.3 (varied between 14-95), with 6 pediatric (<18) patients.

The distribution of primary and secondary findings at each lumbar level, related to 10 different groups, is detailed in Table 2. In other respects, the distribution of sensitivity, negative predictive value (NPV), and accuracy for all combinations of multiple lumbar vertebrae are detailed in Table 3.

When the lumbar vertebrae alone were evaluated, most findings were noted at the L4 level (n:278). However, when all vertebral combinations were evaluated, the combinations containing L1, L2, and L5 together (n:505) were found to have the most findings among all combinations. See Table 4 for the distribution of findings detected for each pathologic condition at these 3 levels.

In 48 of 553 patients, no primary or secondary findings were noted in any of the lumbar levels. Most of them (n:42) were gynecological, while only 6 had appendicitis (n:3) and sigmoid diverticulitis (n:3). Because there were no false positives, positive predictive value (PPV) and specificity for all levels were estimated as 100%. The assessment of CT images at L1, L2, and L5 levels in 1008 patients yielded 91.32% (88.66-93.53% CI) sensitivity, 90.46% NPV (87.85-92.55% CI), and 95.24% (93.74-96.47% CI) accuracy in detecting the cause of acute abdominal pain.

Appendicitis, the most common cause of acute abdominal pain, was found in 95 patients (17.17%), while perforation was present in 13 patients. All patients with perforated appendicitis were also confirmed surgically. In three patients whose appendix was not perforated, no primary or secondary finding in any lumbar level was found as a reason for the pelvic localization.

Hepatobiliary and pancreatic causes of acute abdominal pain presented with imaging findings mostly at upper lumbar levels.

All patients with inflammatory or infectious GI tract conditions presented with primary or secondary findings at L1, L2, and L5 levels.

Of the 22 diverticulitis, 12 were sigmoid diverticulitis and 3 of them showed no primary or secondary findings at any lumbar level. Imaging findings in patients with intestinal obstruction were heterogeneously distributed among lumbar levels, however, %100 of patients in this group had primary or secondary findings at the L1, L2, and L5 levels.

Gynecologic emergencies were the group most commonly recognized at no lumbar level, with 42 (66.6%) of 63 patients. Of these 42 patients, most (n:39, 92.85%) had only secondary findings, while the remaining (n:3, 7.15%) had primary findings in the lumbar levels.

Condensed abdominal CT showed one of the highest performances in peritoneal-retroperitoneal, mesenteric, urinary conditions of non-traumatic abdominal emergencies as well as incidental malignancies and miscellaneous conditions presenting with acute abdominal pain since %100 of these patients in these groups presented with imaging findings at L1, L2 and L5 levels (Table 2 and 4).

The distribution of detected pathological conditions on CT and the number of primary and secondary findings on each vertebral level with the average lengths of these levels together with the DLP values are shown in Table 5.

The median DLP value obtained by scanning the entire abdomen was 404.17 mGy-cm, whereas the median DLP value obtained by scanning 5 lumbar levels was 102.81 mGy-cm. The median DLP value of L1,2,5 levels was found to be 60.68 mGy-cm, which is a significant dose reduction compared to scanning the whole abdomen ($p<0.001$).

Table 2. Distribution of primary and secondary findings

Pathological Conditions		L1	L2	L3	L4	L5
Appendicitis (n: 95)	Primary †	0 (0%)	1 (1.05%)	1 (1.05%)	22 (23.15%)	81 (85.26%)
	Secondary ‡	4 (4.21%)	4 (4.21%)	12 (12.63%)	30 (31.57%)	11 (11.57%)
Hepatobiliary and Pancreatic (n: 72)	Primary	37 (51.38%)	51 (70.83%)	7 (9.72%)	0 (0%)	0 (0%)
• Acute Cholecystitis (n: 42) • Acute Pancreatitis (n: 14) • Choledocholithiasis (n: 10) • Cholangitis (n: 6)	Secondary	25 (34.72%)	16 (22.22%)	24 (33.33%)	4 (5.55%)	1 (1.38%)
Inflammatory/Infectious GI Tract Conditions (n: 104)	Primary	9 (8.65%)	19 (18.26%)	23 (22.11%)	40 (38.46%)	15 (14.42%)
	Secondary	9 (8.65%)	14 (13.46%)	16 (15.38%)	28 (26.92%)	7 (6.73%)
• Ileitis (n: 54) • Non-spesific Colitis (n: 24) • Jejunitis (n: 10) • Duodenitis (n: 6) • Enterocolitis (n: 4) • Pseudomembranous Enterocolitis (n: 3) • Typhilitis (n: 3)						
Diverticulitis and Epiploic Appendagitis (n: 28)	Primary	0 (0%)	3 (10.71%)	0 (0%)	4 (14.28%)	15 (53.57%)
	Secondary	3 (10.71%)	1 (3.57%)	4 (14.28%)	5 (17.85%)	7 (25.00%)
• Diverticulitis (n: 22) • Epiploic Appendagitis (n: 6)						
Intestinal Obstruction (n: 61)	Primary	5 (8,19%)	11 (18.03%)	9 (14.75%)	25 (40.98%)	26 (42.62%)
• Ileal Obstruction (n: 27) • Jejunal Obstruction (n: 22) • Colonic (n: 14)	Secondary	31 (50.81%)	45 (73.77%)	48 (78.68%)	28 (45.90%)	25 (40.98%)
Gynecologic (n: 63)	Primary	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (4.76%)
• Pelvic Inflammatory Disease (n: 33) • Adnexal Cyst Rupture (n: 26) • Adnexal Torsion (n: 3) • Pelvic Neoplastic Tumor (n: 1)	Secondary	0 (0%)	3 (4.76%)	4 (6.34%)	9 (14.28%)	18 (28.57%)
Peritoneal/Retroperitoneal, Mesenteric (n:39)**	Primary	20 (51.28%)	29 (74.35%)	23 (58.97%)	24 (61.53%)	21 (53.84%)
	Secondary	8 (20.51%)	4 (10.25%)	9 (23.07%)	7 (17.94%)	7 (17.94%)
• Peritoneal Carcinomatosis (n: 12) • Sclerosing mesenteritis (n: 9) • Lymphoproliferative Disease (n: 7) • Mesenteric Lymphadenitis (n: 6) • Omental Infarct (n: 4) • Primary Peritonitis (n: 1)						
Urinary Conditions (n: 37)	Primary	10 (27.02%)	20 (54.05%)	8 (21.62%)	3 (8.10%)	4 (10.81%)
• Urolithiasis (n: 19) • Pyelonephritis/Pyelitis (n: 16) • Bladder Cancer (n: 1) • Acute Tubulary Necrosis (n: 1)	Secondary	14 (37.83%)	14 (37.83%)	22 (59.45%)	15 (40.54%)	12 (32.43%)
Incidental Malignancies (n: 21)	Primary	13 (61.90%)	14 (66.66%)	10 (47.61%)	2 (9.52%)	2 (9.52%)
• Liver Metastasis (n: 8) • Colon Cancer (n: 7) • Pancreatic Cancer (n: 4) • Gastric Cancer (n: 2)	Secondary	4 (19.04%)	3 (14.28%)	4 (19.04%)	8 (38.09%)	5 (23.80%)
Miscellaneous (n: 33)	Primary	12 (36.36%)	10 (30.30%)	8 (24.24%)	6 (18.18%)	8 (24.24%)
• Vascular (n: 17) *** • Mesenteric Abscesses (n: 7) • Duodenal Perforation (n: 4) • Intramural Hematoma (n: 3) • Rectus Muscle Hematoma (n: 2)	Secondary	9 (27.27%)	14 (42.42%)	11 (33.33%)	18 (54.54%)	8 (24.24%)
General Distribution	Primary	106 (19.16%)	158 (28.57%)	89 (16.09%)	126 (22.78%)	175 (31.64%)
	Secondary	107 (19.34%)	118 (21.38%)	154 (27.84%)	152 (27.48%)	101 (18.26%)

† The number of the detection of primary findings for each vertebral level

‡The number of the detection of secondary findings for each vertebral level

* Indicates the percentage and number of patients with any primary and/or secondary findings at these levels among patients.

**Secondary peritonitis is considered as the secondary finding of the primary etiologies.

*** 5 were portal venous thrombosis, 4 were superior mesenteric arterial thrombosis, 3 were superior mesenteric venous thrombosis, 3 were non-occlusive mesenteric vascular occlusion, one of them was the rupture of abdominal aorta aneursym and one was the aortic dissection extending infrarenal level.

Table 3. Distribution of sensitivity, negative predictivity and accuracy for all combinations of multiple lumbar vertebrae

	Detected†	Missed‡	Sensitivity*	Negative Predictive Value*	Accuracy*
L1 – L2	n: 278	n: 275	50.27% (46.02 – 54.52%)	62.33% (60.34 – 64.28%)	72.72% (69.86 – 64.28%)
L1 – L3	n: 302	n: 251	54.61% (50.36 – 58.82%)	64.45% (62.33 – 66.51%)	75.10% (72.31 – 77.74%)
L1 – L4	n: 391	n: 162	70.71% (66.72 – 74.47%)	73.74% (71.61 – 76.17%)	83.93% (81.51 – 86.14%)
L1 – L5	n: 474	n: 79	85.71% (82.52 – 88.52%)	85.21% (82.44 – 87.60%)	92.16% (90.33 – 93.75%)
L2 – L3	n: 310	n: 243	56.06% (51.81 – 60.24%)	65.19% (63.02 – 67.29%)	75.89% (73.13 – 78.50%)
L2 – L4	n: 406	n: 147	73.42% (69.53 – 77.06%)	75.58% (72.94 – 78.05%)	85.42% (83.09 – 87.54%)
L2 – L5	n: 498	n: 55	90.05% (87.25 – 92.42%)	89.22% (86.55 – 91.40%)	94.54% (92.96 – 95.86%)
L3 – L4	n: 340	n: 213	61.48% (57.28 – 65.56%)	68.11% (65.78 – 70.36%)	78.87% (76.22 – 81.35%)
L3 – L5	n: 432	n: 121	78.12% (74.44 – 81.50%)	78.99% (76.26 – 81.49%)	88.00% (85.83 – 89.94%)
L4 – L5	n: 372	n: 181	67.27% (63.18 – 71.17%)	71.54% (69.05 – 73.91%)	82.04% (79.53 – 84.37%)
L1 – L2 – L3	n: 311	n: 242	56.24% (51.99 – 60.42%)	65.28% (63.11 – 67.39%)	75.99% (73.23 – 78.60%)
L1 – L2 – L4	n: 407	n: 146	73.60% (69.71 – 77.23%)	75.71% (73.06 – 78.17%)	85.52% (83.19 – 87.63%)
L1 – L2 – L5	**n: 505**	**n: 48**	**91.32% (88.66 – 93.53%)**	**90.46% (87.85 – 92.55%)**	**95.24% (93.74 – 96.47%)**
L1 – L3 – L4	n: 404	n: 149	73.06% (69.15 – 76.71%)	75.33% (72.69 – 77.79%)	85.22% (82.88 – 87.35%)
L1 – L3 – L5	n: 490	n: 63	88.61% (85.66 – 91.33%)	87.84% (85.13 – 90.11%)	93.75% (92.07 – 95.16%)
L1 – L4 – L5	n: 485	n: 68	87.70% (84.67 – 90.32%)	87.00% (84.27 – 89.32%)	93.25% (91.53 – 94.72%)
L2 – L3 – L4	n: 406	n: 147	73.42% (69.53 – 77.06%)	75.58% (72.94 – 78.05%)	85.42% (83.09 – 87.54%)
L2 – L3 – L5	n: 501	n: 52	90.60% (87.85 – 92.90%)	89.74% (87.11 – 91.89%)	94.84% (93.29 – 96.12%)
L2 – L4 – L5	n: 498	n: 55	90.05% (87.25 – 92.42%)	89.22% (86.55 – 91.40%)	94.54% (92.96 – 95.86%)
L3 – L4 – L5	n: 432	n: 121	78.12% (74.44 – 84.50%)	78.99% (76.26 – 81.49%)	88.00% (85.83 – 89.94%)
L1 – L2 – L3 – L4	n: 407	n: 146	73.60% (69.71 – 77.23%)	75.71% (73.06 – 78.17%)	85.52% (83.19 – 87.63%)
L1 – L2 – L3 – L5	**n: 505**	**n: 48**	**91.32% (88.66 – 93.53%)**	**90.46% (87.85 – 92.55%)**	**95.24% (93.74 – 96.47%)**
L1 – L2 – L4 – L5	**n: 505**	**n: 48**	**91.32% (88.66 – 93.53%)**	**90.46% (87.85 – 92.55%)**	**95.24% (93.74 – 96.47%)**
L1 – L3 – L4 – L5	n: 497	n: 56	89.87% (87.05 – 92.26%)	89.04% (86.37 – 91.24%)	94.44% (92.85 – 95.78%)
L2 – L3 – L4 – L5	n: 504	n: 49	91.14% (88.45 – 93.37%)	90.28% (87.67 – 92.38%)	95.14% (93.62 – 96.38%)
L1 – L2 – L3 – L4 – L5	**n: 505**	**n: 48**	**91.32% (88.66 – 93.53%)**	**90.46% (87.85 – 92.55%)**	**95.24% (93.74 – 96.47%)**

† Patients detected with primary or secondary findings
‡ Patients missed with any primary or secondary findings
* Values wthin parenthesis are 95% confidence interval.
Abbreviation: L1: 1st Lumbar vertebrae, L2: 2nd Lumbar vertebrae, L3: 3rd Lumbar vertebrae, L4: 4th Lumbar vertebrae, L5: 5th Lumbar vertebrae.

Table 4. Distribution of detection rate of pathological conditions on L1, L2 and L5

Pathological Conditions	Primary or Secondary Findings Detected†
Appendicitis (n: 95)	92 (96.84%)
Hepatobiliary and Pancreatic (n: 72) • Acute Cholecystitis (n: 42) • Acute Pancreatitis (n: 14) • Choledocholithiasis (n: 10) • Cholangitis (n: 6)	72 (100%)
Inflammatory/Infectious GI Tract Conditions (n: 104) • Ileitis (n: 54) • Non-spesific Colitis (n: 24) • Jejunitis (n: 10) • Duodenitis (n: 6) • Enterocolitis (n: 4) • Pseudomembranous Enterocolitis (n: 3) • Typhilitis (n: 3)	104 (100%)
Diverticulitis and Epiploic Appendagitis (n: 28) • Diverticulitis (n: 22) • Epiploic Appendagitis (n: 6)	25 (89.28%)
Intestinal Obstruction (n: 61) • Ileal Obstruction (n: 27) • Jejunal Obstruction (n: 22) • Colonic (n: 14)	61 (100%)
Gynecologic (n: 63) • Pelvic Inflammatory Disease (n: 33) • Adnexal Cyst Rupture (n: 26) • Adnexal Torsion (n: 3) • Pelvic Neoplastic Tumor (n: 1)	21 (33.33%)
Peritoneal/Retroperitoneal and Mesenteric (n: 39)** • Peritoneal Carcinomatosis (n: 12) • Sclerosing mesenteritis (n: 9) • Lymphoproliferative Disease (n: 7) • Mesenteric Lymphadenitis (n: 6) • Omental Infarct (n: 4) • Primary Peritonitis (n: 1)	39 (100%)
Urinary Conditions (n: 37) • Urolithiasis (n: 19) • Pyelonephritis/Pyelitis (n: 16) • Bladder Cancer (n: 1) • Acute Tubulary Necrosis (n: 1)	37 (100%)
Incidental Malignancies (n: 21) • Liver Metastasis (n: 8) • Colon Cancer (n: 7) • Pancreatic Cancer (n: 4) • Gastric Cancer (n: 2)	21 (100%)
Miscellaneous (n: 33) • Vascular (n: 17) *** • Mesenteric Abscesses (n: 7) • Duodenal Perforation (n: 4) • Intramural Hematoma (n: 3) • Rectus Muscle Hematoma (n: 2)	33 (100%)

† The number of the detection of primary or secondary findings for each vertebral level

**Secondary peritonitis is considered as the secondary finding of the primary etiologies.

*** 5 were portal venous thrombosis, 4 were superior mesenteric arterial thrombosis, 3 were superior mesenteric venous thrombosis, 3 were non-occlusive mesenteric vascular occlusion, one of them was the rupture of abdominal aorta aneursym and one was the aortic dissection extending infrarenal level.

Table 5. The number of primary and secondary findings at each vertebral level with the average lengths of these levels and DLP values

	L1 (2.27±0.25 cm)	L2 (2.41±0.26 cm)	L3 (2.5±0.25 cm)	L4 (2.64±0.27 cm)	L5 (2.73±0.32 cm)	L1,2,5 (7.41±0.71 cm)
Primary Finding	n = 106	n = 148	n = 89	n = 126	n = 243	n = 391
Secondary Finding	n = 107	n = 107	n = 154	n = 155	n = 103	n = 317
Primary and Secondary Finding	n = 213	n = 255	n = 243	n = 281	n = 346	n = 708
Median DLP Value† (mGy-cm)	18.67 (4.46-69.37)	19.60 (5.37-72.15)	20.33 (5.40-77.69)	21.44 (5.81-83.25)	22.45 (5.50-88.80)	60.68 (15.51-230.32)
Median CTDIvol: 8.17 mGy (2.35 – 27.75)						
Median DLP of Total Lumbar: 102.81 mGy-cm (26.79 – 391.27)						
Median DLP of Whole Abdomen: 404.17 mGy-cm (115.10 – 1490.81)						

† Values wthin parenthesis are minimum and maximum values, respectively.
Abbreviation: DLP: Dose-Length Product, CTDIvol: Computed Tomography Dose Index Volume, L1: 1st Lumbar vertebrae, L2: 2nd Lumbar vertebrae, L3: 3rd Lumbar vertebrae, L4: 4th Lumbar vertebrae, L5: 5th Lumbar vertebrae.

DISCUSSION

In this study, we found that the vast majority of primary, secondary, and total CT findings of common causes of acute abdominal pain were detected in specific lumbar levels. High sensitivity, NPV, and accuracy values were obtained when evaluation areas were restricted to certain vertebral levels.

Interrogation of specific pathologies belonging to the upper or lower abdominal region revealed that 96.1% of primary and 83.6% of total CT findings of pathological causes of the lower abdominal region including appendicitis, diverticulitis, and epiploic appendagitis were located together with gynecological diseases in L4 and L5 levels (Table 2). The 92.6% of primary and 78.1% of total CT findings of hepatobiliary and pancreatic causes that could be considered as a specific cause of acute upper abdominal pain were found in the L1 and L2 levels. These conditions, occurring mainly in the upper and lower regions of the abdomen, accounted for 54% of all included patients. Apart from the abovementioned causes of acute abdomen assigned to upper and lower abdominal pathologies, inflammatory and infectious GI tract diseases, intestinal obstruction, peritoneal/ retroperitoneal and mesenteric diseases, urinary tract diseases, incidental malignancies, and miscellaneous conditions were also considered. These conditions can not be confined exclusively to the upper or lower abdominal region, as these diseases are common and it is difficult to localize them on the basis of physical examination findings. In 48 patients, no CT findings were noted in any of the lumbar levels. A significant proportion of these patients were gynecological. At this point, it should be noted that the diagnostic reliability of CT in gynecological patients is quite low compared to US and MRI. Therefore, the US may be performed first if the gynecologic disease is considered. Since the US is a crucial companion exam for both radiation dose saving and improved diagnostic performance to detect gynecologic emergencies [9-11].

It was observed that more findings were found in the lower lumbar regions such as L4 and L5. One of the reasons for this aspect is that the vast majority of acute abdominal causes originate from the lower regions [12-14]. In addition, the fact that these vertebrae are longer in the z-axis could also be a factor.

In our study, no finding was found in 455 patients to explain acute abdominal pain. The high percentage of unnecessary CT and hence unnecessary radiation exposure in the evaluation of acute abdomen may result from the clinician's tendency to avoid both medicolegal responsibility and clarification of the clinical situation. This study has important findings because it demonstrates the aggregation of lumbar level findings for the diagnosis of cause in certain patients with acute abdomen admitted to the emergency department over a 2-year period. In addition, one of the valuable findings of this study is that the primary and secondary findings for diagnosis are actually evaluated from a much shorter z- axis compared to the whole examination. Consequently, while this is not a perfect indicator of dose measurement, it is an indicator of how much

less DLP value we can ideally use for diagnosis than is actually the case. Decent communication between the clinician and the radiologist plays a key role in determining the appropriate modality and avoiding radiation overexposure, as previous studies have shown [3,15,16]. Topographic classification of acute abdominal pain (pain in one of the four abdominal quadrants, diffuse abdominal pain, flank, or epigastric pain) contributes to the selection of the most appropriate imaging technique in the evaluation of acute abdominal pain [17]. Since appendicitis is one of the most common causes of acute abdomen, previous reports indicated that focused CT examination with scanning the lower abdomen only is efficient in assessment of acute appendicitis [18, 19]. In our study, which covers a broad concept of pathologic conditions, the area we evaluated in making the diagnosis on the z-axis actually appears to be a quite short segment in determining the cause of the acute abdomen. These points could be used in the future to develop focused CT protocols that cover the L1, L2, and L5 levels, or at least to complement artificial intelligence studies that consider these levels as "hotspots".

Our study has limitations. First, this was a retrospective study. Second, very few pediatric patients were included in this study because CT is rarely scanned for this group in our hospital, especially for non-traumatic patients. Third, because automatic exposure control was used in the CT studies, we were unable to obtain accurate DLP values for each lumbar level. The fourth limitation was the lack of comparison between the two reviewer's CT image assessments. Consensus reading and analysis are limitations of this study. Further studies may be needed for more robust conclusions, including multicenter studies with interobserver variability. The final limitation is that there were few patients for some of the miscellaneous pathologic conditions in this study, which spanned a 2-year period.

In conclusion, in patients without trauma or a history of abdominal surgery, acute abdominal causes tend to aggregate at certain lumbar levels. It should also be noted that when examining the abdomen CT, a radiologist should spend the most time to examine the L1, L2, and L5 levels, even if the examination includes the entire abdomen.

Author contribution

Study conception and design: AGE and MRO; data collection: AGE; analysis and interpretation of results: İSİ; draft manuscript preparation: AGE and EA. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

Approval for this study was granted by the Institutional Ethics Board (Hacettepe University, Medicine School - approval code: GO 19/800). The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

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Conflict of interest

The authors declare that there is no conflict of interest.

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ORIGINAL ARTICLE

Serum neopterin, YKL-40, IL-6, and TNF-α in patients with obstructive sleep apnea

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ABSTRACT

Objective: Obstructive Sleep Apnea (OSA) is characterized by recurrent episodes of complete or partial upper airway obstruction during sleep. The role of locally increased inflammation in the upper respiratory tract is known in the pathogenesis of OSA. This study aimed to examine the role of some inflammatory markers in OSA.

Materials and Methods: Adult patients who underwent diagnostic polysomnography (PSG) for the first time, were evaluated in this prospective study. Serum levels of neopterin, human cartilage glycoprotein 39 (YKL-40), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) were examined in the study group.

Results: The study included 165 adult patients. Individuals of 56 who were did not have OSA were taken as the control group. Patients diagnosed with OSA (n=109) were included in the study and divided into two groups mild-moderate and severe based on the OSA apnea-hypopnea index (AHI). The study groups were divided into 3 cathegories: AHI <5 (control) Group 1, AHI 5-15 (mild), and AHI 15-30 (moderate) Group 2, AHI>30 (severe) Group 3. Individuals of 165 who completed the study [Group 1 (n=56), Group 2 (n=69), Group 3 (n=40)] were included in the statistical evaluation. There was a significant difference between the groups in terms of neopterin, YKL-40, and IL-6 values ($p<0.05$). The median value of IL-6 and neopterin measured in the severe group was significantly higher than the value measured in the control and mild-moderate groups ($p<0.001$). The mean value of YKL-40 measured in the severe group was significantly higher than the value measured in the control group ($p=0.012$). No significant difference was observed in TNF-α serum levels of groups.

Conclusion: Serum levels of neopterin, YKL-40, and IL-6 were found to be elevated in OSA patients (especially in the severe group).

Keywords: Obstructive Sleep Apnea, Neopterin, YKL-40, IL-6, TNF-α.

INTRODUCTION

Obstructive Sleep Apnea (OSA) is a syndrome characterized by recurrent episodes of complete (apnea) or partial (hypopnea) upper respiratory tract obstruction during sleep and often a decrease in blood O2 saturation [1,2]. Currently, many different types of sleep disorders have been described. Among these, OSA takes the most important place with an average incidence of 1-5% [3].

As a result of apnea and hypopnea periods, hypercarbia and hypoxemia occur, leading to pathologies that affect various organs and systems such as intrathoracic pressure fluctuations, baroreceptor dysfunction, oxidative stress, and endothelial dysfunction [4,5]. The role of locally increased inflammation in the upper respiratory tract is known in the pathogenesis of OSA. It is also believed that hypoxia, asphyxia, hypercapnia, and respiratory acidosis, which occur with a decrease in the intensity of regular breathing that should continue spontaneously, lead to the development of local and systemic inflammation [6,7].

In various studies, increased levels of biomarkers such as C-reactive protein (CRP), interleukin 6 (IL-6), vascular endothelial growth factor (VEGF), leptin, tumor necrosis factor-alpha (TNF-α), reactive O2 radicals, intracellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) indicate that systemic inflammation is increased in OSA [8-10].

Neopterin which is a pyrazinopyrimidine compound, is used as a biomarker, especially in important pathologies in which cellular immune mechanisms are activated. An increased concentration of neopterin is associated with all conditions in which the immune response is active, including malignancies and autoimmune diseases [11]. YKL-40 is also a biomarker used in systemic inflammation. It is released from activated macrophage, neutrophils and vascular smooth muscle cells in the inflammatory process. High levels of YKL-40 have been detected in diseases such as atherosclerosis, diabetes, obstructive pulmonary diseases, cancer, and asthma that involve inflammation and tissue remodeling [12]. IL-6 is a cytokine produced in both adipose tissue and skeletal muscle. It is involved in both inflammatory and anti-inflammatory processes. It plays an important role in the pathogenesis of inflammatory diseases in terms of both its effects on the innate and acquired immune systems and its systemic effects [13]. TNF-α is primarily produced by macrophages. IL-6 and TNF-α are often called proinflammatory cytokines and they contribute to exacerbatin of the inflammatory response [14].

In this study, besides the classical markers of inflammation, inflammatory biomarkers that have not yet been used routinely such as neopterin and YKL-40 were also studied. It was aimed to evaluate the importance of inflammatory markers in OSA and whether they would be beneficial in the diagnosis of the disease. Also, it was aimed to evaluate the similarities and differences in inflammatory markers between patients with OSA and individuals without OSA who have the same characteristics features.

MATERIALS AND METHODS

Study group

210 adult patients who underwent polysomnography (PSG) for the first time for diagnostic purposes between December 2016 and July 2017 in the Sleep Laboratory of Duzce University Medical Faculty Hospital were evaluated. The patients were taken to the rooms two hours before their usual bedtime, allowing them to get used to the room they would sleep. After connecting the electrodes to the patients, the patients were left alone while all the electrodes were controlled from the computer.Three-channel electroencephalography, two-channel electrooculography, chin, right and left tibialis anterior electromyography, body position, oro-nasal thermal sensor, nasal pressure sensor, electrocardiography, respiratory sounds recording, thoracic and abdominal respiratory movements, O2 saturation and synchronous video recording were performed with all-night sleep analysis. All records were scored manually on the computer. 45 of the 210 patients were excluded from the study due to comorbidities. 165 patients with a mean age of 45.47±10.4 years who met the study criteria, were evaluated without gender discrimination. The study group consisted of 49 women and 116 men. In this group, 56 persons who were not found to

have OSA after PSG and met the study criteria were taken as the control group. Patients diagnosed with OSA (n=109) were included in the study in two groups mild-moderate and severe, based on the OSA severity criterion apnea-hypopnea index (AHI) [15]. Persons with an AHI index of <5 after PSG were included in the control group, while patients with AHI≥5 were included in the study by grouping among themselves. AHI 5-15 were grouped as "mild", AHI 15-30 as "moderate", and AHI>30 as "severe" OSA. The age, gender, height, and body weight measurements of the people included in the study were made on the night of the sleep test. People were questioned verbally in terms of acute and chronic diseases.

The study was approved by the ethics committee of Duzce University ethics committee (2016/104). Informed consent was obtained from all individual participants included in the study.

Exclusion criteria

Patients with a diagnosis of diabetes, heart failure, coronary artery disease, chronic renal failure, autoimmune disease, malignancy, inflammatory diseases, chronic lung diseases, or asthma and patients who refused to participate in the study were excluded from the study.

Biochemical analysis

Patient blood samples were taken just before PSG into serum tubes containing clot activator. After the appropriate amount of sample was left for 20 minutes, it was centrifuged for 10 minutes at 4000xg, the serum was portioned into eppendorf tubes, and stored at -80 °C. The IL-6, TNF-α, neopterin, and YKL-40 tests were studied at once in the fully automatic ELISA device of Triturus (Grifols Triturus, Spain) using the ELISA (Enzyme-Linked Immunosorbent Assay) method.

Statistical analysis

Descriptive statistics (mean, standard deviation, median, minimum, maximum) of all variables in the study were calculated. The normality assumption of continuous quantitative variables was checked with Kolmogorov-Smirnov and Shapiro-Willk tests. One Way ANOVA (post hoc LSD test) was used for the intergroup comparisons of normally distributed variables, and Kruskal-Wallis (post hoc Dunn test) analysis was used for intergroup comparisons of non-normally distributed variables. Relationships between categorical variables were determined by the Pearson Chi-Square test, and relations between quantitative variables were determined by Spearman Correlation analysis. Statistical evaluations were made in the SPSS 22 program and $p<0.05$ was considered statistically significant.

RESULTS

165 people were included in the study as three groups. 40 (% 24.3) of them were severe (group 3) OSA, 69 (% 41.8) of them were mild-moderate (group 2) OSA, and 56 (% 33.9) of them were as control group without OSA (group 1). The general characteristics of the patients are summarized in Table 1.

There were no significant differences between the groups in terms of age, gender, and smoking habits ($p>0.05$). But, it was observed that the measurement values in body mass index (BMI) were significantly different between the groups. The median BMI value of the individuals in the mild-moderate and

Table 1. General characteristics of the patients

Characteristic	OSA Group			Total	p
	Control	Mild/Moderate	Severe		
N (%)	56 (33.9)	69 (41.8)	40 (24.3)	165 (100)	
Male (%)	33 (28.4)	51 (44)	32 (27.6)	116 (100)	0.058
Female (%)	23 (46.9)	18 (36.8)	8 (16.3)	49 (100)	0.058
Age (years) mean (min-max)	43.02 (17-65)	46.1 (22-67)	47.8 (26-77)	45.47 (17-77)	0.069
BMI (kg/m^2) mean (min-max)	29 (18.3-40.9)	35.9 (22.5-323)	42.3 (17.6-352)	335.04 (17.6-352)	**<0.001**
% O_2 Saturation mean (min-max)	90 (65-95)	83.9 (71-96)	71.9 (21-92)	83,2 (21-96)	**<0.001**
Smoking (+) (%)	22 (34.4)	23 (35.9)	19 (29.7)	64 (100)	>0.05
Smoking (-) (%)	34 (33.7)	46 (45.5)	21 (20.8)	101 (100)	>0.05

OSA : Obstructive sleep apnea syndrome, BMI: Body mass index

severe groups was found to be significantly higher than the median value of the BMI measured in the individuals in the control group (p<0.001).

It was determined that the lowest O2 saturation measurement values were significantly different between the groups. The median value of O2 saturation of patients in the mild-moderate and severe groups was significantly lower than the value measured in the control group. In addition, the median O2 saturation value of the patients in the severe group was significantly lower than the value measured in the mild-moderate group (p<0.05).

There was a significant difference between OSA groups at the IL-6 level. The median value of IL-6 measured in the severe group was significantly higher than the value measured in the control and mild-moderate groups (p<0.05). In the study, as the OSA severity of the patients increased, an increase in IL-6 levels was also noticed (Table 2). No significant difference was observed between the groups in terms of TNF-α.

At the YKL-40 level, there was a significant difference between the groups. The mean value of YKL-40 measured in the severe group was significantly higher than the value measured in the control group (p<0.05). Neopterin level measurements were also found to be significantly different between the groups. The median value of neopterin measured in the severe group was significantly higher than the values measured in the control and mild-moderate groups (p<0.05). As OSA severity increased, the patient's neopterin levels were observed to also be increasing. The serum levels of the studied inflammation markers are summarized in Table 2.

Table 2. Comparison of serum levels of IL-6, TNF-α, YKL-40, and neopterin

		OSA Group			Total	p	Post Hoc test	
		Mild-moderate	Severe	Control			Groups compared	p'
IL-6	*N*	69	40	56	165	**<0.001**	(Mild-moderate) / (control)	0.001
	Average	43.10	160.88	45.86	72.59			
	Std Deviation	72.74	144.77	60.49	111.13		(Severe) / (control)	**<0.001**
	Median	114.76	169.30	132.13	143.94			
	Minimum	30	32	44	30		(Mild-moderate) / (severe)	**<0.001**
	Maximum	331	852	354	852			
TNF-α	*N*	69	40	56	165	0.782		
	Average	0.18	0.20	0.18	0.18			
	Std Deviation	0.21	0.20	0.21	0.21			
	Median	0.13	0.14	0.13	0.13			
	Minimum	0.02	0.01	0.01	0.01			
	Maximum	1.13	0.72	0.87	1.13			
Neopterin	*N*	69	40	56	165	**<0.001**	(Mild-moderate) / (control)	0.115
	Average	8.47	9.94	7.53	8.51			
	Std Deviation	3.00	2.84	2.52	2.93		(Severe) / (control)	**<0.001**
	Median	8.07	9.54	7.03	8.16			
	Minimum	2.74	5.30	2.94	2.74		(Mild-moderate) / (severe)	**0.018**
	Maximum	17.60	20.20	17.00	20.20			
YKL-40	*N*	69	40	56	165	**0.012**	(Mild-moderate) / (control)	0.102
	Average	153.09	176.05	132.21	151.57			
	Std Deviation	72.25	67.28	70.78	72.08		(Severe) / (control)	**0.003**
	Median	159.00	165.50	130.00	152.00			
	Minimum	5	25	15	5		(Mild-moderate) / (severe)	0.104
	Maximum	302	299	276	302			

DISCUSSION

Local and systemic inflammation is important in the course of the disease and the occurrence of complications in OSA. It has been observed that increased systemic inflammation is effective in OSA, especially in cardiovascular events [16]. Several studies had shown an increase of numerous inflammation markers such as CRP, leptin, TNF-α, IL-6, VEGF, reactive O2 radicals, ICAM-1, and VCAM-1 in OSA [8,17]. There are studies showing that TNF-α and IL-6 levels decrease after surgical treatments in patients with OSA. At the same time, the decrease in inflammation biomarkers in parallel with clinical relief after "Continuous Positive Airway Pressure (CPAP)" treatments shows that systemic inflammation is an important factor in the emergence of OSA or as a result of the disease [17,18].

The following results were obtained for O2 saturation; the lowest O2 saturation measurement values were significantly different between the groups. The median O2 saturation value of the individuals in the mild-moderate and severe groups was significantly lower than the value measured in the control group. In addition, the median O2 saturation value of individuals in the severe group was significantly lower than the value measured in the mild-moderate group ($p<0.001$) (Table 1).

It was observed that the group with severe OSA had more hypoxic values during sleep (72.73%). Of course, while the patients are awake, their O2 % saturation is higher. The lowest O2 saturation parameter that we evaluated in the study is the lowest O2 saturation measured from the fingertip of the patient during sleep, including recurrent apnea periods. This means that patients with OSA go through severe hypoxic phases during sleep. These fluctuations in O2 saturation cause hypoxic tissue damage as a result of the recovery of impaired blood O2 saturation, as in ischemia-reperfusion injury [20]. Intermittent hypoxia observed during sleep initiates systemic inflammation and causes an increase in serum IL-6 level, which is an important proinflammatory cytokine in severe OSA [1]. In a meta-analysis of 39 studies measuring serum IL-6 levels, IL-6 levels of 2558 OSA patients and 1897 control volunteers were examined. It has been reported that serum IL-6 levels were found to be statistically significantly higher in OSA cases [21].

In addition to studies with high serum TNF-α levels in OSA patients, there are also studies with no significant difference [22]. Fornadi et al. found no association between TNF-α and AHI in a study of 100 kidney transplant patients [23]. Similarly, no significant difference was found between the groups with TNF-α in the current study. The serum cytokine levels are dynamic and depends on some conditions: for example, a rapid increase in TNF-α was observed immediately after apnea events [24]. So observing no significant difference in TNF-α can be depending on the time of blood collection.

Otherwise, serum levels of IL-6, YKL-40, and neopterin were found to be significantly higher in the patient groups compared to the control group without OSA ($p<0.05$). This elevation was more pronounced in the group with severe OSA ($p<0.001$).

Studies examining serum neopterin levels in OSA patients are few. Ursavas et al. analyzed serum neopterin levels of 22 patients with OSA and 18 patients without OSA. They found no statistically significant difference between patients with and without OSA, but they found that neopterin levels were positively correlated with BMI [25]. In another study, plasma neopterin levels were found to be elevated in hypertensive patients with OSA and were positively correlated with the severity of OSA [26]. In this study the median value of neopterin measured in the severe group was significantly higher than the values measured in the control and mild-moderate groups ($p<0.05$; Table 2).

In a study by Sui X et al. serum YKL-40 levels were measured in OSA patients by forming two groups with and without coronary artery disease. Serum YKL-40 levels were found to be high in OSA patients with coronary artery disease and also serum YKL-40 levels were correlated with the severity of the disease [27]. There are also different studies stating serum YKL-40 levels may be an indicator of early atherosclerosis in OSA [28]. In a study conducted on 156 patients diagnosed with OSA and 104 healthy volunteers; serum YKL-40 levels were found to be significantly higher in patients compared to the control group, and a positive correlation was found between increased OSA severity and serum YKL-40 levels [27]. Li et al. found YKL-40 levels to be significantly higher in patients with severe OSA. They also found a correlation between serum CRP level, AHI, BMI, insulin resistance, and YKL-40

levels [29]. There is a difference in YKL-40 levels in our severe patient group when compared to mild-moderate and control groups. The mean value of YKL-40 measured in the severe group was significantly higher than the value measured in the control group ($p<0.01$). Even though the average YKL-40 levels in the mild-moderate patient group differ from those in the control group, this difference did not reach statistical significance ($p=0.102$).

In a study conducted by Bulcun et al. serum CRP, hs-CRP, IL- 6, and TNF-α levels were compared in obese OSA patients (n: 63), OSA patients with normal BMI (n: 49), and control group without OSA (n: 21). As a result, they found no significant difference between the obese and nonobese groups with OSA. Although inflammatory markers were high in patients with OSA, there was no statistically significant difference from the group without OSA [30]. The statistically significant difference in terms of BMI in the patient groups we selected is the inevitable result of the very common association between obesity and OSA. BMI is higher than in other groups, especially in group 3; where there are patients with severe OSA. However, the fact that obesity is an independent risk factor for OSA, and that inflammation increases as OSA and weight increases (in correlation with AHI) independently of obesity in various studies suggest that high BMI in patient groups does not adversely affect our study [30].

Vgontzas et al. studied serum IL-1β, TNF-α, IL-6 levels in OSA, narcolepsy, and hypersomnia. They found that serum TNF-α levels were significantly higher in OSA and narcolepsy, associated with excessive daytime sleepiness. Serum IL-6 level was found to be significantly higher only in OSA and it was shown to be correlated with BMI. When IL-6 and TNF-α were given to individuals, increased sleep desire and weakness occurred; suggesting that these two cytokines mediated the main symptoms of OSA with these findings. The same researchers found IL-6 and TNF-α to be high in obese individuals with OSA. However, they showed that high cytokine levels in OSA are factors independent of tissue adiposity and insulin resistance [31,32].

In this study, no significant difference was found between the groups in terms of age, gender, and smoking habits. There were approximately similar proportions of smokers and non-smokers in all three study groups. The handicap that we had in this regard was that the exposure times of patients who smoke had not been evaluated in the study, and patients who quit smoking had also been evaluated as active non-smokers, despite having previously been exposed to tobacco. With a more comprehensive study, the relationship between OSA smoking and inflammation can be evaluated by considering the smoking history and exposure time of the patients. In this study, the inflammatory markers investigated were compared between the OSA groups and the relationship between AHI or BMI and inflammatory markers was not examined. This is another limitation of the study.

There are serious complications and increased mortality in OSA with AHI>20 and above. As can be seen in the studies, the presence of subclinical inflammation increases as the severity of OSA increases, and the risk of complications increases. Inflammation, which is one of the factors that should be blamed for the aggravation of OSA and the development of complications, may also play a key role in the treatment. Further on it is a big problem that CPAP and other devices used in the treatment of OSA today are difficult to use and provide palliative treatment. When patients can not use or refuse to use their devices, there are no treatment alternatives available. In addition to being able to diagnose patients with OSA, the predictability of possible complications is also important for patient survival and quality of life. For this reason, it seems that biochemical-based studies can be a light for understanding the pathophysiology of OSA, producing alternative options for its' treatment and prevention of complications.

In conclusion, local and systemic inflammation has an important role in the course of the disease and the emergence of complications in OSA. In particular, there is increasing evidence that inflammation plays a major role in the cardiovascular pathophysiology of OSA. In the light of these data, more studies are needed on the place of inflammation in the diagnosis and treatment process of OSA, whether or how inflammatory markers can be used in diagnosis and treatment follow-up. In this study, a general and broad view of the subject was aimed by evaluating the newly found markers thought to be an indicator of inflammation and classical markers of inflammation together.

Author contribution

Study conception and design: ÖA, İEŞ, and EGB; data collection: ÖA; analysis and interpretation of results: ÖA and ŞC; draft manuscript preparation: ÖA, İEŞ and EGB. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

Ethics committee approval was received for this study from the ethics committee of Duzce University (2016/104). Informed consent was obtained from all individual participants included in the study.

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Conflict of interest

The authors declare that there is no conflict of interest.

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acta medica

ORIGINAL ARTICLE

Lessons learned in medical students' remote social responsibility experiences during COVID-19 pandemic

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ABSTRACT

Background: With the declaration of the COVID-19 pandemic in March 2020, face-to-face medical education was interrupted. To make first-year students a part of the fight against the pandemic, "We, as well, are in the Fight Against Novel Coronavirus Disease (COVID-19)!" remote social responsibility project was implemented in May 2020.

Aim: The aim of the study was to evaluate the perceptions and opinions of medical students regarding their remote social responsibility experiences during the COVID-19 pandemic.

Methods: Thematic content analysis of short-written reflection data collected to provide a deep insight into the experiences of the medical students was carried out. Results: The meaning and achievements of the remote social responsibility project revealed seven themes: "perception of social responsibility", "perception of being medical students", "perception of being a physician", "fight against the pandemic", "affective achievements", "acquisition of generic skills" and "corporate commitment". The findings draw attention to understanding the meaning of social responsibility, medical students' and physicians' role and responsibility in protecting health during the pandemic.

Conclusion: Medical students providing social responsibility opportunities for their students during the pandemic enables them to be a part of the fight against the pandemic and ensure important gains. These gains also valuable in preparing them for pandemics and similar emergencies in their future professional lives. In addition, competencies such as communication in crisis environment, access to evidence-based information, informing the society that will prepare for crisis situations should be gained in the medical education.

Keywords: Covid-19 pandemic, social responsibility, medical students.

INTRODUCTION

Physicians' social responsibilities have become a prominent topic in the fight against health problems and crises on a global scale. The motivation of physicians to feel responsible and accountable to society has led to the increasing importance of the issue in medical education. Medical education and practice should be sensitive to the social determinants of health and vulnerable and marginalized people [1]. Medical schools should offer opportunities during medical education so that the students internalize the concept of social responsibility and engage in behaviors that will benefit society. Students can learn by experiencing the needs and expectations of society in different social environments and fulfill their responsibilities in an organized manner. Social responsibility training ensures not only students but also medical schools are accountable to the society, integrating with the society [2].

The New Coronavirus Disease (COVID-19) was declared as a pandemic by the WHO on March 11, 2020. The COVID-19 pandemic has directly affected medical education. Lockdowns, meeting restrictions and the withdrawal of students from educational and clinical environments have forced medical schools to produce a series of educational innovations. One is the continuation of voluntary social responsibility activities to support health systems [3]. Students working on volunteer projects emphasized that making an active contribution in this challenging time rather than staying at home made them feel good [4].

In Hacettepe University Faculty of Medicine (HUFM), the "Becoming a Physician and Social Responsibility" (BPSR) program has been implemented for first-year medical students since 2018. The program aims to develop social responsibility awareness and sensitivity by planning and implementing a social responsibility project (SRP). SRPs are planned and implemented in small groups under the guidance of a facilitator. In the 2019-2020 academic year, by the declaration of the COVID-19 pandemic, face-to-face education was interrupted and first-year students had to stay at home due to legal legislation. Most of the SRP groups could not complete the field study of the SRPs they planned. "*We, as well, are in the Fight Against Novel Coronavirus Disease (COVID-19)!*" project has been developed as part of the BPSR Program to make first-year medical students a part of the fight against the COVID-19 pandemic.

It was aimed to evaluate the perceptions and opinions of medical students regarding their remote social responsibility experiences during the COVID-19 pandemic.

MATERIALS AND METHODS

Participants

The study group comprised first-year students (n= 479) at HUFM in the 2019-2020 academic year.

Remote Social Responsibility Project

The objectives of the Project were to inform the students about pandemics, enable them to understand the importance of evidence-based information and allow students to have an experience of informing the public about the pandemic by using social media platforms [5]. Project activities were planned in the SRP groups under the guidance of facilitators, but the target groups were informed simultaneously by the students individually. It was aimed to inform four target groups:

- general public
- 65 years and older and individuals with chronic diseases
- teenagers (children staying at home-adolescent)
- women who have just given birth

Reminders (14 pieces), infographics (5 pieces), video (Arabic with English subtitle), and story visualization materials have been prepared by an expert group. The translation of infographics into six different languages (English, Arabic, Albanian, Greek, Azeri, and Swahili) was carried out with the students' contribution to enable international students to share in their own countries (Annex-1). Due to social life constraints, all students informed the target groups using these materials in May 2020 through social media communication channels such as Instagram, Facebook, and WhatsApp. When students couldn't reach the target groups via social media, students informed them by

phone. All materials were shared simultaneously on the HUFM website. After completing the project activities, the students were asked to complete a written reflection assignment about their project experiences. The students were asked to answer the following question:

- Explain the meaning of the "We, as well, are in the Fight Against Novel Coronavirus Disease (COVID-19)!" project experiences and its contributions (To include both your feelings and thoughts).

Four hundred twenty-eight students (89.3%) submitted their written reflections.

Analysis of written reflection data

Written reflection allows students to relate their experiences with their thoughts, feelings, and values in a meaningful way. It also enables educators to examine learners' emotions, exciting experiences, and learning areas [6]. In the study, qualitative analysis (thematic content analysis) of written reflections was conducted to gain deep insight into students' experiences.

Two researchers (MD and BA) conducted the analysis. The dataset was created by combining all written reflections. Thematic content analysis was performed, as described in Table 1 [7].

Ethical consideration

The written reflections of the students were retrospectively analyzed after completing the BPSR program. Reflective writings were accessed through the educational portal (Blackboard) with institutional written permission. Identity information was kept confidential at all stages of the study.

Table 1. Thematic content analysis stages

Phase	Description of the process
1	Reading of the raw data (dataset) and gaining a general understanding
2	Coding interesting features of the data in a systematic fashion
3	Collating codes into potential themes
4	Generating a thematic 'map' of the analysis
5	Generating clear definitions and names for each theme
6	Producing a scholarly report of the analysis.

RESULTS

As a result of the thematic content analysis, we identified seven themes, 53 codes, and 806 data extracts. The explanations of the participants are related to the following themes, respectively: "perception of social responsibility ", "perception of being medical students", "perception of being a physician", "fight against the pandemic", "affective achievements", "acquisition of generic skills" and "corporate commitment" (Figure 1).

We used the SRP group (G) and participant (P) numbers when sharing the quotations.

Perception of social responsibility

It was widely stated that experiencing SR in an extraordinary situation helped them understand the importance of SR during the pandemic and showed that SR activities could be carried out in any environment and at any time, even without face-to-face. Students perceived this experience as a satisfying experience. The fact that students receive positive feedback from the target groups after sharing and that more information is requested from them can be effective in this perception.

It is very proud to be doing such a project in these days when it is essential to inform people correctly. I think that each feedback is proof for us of how valuable and important this Project is. I realized how human life can be saved with a simple precaution and sensitivity. (G33, P6)

Perception of being a medical student

Medical students who must stay at home and continue their education remotely perceive their

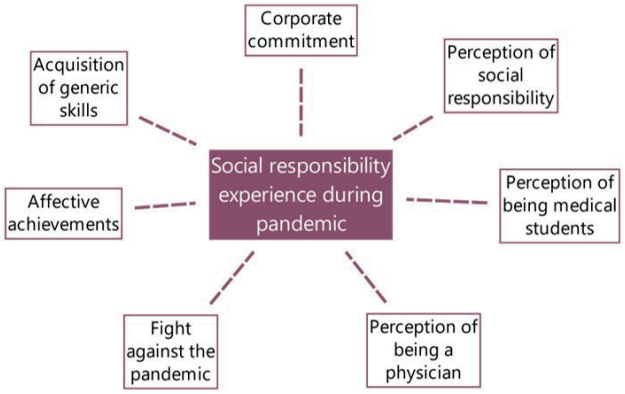

Figure 1. Meaning and gains of the SRP experience during the COVID-19 pandemic

SR experience as contributing to the fight against the pandemic. They stated that this experience improved the medical students' understanding of their role and responsibilities in informing society in extraordinary situations. The positive feedback from the target groups made them feel that medical students are trusted in society. They think that SRP experiences contributed to the development of medical student identity.

As a medical student, I felt in a status that informs and raises awareness of society for the first time. It was good preparation as I will continue in this way when we become a doctor in the future. (G2, P11)

Perception of being a physician

The students mentioned this experience helped them better understand the principle of the medical profession is to protect and improve the health of people/society and the responsibility of physicians toward society as well as the patients (individually). They emphasized the importance of sharing evidence-based information against misinformation and that this project increased their awareness of the physician's role in informing society.

The most crucial gain that the social responsibility project brought me is that I realized that the physician's mission is not just to treat. At such times when information pollution is at its peak, it is our responsibility show the society how to protect themselves and their lives while preventing panic and allowing the public to access reliable information. (G3, P13)

Fight against the pandemic

Students stated that their SRP experiences primarily contributed to their knowledge about the COVID-19 pandemic. Some students also emphasized that it motivates more research on COVID-19. Students understood the power of social media in reaching target groups (elderly, adolescents, etc.) and informing them about the pandemic. Their awareness of the use of these platforms for educational purposes has increased. This experience was valuable as it allowed them to communicate with people, understand them and socialize simultaneously during isolation at home.

I enjoyed using the educational part of social media during the pandemic. I don't think social media is a completely time-consuming platform anymore. (G4, P9)

Affective achievements

The SRP experience has been perceived as a valuable experience that supports the health care providers working actively in the field. Students defined their affective achievements by using expressions such as enjoying and feeling like a physician. They stated that it increased their self-confidence, sensitivity to people and the environment and created a desire to better prepare for their profession. One student commented that her experiences made her realize the difficulty of changing people's behavior (wearing a mask).

While we were staying at our homes, residents, professors, nurses, health technicians, and many more people in the field of health risked their lives and worked for us. While working in those difficult conditions, I participated in such a social responsibility project. Although we could not inform people face-to-face, at least it is precious to lighten the burden health care system by raising awareness through informing remotely. (G6, P12)

Acquisition of generic skills

Students think that they will be able to use some gains experienced in SRP, such as communicating with people (written and verbal), informing society, and approaching target groups (elderly, pregnant, etc.) in their professional life in the future. Some students express it as an important gain to experience remote planning and execution of SRPs that aim to reach large target audiences in extraordinary situations such as pandemics.

Raising my father's awareness was what the Project indirectly contributed to me. In that process, I realized that some people who do not follow the rules know what they are but do not want to accept them. I learned how to explain (G13, P2)

Corporate commitment

Students perceive it as effective and reliable to share the information prepared by experts for society with the logo of their school. This approach was described as proud by some students. At the beginning of the pandemic, their school's effort to inform society with its social responsibility approach and the inclusion of its students were expressed that increased corporate commitment.

I am still a first-year medical student, and due to the pandemic, I could not spend any time in medical school, so I could not feel enough commitment to the faculty. During the Corona pandemic, my being seen as a member and representative of this faculty and being given a task made me feel the sense of belonging that I felt lacking. (G30, P6)

Two students emphasized some negative experiences while informing the public. These are "people not to care enough; failure to prevent the spread of misinformation; to be late in sharing and in being passive in the project."

DISCUSSION

It was aimed to evaluate the meaning and gains of a SRP developed rapidly in the context of the pandemic and implemented remotely for medical students.

Students think their experience to be purposeful, valuable and fulfilling although they are more passive than their active roles in face-to-face SR training. Informing the people about the pandemic is seen as an important gain. For example, wearing a mask is necessary for the safety and health of other people around and personal protection. There are values and perceptions that shape the personal decisions of individuals in society about whether to wear a mask [8]. Complex/complicated, contradictory, or false information can also be spread during the pandemic. The fight against infodemi, which is also defined as misinformation arising from the abundance of information that needs to be dealt with in society, comes to the fore as a requirement of health advocacy [9]. This becomes even more important if the health literacy of the population is low [10]. In the study, students primarily understood that it is important for society to have evidence-based knowledge in terms of showing safe behaviors in the information pollution experienced in the case of a pandemic. Students experiences on informing the target groups enables them to be a part of the fight against the pandemic.

The COVID-19 pandemic has led to the debate of the role of medical students during the pandemic [11]. Health sciences students stated that voluntary participation in a pandemic is a moral/ethical/professional obligation, and it is necessary to be encouraged to volunteer [12]. The active participation of medical students voluntarily with an individual choice can provide useful knowledge and skills about flexibility, tolerance to uncertain situations, professional identity development, motivation to learn, and awareness and risk assessment [13]. However, it should not be forgotten that participation with limited knowledge may lead to psychological concerns, ethical problems, and even legal obligations [14]. Voluntary student participation in SR activities during the COVID-19 pandemic positively affected students' social skills and made them feel happy, enthusiastic, valuable and useful to society [15]. Such activities make the individuals in the society feel safe and increase the feeling of trust and gratitude towards the physician [16,17]. Differences between volunteering and service-learning approach are discussed in the literature. While the main focus in volunteering is the service provided, in service-learning, it is the learner's experiential learning [18]. We tried to establish a balance between providing service to society and reaching the learning goals of the learners. The findings of the study support that the learning outcomes were achieved in terms of students. They also support the literature findings [15-17,19].

Students think that their experience improves their awareness and understanding of the physician's responsibility to society and his role in protecting health. These achievements are primarily related to the physician's health advocacy role. Health advocacy becomes even more critical in extraordinary situations such as the pandemic. By implementing purposeful programs on advocacy for ordinary and extraordinary situations, medical schools can better prepare students to meet the challenges they may face as physicians [20].

The study group consists of first-year students restricted from leaving home due to their age and continuing their medical education remotely online. These limitations and conducting education remotely lead to isolation, decrease in social interaction and communication [21]. In the study, students see their experiences as enhancing their social interactions by communicating with people on social media and by phone when necessary. Students' social interaction led them to understand how people were affected by the pandemic and think about how to reach and communicate with target groups (elderly, adolescents, and pregnant

women). Social empathy is 'the ability to understand people more deeply by perceiving or experiencing life situations and, as a result, gain insight into structural inequalities'. Social empathy consists of three elements: individual empathy, contextual understanding, and social responsibility [22]. The COVID-19 social responsibility experience of the students can be considered as an opportunity provided to them in this context.

Pandemic is a period when people tend to unite and support each other in society and learn to do this from a distance. Social media allowed learning communities to share their experiences and good practice examples [23-26]. The use of social media for educational purposes has the benefits of interaction in information transfer, equal participation of the parties in the education processes and real-time information exchange [27,28]. Characteristics of Generation Z (born in 1997 or later) that make up the study group; "hyper-connected to computers and the internet," "have global expectations of learning opportunities," and "active problem-solvers, independent learners, and social justice advocates" [29]. Health professional educators need to accept this generation's unique characteristics and carry social media into learning environments [30]. In the study, first-year medical students were encouraged to use social media to disseminate accurate information. Experience in using social media for SR activities can be considered a significant gain.

In the study, sharing the information with the corporate logo was seen as self-confidence and strengthening corporate belonging. It is thought that SR activities will increase the corporate commitment of not only students but also educators [31].

Medical shools should prepare graduates for clinical and non-clinical roles in ordinary and extraordinary situations [21,32]. However, public health emergency preparedness education is not sufficiently included in medical education [33]. In most public health emergencies, such as the pandemic, event-specific "spontaneously" training is not always possible [34]. Hall et al. drew attention to adaptive strategies for learning and expanding learning paths using digital technologies in maintaining medical education during the pandemic [35].

CONCLUSION

As a result, the gains of the SRP, which aims to make first-year medical students a part of the fight against the COVID-19 pandemic; can be summarized as understanding the importance of social responsibility, the role of the medical student and physician during the pandemic, the fight against the pandemic, acquisition of generic skills and affective achievements and strengthening corporate commitment. In the study, students think that their medical school act quickly and responsibly towards society during pandemic, and they find it very valuable that they are involved in these process. It is also valuable in preparing them for pandemics and similar emergencies in their future professional lives.

Research findings drew attention to the development of medical curricula for graduates who are better prepared for public health emergencies. Competencies such as communication in crisis environment, access to evidence-based information, informing the society that will prepare for crisis situations should be gained in the medical education.

The study's strength is a qualitative study that allows students to reflect on their perceptions and thoughts. However, it was conducted only in one medical school and only included the students' opinions.

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Author contribution

Study conception and design: MD, BA, and DA; data collection: MD, BA, and DA; analysis and interpretation of results: MD, BA, and DA; draft manuscript preparation: MD, BA, and DA. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Hacettepe University Non-Interventional Clinical Research Ethics Board (Protocol no. GO 21/360 / March 16, 2021).

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Conflict of interest

The authors declare that there is no conflict of interest.

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Annex 1. Materials of the "We, as well, are in the fight against COVID-19!" Project

Material type (number)	Content	Language
Reminder (14)	1. General Information on COVID-19 2. Proper Hand Washing 3. Social / Physical Distancing! 4. Wearing Face Masks Properly 5. Risk Groups 6. COVID-19 and Adolescent-1 7. COVID-19 and Adelosanes-2 8. COVID-19 and Adolescent-3 9. COVID-19 and Tobacco Use 10. COVID-19 and 65 Years of Age and Older People 11. 10 Recommendations for Children, Adolescents and Parents 12. COVID-19 and Breastfeeding 13. COVID-19 and Foods 14. COVID-19 and Correct Information Sources	Turkish
Infographics (5)	1. Social / Physical Distancing! 2. Wearing Face Masks Properly 3. COVID-19 and Tobacco Use 4. COVID-19 and 65 Years of Age and Older People 5. 10 Recommendations for Children, Adolescents and Parents	Turkish English Arabic Albanian Greek Azeri Swahili
Video (1)	Messages from HUFM Phase I Students	Arabic with English subtitle
Video (2)	Narrative; "Pathetic End of Novel Coronavirus"	Turkish

acta medica

ORIGINAL ARTICLE

Hedera helix (Wall Ivy) leaf ethanol extract shows cytotoxic and apoptotic effects in glioblastoma cells by generating reactive oxygen species*

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ABSTRACT

Aim: Glioblastoma, known for its aggressiveness, accounts for most malignant gliomas and the efficiency of its treatment is still not enough showing remarkably poor prognosis even though a complex treatment approach. *Hedera helix* is an evergreen plant mostly known for its effects on respiratory function, especially in chronic bronchial asthma, as well as its antimicrobial, anti-inflammatory, antioxidant, and potential antitumor properties, while its effect on glioblastoma cancer and the underlying mechanism has not been elucidated yet. The aim of this study was to examine the anti-cancer properties of *Hedera helix* (common ivy) in relation to its ability to induce cytotoxicity, apoptosis, and reactive oxygen species production in glioblastoma (U87) cells.

Material and Methods: The leaves' ethanol, methanol, and water extracts were analyzed for phenol, flavonoid, and antioxidant levels through photometric methods. Then, different concentrations of ethanol extract were applied to U87 cells for 24 hours and analyzed for cytotoxic, apoptotic, and ROS-generating effects by measuring the luminometric intracellular ATP amount, Acridine Orange/Ethidium Bromide double staining, and DCFH-DA methods, respectively.

Results: Phenol, flavonoid, and antioxidant results of all three extracts show that ethanol was best for extraction. The results showed that *Hedera helix* ethanol extract had dose-dependent cytotoxic and apoptotic effects with increased intracellular ROS levels.

Conclusion: These results concluded that high doses of *Hedera helix* ethanolic extract may exhibit anti-cancer effects through pro-oxidant activity.

Keywords: Hedera helix, common ivy, anti-cancer, pro-oxidant, glioblastoma.

INTRODUCTION

Glioblastoma is the prevailing and highly aggressive form of primary brain tumor in adults, comprising 57.3% of all gliomas [1] and 45.6% of all primary malignant brain tumors [2]. Glioma is the most prevalent malignant neoplasm of the central nervous system (CNS) when considering incidence and mortality rates [3]. The incidence of glioblastoma multiforme (GBM) tends to increase with age [4], and the occurrence is increasing potentially due to multifactorial factors, including an aging population, exposure to ionizing radiation, air pollution, and other possible causes [5]. Although surgical resection, radiotherapy (RT), and simultaneous chemotherapy are applied in conventional treatment [6], the success rate is low, the side effects are high and the survival time is around 15 months. [7]. Therefore, the search for alternative treatments continues, and one of them is the use of medicinal plants, described as phytotherapy.

Hedera helix L. (*H. helix*), commonly known as English ivy or common ivy, is an evergreen woody vine belonging to the family Araliaceae, and one of 15 species in the Hedera genus [8]. *H. helix* has a rich historical background in traditional medicine, where it has been widely utilized to address a diverse range of health conditions including respiratory infections, arthritis, and skin disorders, among others. [8]. The Hedera leaves have been used for treating cough and respiratory problems and the German Commission E has approved the use of *H. helix* for the treatment of catarrhs (excessive mucus production) affecting the respiratory tract and for alleviating symptoms associated with chronic inflammatory bronchial conditions [9]. *H. helix* extract is also often used in cosmetics and skin care products due to its emollient and antipruritic properties. Creams, lotions, and shampoos containing *H. helix* extract are commonly used to soothe and moisturize dry, pruritic skin and to relieve symptoms of skin disorders such as eczema and psoriasis [10]. In homeopathy, the *H. helix* is believed to have therapeutic properties that can be used to treat a range of conditions, including hyperthyroidism, rheumatic disorders, and respiratory tract inflammation [8]. Anti-inflammatory, antimicrobial, and anthelmintic activities of the *H. helix* were shown in various studies in the literature [11-13]. Besides all these pharmacological effects, the anti-tumor effects of *H. helix* extracts also have been investigated and shown [14-16]. However, previous studies revealed a lack of research on the potential anti-tumor effects of *H. helix* on glioblastoma. Therefore, the current investigation was carried out to fill this knowledge gap and explore the potential cytotoxic, apoptotic, and Reactive Oxygen Species (ROS) production effects of *H. helix* in the U87 glioblastoma cell line.

MATERIAL AND METHODS

Cell culture

U87 cells were sourced from the American Type Cell Culture Collection (ATCC, Germany). These cells were regularly maintained in DMEM culture medium (Biochrom, Germany) supplemented with 10% fetal bovine serum (Biochrom, Germany) and 1% penicillin/streptomycin (100 U/ml penicillin and 100 µg/ml streptomycin) (Biochrom, Germany). The cells were cultured in a humidified atmosphere with 5% CO_2 at a temperature of 37°C.

Plant extraction

The ivy leaves collected from the inner Anatolia region were subjected to extraction using ethanol, methanol, and water. The dried samples (100 g) were mechanically powdered and then extracted either with 70% ethanol (Absolute; Merck, Germany), 70% methanol (Merck, Germany), or 100% water. The extraction processes were carried out on an orbital shaker at room temperature for a minimum of 24 hours. The resulting ethanol, methanol, and water extracts were filtered using Whatman filter paper, and the alcohol was removed using a rotary evaporator (Heidolph, Germany). The remaining supernatant was subsequently lyophilized under a vacuum at -82°C using a freeze dryer (Labconco, USA).

Total phenolic and flavonoid contents

The phenolic and flavonoid contents of the ethanol, methanol, and water extracts were assessed using photometric methods. The total phenolic contents (TPC) were measured using the Folin-Ciocalteu method with slight modifications [17]. To determine the TPC, the absorbance of the samples

was measured at a wavelength of 760 nm using a spectrophotometer (Varioskan Flash, Multimode Reader, Thermo Scientific, USA). A standard curve was created by using various concentrations of gallic acid. The TPC values were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry plant material (mg GAE/g plant material).

To quantify the total flavonoid compounds (TFC), a colorimetric assay was performed according to the described method [18, 19]. After the addition of 75 µL of 5% sodium nitrite ($NaNO_2$) to the mixture of 250 µL sample and 1 mL of distilled water in a volumetric flask, 75 µL of 10% aluminum chloride ($AlCl_3$) was added after 5 minutes. Subsequently, after 6 minutes, 500 µL of 1 N sodium hydroxide (NaOH) was added to the mixture. Following the addition of 500 µL of 1 N sodium hydroxide (NaOH) to the mixture, it was diluted with 2.5 mL of distilled water and the resulting samples were vortexed and kept at room temperature. The absorbance of the resulting mixture was measured directly at 510 nm using a Varioskan Flash Multimode Reader. A calibration curve was established using a diluted solution of quercetin to determine the TFC. The TFC values were expressed in milligrams of quercetin equivalent per 100 grams of dry matter (mg QuE/100 g DM).

DPPH assay

The DPPH assay is commonly used to measure the ability of a substance to scavenge free radicals. This is done by observing the reduction in the absorption of a methanolic solution of DPPH, a stable radical after it is neutralized by an antioxidant This is indicative of the antioxidant's radical scavenging activity. To carry out the assay, 20 µL of diluted extract is added to 980 µL of 100 µM DPPH solution, followed by a 30-minute incubation period. The absorbance of the resulting mixture is then measured at 517 nm using a microplate reader (Varioskan Flash, Multimode Reader, Thermo Scientific, America). The percentage inhibition of the DPPH radical was determined using the following formula: I = 100 (Ac - As)/Ac, where I represents the percentage of DPPH inhibition, Ac is the absorbance value of the control sample, and As is the absorbance value of the sample being tested [20, 21].

ATP Cell Viability Assay

Cytotoxicity of *H. helix* ethanol extract on glioblastoma cells investigated through ATP cell viability assay. In this assay, ATP is measured using a luciferase-based assay system. The assay relies on the fact that luciferase, an enzyme found in fireflies, catalyzes the reaction between ATP and luciferin, resulting in the emission of light. The amount of light emitted is proportional to the amount of ATP present in the sample. For performing the ATP test, $5x10^3$ U87 cells were plated in an opaque white 96 well plate and treated with different concentrations of ethanol extract for 24 hrs. Then, the ATP cell viability kit (Promega, USA) was applied according to the manufacturer's protocol. Luminometric light intensity was measured through a multiscan plate reader (Varioskan Flash, Multimode Reader, Thermo Scientific, USA), and intracellular ATP levels were determined. Cell viability of extract-treated cells was compared to non-treated cells and half maximal inhibitory concentration (IC_{50}) of the extract was calculated.

DCFH-DA for ROS analysis

DCFH-DA, 2',7'-dichlorodihydrofluorescein diacetate, is a fluorescent dye that is frequently utilized to quantify the levels of intracellular ROS. When DCFH-DA is taken up by cells, it is hydrolyzed by intracellular esterase to form the non-fluorescent 2',7'-dichlorodihydrofluorescein (DCFH). DCFH is oxidized by ROS and forms the 2',7'-dichlorofluorescein (DCF) which is a fluorescent compound. The intensity of the DCF fluorescence is proportional to the amount of ROS generated in the cell. To perform ROS analysis using DCFH-DA, $5x10^3$ U87 cells were plated in a black 96 well plate and treated with the same concentrations of the ethanol extract used in the cytotoxicity test for 24hrs. Then the cells were washed with 1xPBS one time and incubated with 5 µM H2DCF-DA for 30 minutes at 37°C in the dark. After 30 min. incubation, the absorbance was measured using a multi-plate reader (Varioskan Flash Multimode Reader, Thermo Scientific) to measure the DCF fluorescence intensity. At excitation and emission wavelengths 485 nm and 535 nm, respectively. The results were normalized to the viable cells.

Acridine Orange/Ethidium Bromide (AO/EB), Double Staining Method

The apoptotic effect was studied by AO/EB technique which is a simple and inexpensive method used to differentiate apoptotic cells from living cells. According to the cytotoxicity

experiments, the concentrations around and below the IC_{50} value (300, 350, 400, 450, and 500 µg/ml) were applied to the cells for 24 hours. The cells were then removed from the plates and stained with the AO/EB dye (Sigma Aldrich, USA). Stained cells were examined through the fluorescent microscope (Leica Microsystems, Germany) and apoptotic cell ratios were determined by evaluating at least 100 cells for each sample. Green-dyed cells were counted as live cells due to AO staining and dead cells were red due to EB staining.

Statistical analysis

All experiments were performed in triplicate and the results were expressed as mean value ± standard deviation (mean ± SD). The conformity of the quantitative variables to the normal distribution was examined using the Shapiro Wilk test. One-way analysis of variance (One-Way ANOVA) was used to compare the mean of related variables between groups. The Dunnett test was used as a post hoc analysis for pairwise comparisons. The statistical significance level was taken as 0.05 and the calculations were made using IBM SPSS Statistics for Windows, Version 26.0. (Armonk, NY IBM Corp).

RESULTS

Comparison of ethanol, methanol, and water extracts for antioxidant activity with phenolic and flavonoid contents

The extracts were prepared using three different solvents; ethanol, methanol, and water, and their TPC, TFC, and antioxidant capacity were compared to choose the best extraction solvent. Our results showed us that phenolic and flavonoid content was highest in ethanol extraction of the ivy (Figure 1).

The antioxidant activity of the extracts was analyzed by DPPH assay which is commonly used to assess the ability of antioxidants to scavenge free radicals. The disappearance of the DPPH absorption reflects the antioxidant's ability to react with the radical. Therefore, a higher reduction in the DPPH absorption indicates a stronger antioxidant effect. Our DPPH assay results showed that higher antioxidant activity belonged to the ethanol extract of the ivy that was also compatible with the extracts' phenolic and flavonoid contents (Figure 1).

Cytotoxicity of *H. helix* extract on glioblastoma cells and apoptosis

Cytotoxicity analysis of *H. helix* ethanol extract was carried out using the ATP cell viability method. ATP assay, based on the measurement of ATP levels in cells, is a good indicator of cell viability and a widely used method for evaluating cytotoxicity. The viability test demonstrated that the *H. helix* extract had a dose-dependent cytotoxic effect, and the IC_{50} was approximately 475 µg / ml (Figure 2).

Apoptosis-inducing effect of the extract was studied using the AO/EB double staining method. This method is a frequently used technique to analyze apoptosis since it is fast, cheap, and easy to perform. The AO/EB assay results indicated that the ethanol extract of *H. helix* leaves induced apoptosis in glioblastoma cells at doses approximately equal to or below the IC_{50}. Furthermore, the level of apoptosis increased with higher concentrations of the extract (Figure 3).

ROS Generating effect of *H. helix*

ROS are molecules that are naturally produced within cells during metabolic processes. While ROS plays important roles in various cellular functions, including signaling and regulation of gene expression, high levels of ROS can cause cellular damage, leading to cell death. In the current study, we also investigated the connection between *H. helix* and ROS generation. DCFH-DA results showed that high doses of *H. helix* increased intracellular ROS levels in glioblastoma cells and exhibited pro-oxidant activity even though the plant has antioxidant properties with lower doses (Figure 4).

DISCUSSION

Glioblastoma is a highly aggressive and malignant type of brain tumor that originates from the astrocytes, which are supportive cells in the brain. It is the most common and deadliest form of primary brain tumor [22]. Due to the aggressive nature of glioblastoma, there is ongoing research aimed at developing new treatments for the disease, including immunotherapy, targeted therapies, and gene therapy [23, 24].

Over the past few decades, there has been an exponential increase in the field of herbal medicine, and it is becoming increasingly popular

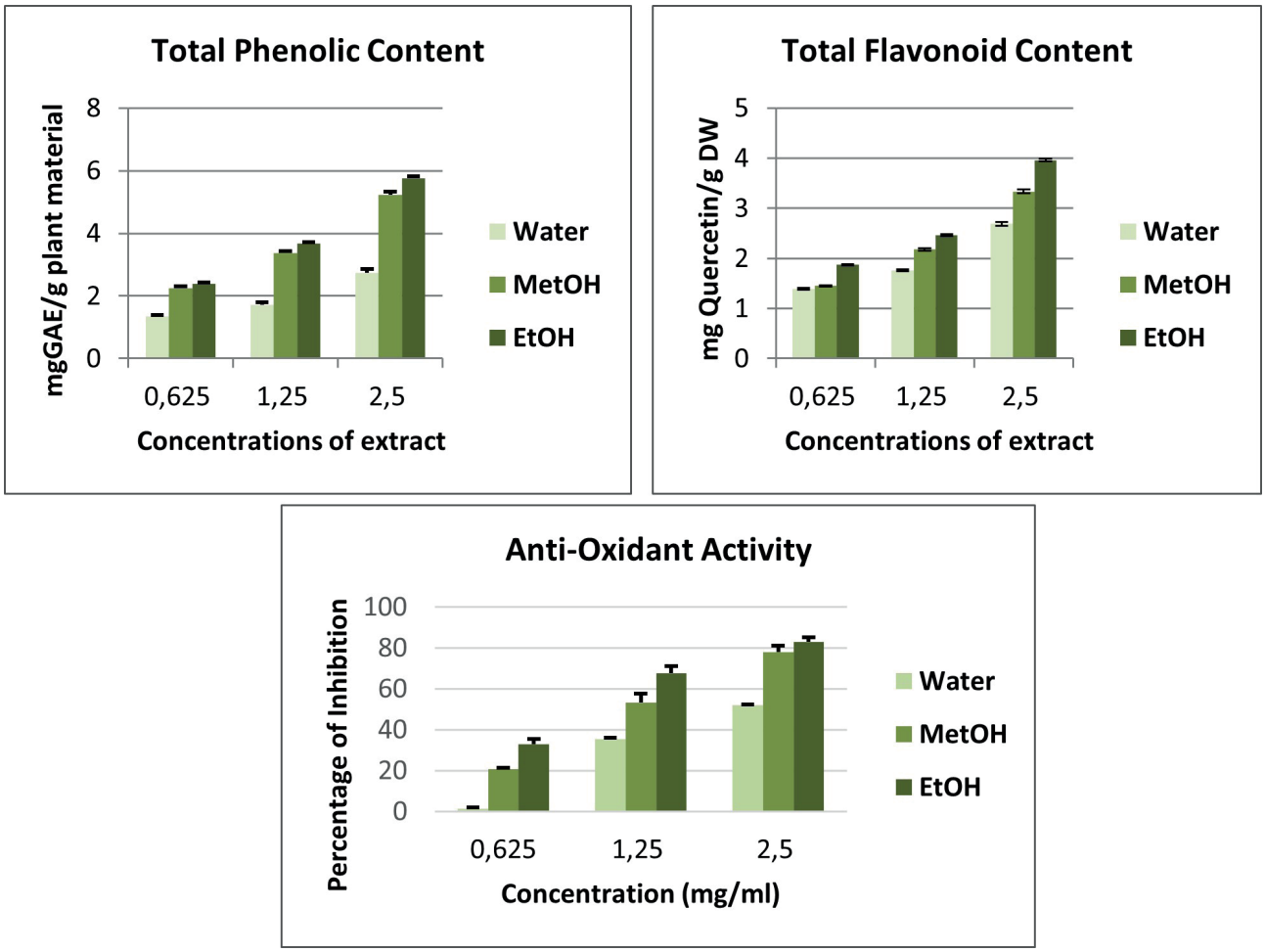

Figure 1. Comparison of phenol, flavonoid, and anti-oxidant analysis of ethanol, methanol, and water extracts showed that ethanol was the best solvent for extraction of *H. helix*.

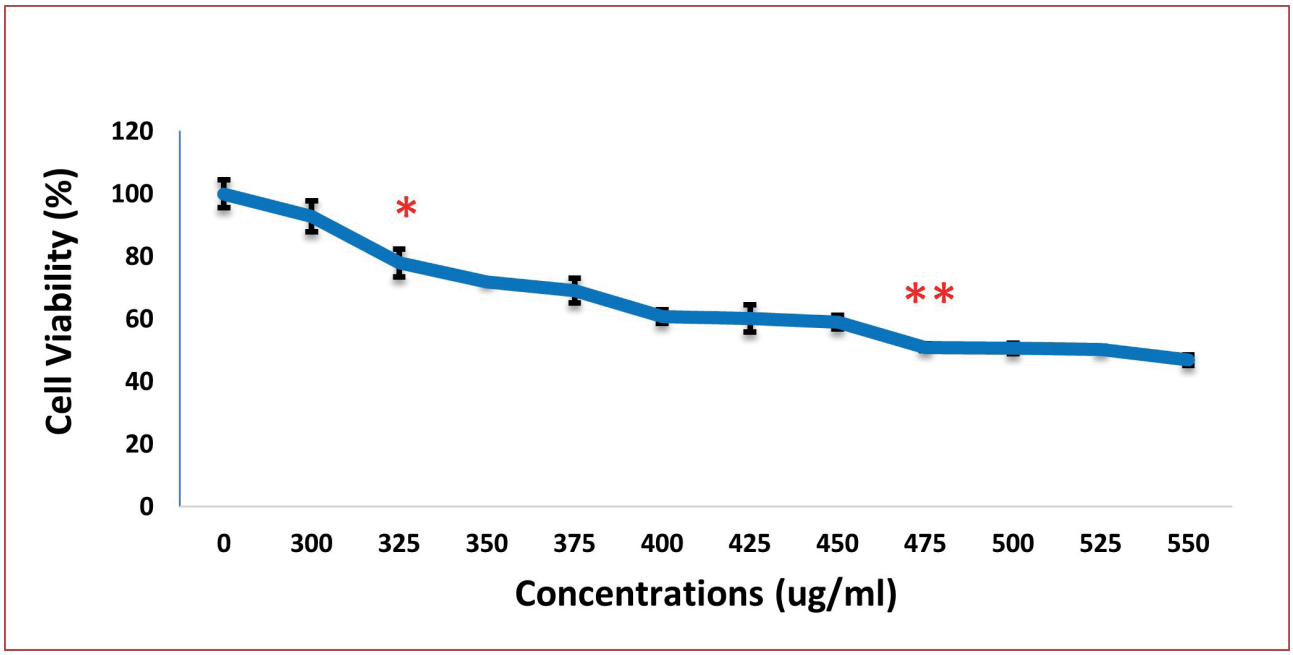

Figure 2. Viability test results of *H. helix* ethanol extract on glioblastoma cancer cells. The difference in cytotoxicity efficacy compared to the control was statistically significant from 325 µg/mL. Data are presented as mean ± SD. The significant difference compared to the control is indicated by *$p < 0.05$ and ** $p < 0.01$.

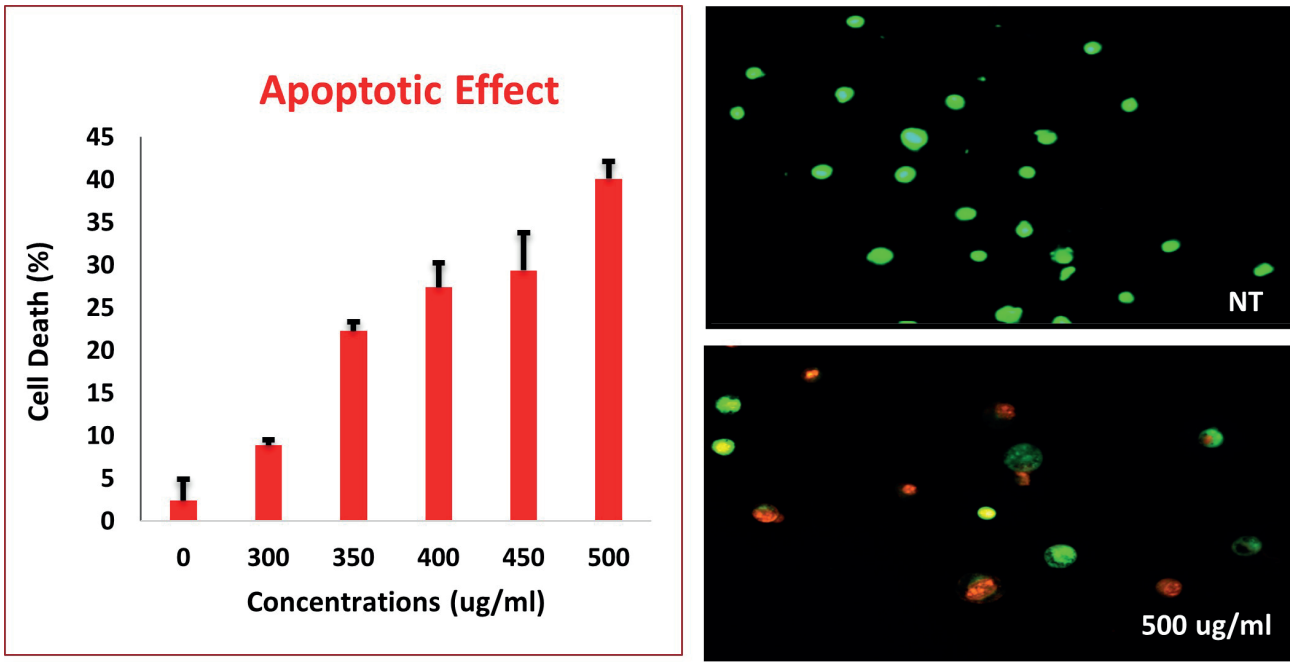

Figure 3. The cell death comparison of different concentrations of *H. helix* extract on glioblastoma cells was analyzed using AO/EB method. Dead cells (red) and live cells (green) were examined by fluorescence microscopy.

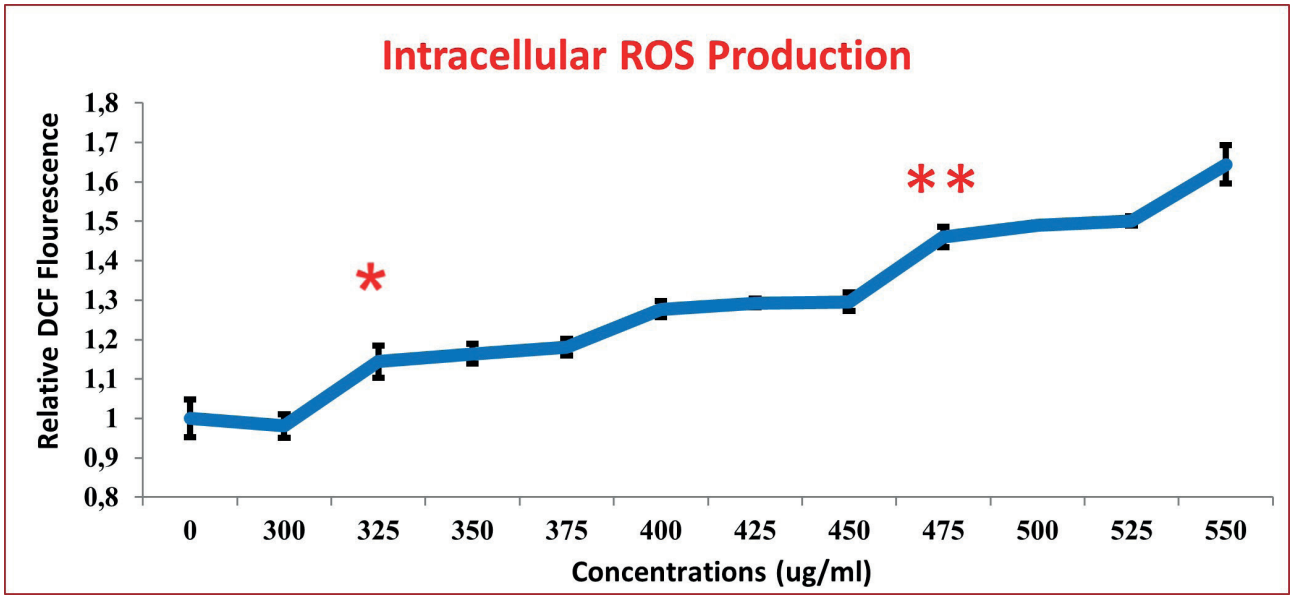

Figure 4. ROS generating effect of *H. helix* ethanol extract in glioblastoma cells. Extract started to increase ROS generation from the doses of 325 µg/ml. Results are presented as mean ± SD. The significant difference compared to the control is indicated by *p < 0.05 and ** p < 0.01.

in both developing and developed countries due to its natural origin and fewer reported side effects [25]. H. helix, commonly known as ivy leaves, is a plant that contains a diverse range of metabolites and has been traditionally used for treating the common cold, cough, and bronchial disorders [26]. In addition, ivy leaves have been explored as a potential alternative medicine for managing rheumatoid arthritis [26]. Although there are many studies on the therapeutic effects of *H. helix*, the best effect has been shown for reducing the frequency and intensity of cough [27]. Rai demonstrated the anti-inflammatory properties of ivy leaves, where intraperitoneal injections of 7.5 ml/kg of ethanol extract resulted in 88.89% inhibition of formalin-induced paw edema in Swiss Albino mice. The anti-inflammatory activity of the ivy leaf extract was comparable to that of the reference drug diclofenac, which exhibited 94.44% inhibition [11]. In another study, the *H. helix* extract inhibited IL-6 and IL-8 secretion and blocked the VEGF pro-angiogenic factor after inflammation

was induced by lipopolysaccharide (LPS) to mimic the bacterial infection and TNF-α for the acute phase systemic inflammation [28]. According to a recent article by Shokry et al. (2022), extracts of ivy leaves may be a promising option for treating rheumatoid arthritis. The researchers proposed that the flavonoid content in ivy leaves may be responsible for their potential therapeutic effects. The study found that the ivy leaf extract was able to suppress biochemical, oxidative, and pathological changes associated with Adjuvant-induced arthritis [29]. Roşca-Casian also reported the antifungal activity of *H. helix* leaf ethanolic extract against phytopathogenic fungi [30].

In the current study, we primarily extracted *H. helix* leaves in three solvents; ethanol, methanol, and water, then compared these extracts for phenolic and flavonoid contents besides antioxidant activity since a positive correlation was shown between antioxidant effects, polyphenolic and flavonoid contents for *H. helix* ethanolic extract [12]. Our results showed that the ethanol and methanol extracts gave higher results for phenol and flavonoid contents together with antioxidant activity compared to the aqueous extract, and ethanol was the best among them. These results were also compatible with the literature that the hydro-alcoholic extract of *H. helix* showed better in vitro activity against adult parasites compared to the aqueous extract [13].

In literature, *H. helix* was also investigated for its antitumor effects besides all its pharmacological and therapeutic activities. *H. helix* ethanolic leaf extract was shown to suppress the migration of Mat-LyLu cells (strongly metastatic rat prostate cancer cells) together with proliferation and mitotic activity [15]. Rehman et al also showed that *H. helix* leaf extracts decrease cell viability on HT-29 colon cancer cells [14]. The previous studies revealed a lack of research on the potential anti-tumor effects of *H. helix* leaf extract on glioblastoma cells. The antitumor effects of Hederagenin, a triterpenoid acid abundant in ivy leaves, on glioma were first investigated by Dai et al., who observed that Hederagenin inhibited cell proliferation, invasion, and migration in U87 and U251 glioma cell lines [31]. In our current study, we investigated the effects of ethanolic extract of ivy leaves on U87 cells for the first time. Our results showed that the extract decreased cell viability and induced apoptosis.

Although *H. helix* has antioxidant phenolic compounds [8], it shows pro-oxidant activity and generates ROS due to the Fenton reaction in the presence of transition metals such as iron and copper [32]. While low levels of ROS play important roles in various cellular functions including signaling and regulation of gene expression [33], elevated levels of ROS can cause cellular damage, leading to cell death [34]. Excessive ROS can lead to a state of oxidative stress within cells, which can damage cellular components such as lipids, proteins, and DNA. This damage can trigger several mechanisms of cell death, including apoptosis, necrosis, and autophagy [35-37]. In this study, it was observed that the extract of *H. helix* increased intracellular ROS generation in U87 cells in a dose-dependent manner. This finding is consistent with previous literature, which demonstrated that α-Hederin, a monodesmosidic triterpenoid saponin isolated from Hedera helix, induces apoptosis and autophagic cell death in gastric [38] and colorectal cancers [39] through generating ROS.

To the best of our knowledge, this study is the first to investigate the effects of *H. helix* leaf ethanol extract on cytotoxicity and apoptosis in glioblastoma (U87) cells and to explore the relationship between these effects and ROS production. Its cytotoxic and apoptotic effect on glioblastoma cells may be due to its ROS-generating effect in a dose-dependent manner. While this study is intriguing and sheds light on the potential anti-cancer properties of *H. helix* on glioblastoma, this study focused on the U87 glioblastoma cell line, which represents only one type of cancer cell line. In addition, glial cells have not been examined for the effects of *H. helix* on normal cells. Further research is needed to address these limitations to show the potential anti-cancer effects of Hedera helix ethanol extract on glioblastoma.

CONCLUSION

According to the results obtained, Hedera helix ethanol extract was found to have dose-dependent cytotoxic and apoptotic effects in the U87 glioblastoma cell line. It has been concluded that high doses of *H. helix*, an antioxidant plant, may exhibit anti-cancer effects through pro-oxidant activity.

Author contribution

Study conception and design: AK; data collection: VBY, ED, FK; analysis and interpretation of results: AK and VBY; draft manuscript preparation: VBY and EK. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

This study does not require ethical approval since it does not contain any studies with human or animal subjects.

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Conflict of interest

The authors declare that there is no conflict of interest.

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acta medica

ORIGINAL ARTICLE

Thirty years in anesthesiology: A bibliometric analysis*

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ABSTRACT

Background: The number of publications on anesthesiology continues to increase. Although there are studies conducted about this increase, they are insufficient to show the direction and structure of the changes. This study aimed to investigate the conceptual structure and thematic evolution in publications about anesthesiology over the last 30 years.

Methods: In this study we made a systematic reviews and bibliometric analyses of observational and Randomized Controlled Trial (RCT) studies. Bibliometric analysis was used to reveal thematic clusters by associating keywords and relationship patterns in three periods. Thematic clusters were revealed using a science mapping analysis based on co-word networks and strategic diagrams created according to centrality. Density values were used to determine the positions of the clusters. A total of 119,842 articles published between 1990 and 2022 in the anesthesiology category of the Web of Science (WOS) Core Collection were analyzed. Articles published before 1990 and/or not in the WOS Collection were excluded from the study.

Results: Seven thematic areas were identified over three time periods: Pain, Medications, Regional Anesthesia, Cardiovascular Anesthesia, Pediatric Anesthesia, Critical Care, and Airway Management. Over the years, motor themes have been shaped by new anesthetic agents and techniques. Pain and Regional Anesthesia have been the most researched topics. Patient Safety, Elderly Patients, and Palliative Care were the emerging themes in the last years. Critical Care thematic area moved to Mortality, Mechanical Ventilation, and COVID-19.

Conclusion: Pain is the most popular topic over the last 30 years. Increasing of themes like Patient Safety and Palliative Care in the last years may be related to the increasing quality management awareness and the older population. The fact that the COVID-19 thematic area has a large place in last period shows the effect of the pandemic in the field of anesthesiology.

Keywords Anesthesia, bibliometrics, science mapping.

INTRODUCTION

Since the first written document on anesthesiology in 1847 [1], the number of publications on anesthesiology has increased. Currently, there are more than 250,000 scientific studies on this topic in the literature. Along with technological developments in medicine and medical engineering, the agendas and interests of anesthesiologists have constantly changed over the last 50 years. Although various studies have shown this change in anesthesiology literature, the number and content of these changes remain insufficient to show the direction and structure of these changes. It is possible to trace the transformation of conceptual and thematic flows in the literature using bibliometric analysis.

Bibliometrics is the application of mathematical and statistical methods in scientific communication environments [2]. It is an important tool for the discovery of tacit knowledge among scientific publications and offers methods and metrics for explaining the complex structure of scientific communication [3]. Bibliometrics examine different bibliographic elements, such as keywords [4,5], citations [6,7], authors, and institutions of publications [8-10] in a scope that differs according to the purpose of the studies [11]. One of the main approaches in bibliometric literature addresses the field of science [12].

This study aimed to understand the conceptual structure and thematic evolution of anesthesiology over the last 30 years through detailed bibliometric techniques. We hope to guide readers on their scientific paths by showing how trends and topics change in anesthesiology.

MATERIALS AND METHODS

The type of this study is a bibliometric data analysis. Hence, no ethical approval or waiver document was required. The methodology of this study was based on two basic approaches to bibliometrics: performance analysis and science mapping [13-15]. A performance analysis was conducted to measure the contribution and impact of the analysis elements in the research field, and science mapping was performed to reveal the cognitive structure of the research field and determine research themes based on data visualization [14]. Both methods were adopted in our study to examine the conceptual structure and thematic evolution of research themes in the anesthesiology field. The workflow of this study is illustrated in Figure 1.

Data

In the first stage, it was necessary to retrieve publications from the anesthesiology field. Academic publications are the primary source of data for bibliometric studies. For this reason, bibliographic databases were used as data sources in the bibliometric analysis. Currently, there are many bibliographic databases in which academic journals are systematically indexed. We used Web of Science (WoS) in our study, the main reason for this is that the anesthesiology research field is defined categorically in WoS. Keywords are one of the most significant tools for the presentation of academic publications in the digital environment [16]. Efficient use of keywords is one of the main tools for readers to access articles. Keywords are determined by the author of the article to include the outline, subject, interesting aspects and sub-

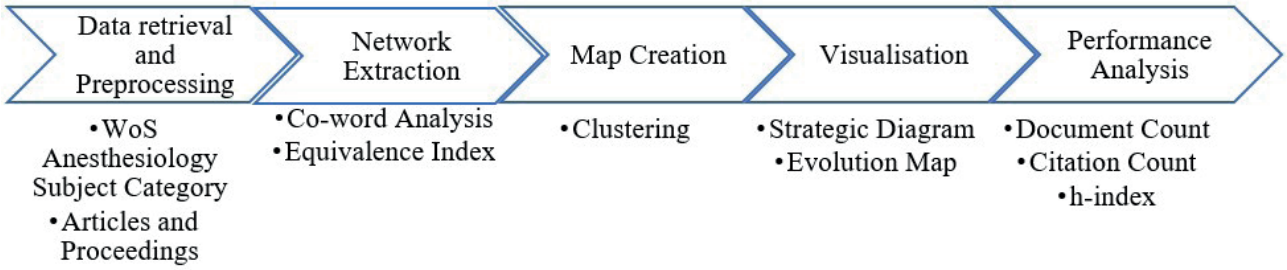


Figure 1. Analysis workflow.

fields of the article [17]. In this respect, it has the aspect of representing an article in its most concentrated form.

Bibliometric analyses were conducted using the keywords of publications in the anesthesiology research field. To analyze publications using keywords, the search was limited to two main publication types: articles and proceedings. Articles and proceedings are main academic products. While preliminary findings of the on-going researches and contemporary academic activities of the field are discussed through proceedings at the conferences, a more comprehensive and long term production period is in question with articles [18]. Both types of studies are at the center of academic communication in terms of representing academic performance in a field [19-21]. Therefore, these two publication types are frequently used in bibliometric studies examining the performance in a field [20,22,23].

Another limitation is the year range. The starting point was taken as 1990, when keywords were regularly indexed in publications.

Bibliometric analyses are conducted by examining academic publications in a certain period. In this context, the choice of periodization can be shaped according to the purpose of the study. There are studies to monitor the reflection of certain events affecting a field in the literature [24,25] and studies that monitor the transformation of a field in the process in periods [26,27]. In our study, since we wanted to follow the conceptual structure and thematic evolution of anesthesiology over the scientific publications, 30 years of academic production was considered in 3 periods of 10 years each.

Analyses were performed for three periods to observe the periodic evolution:1990-1999, 2000-2009, and 2010-2021. Data preprocessing is the most important step in bibliometrics to obtain accurate results. Therefore, it is necessary to combine the same concepts and eliminate typographic errors in analysis elements. Data preprocessing was conducted by field experts using distance algorithms [28].For this purpose, 80252 keywords were reviewed by experts. A systematic approach was followed at this stage and firstly similar keywords were brought together with distance algorithms. Then, by using a thesaurus approach the keywords were viewed as broader and narrower terms [29] and only similar keywords were brought together to maintain the balance between levels and to maintain this level for different terms [30]. The SciMAT [28], bibliometric analysis tool and Openrefine [31] data cleaning tool were used to prepare the data and perform the analyses.

Methodology

In our study, keywords were used as analysis items. Co-word analysis was used to reveal thematic clusters in the field. Co-word analysis is a method for exploring topics in a related scientific field by matching keywords that are frequently used together. The co-word analysis technique uses the association of keywords according to their co-occurrence in a publication to identify relationship patterns among study subjects [4,32].

In this method, all keywords in a set of publications are matched, and keywords that are used more frequently with each other, form certain densities within this relationship network. These densities are used to identify the thematic clusters studied in the field.

After the links between the analysis elements are established, the links within the created network must be normalized to derive similarities [33]. Normalization is a process of arranging data to establish stable data structures, reduce data anomalies, and construct a consistent data framework. [34,35]. In co-word analysis, keyword links are weighted by normalization according to their co-occurrence in the publications. In our study, normalization was performed with the equivalence index, which is proportional to the ratio between the co-occurrence of two keywords in the same document and the number of documents in which these keywords appear [4,13,32,33,36]. After the normalization of links between keywords, intensely related concepts emerged as research themes in the field (Figure 2-a).

After the thematic densities emerge in the keyword network created for the co-word analysis, the clustering and visualization of these themes is started. Thematic clusters are formed by bringing together the keywords that are most frequently used together. Each thematic cluster consists of the keywords that are most frequently used together in the keyword network. The representation of the clusters is provided by the most central keyword within this thematic density (Figure 2-a).

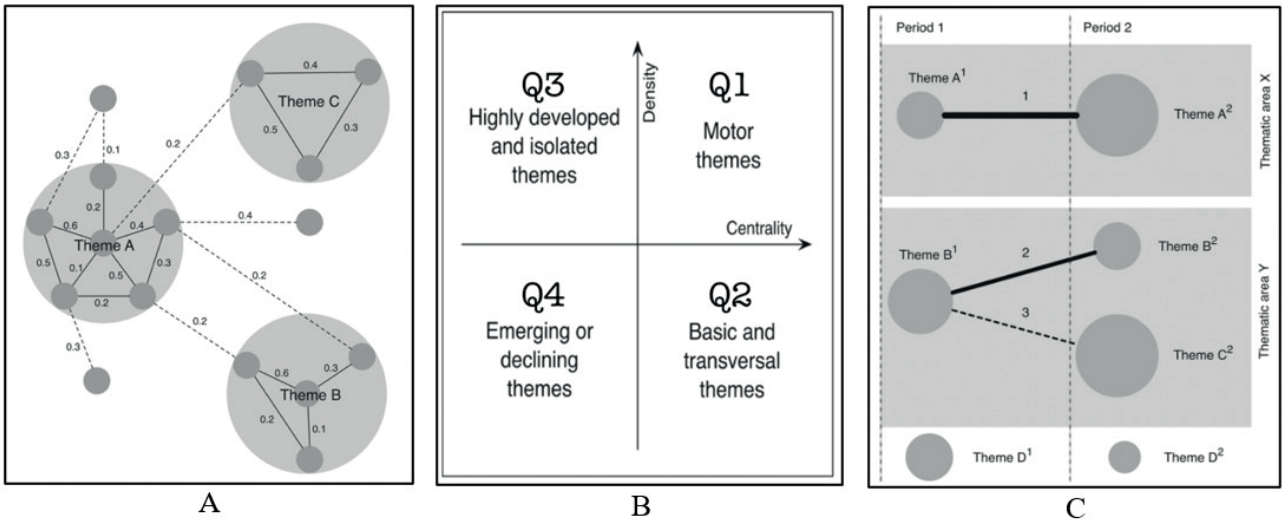


Figure 2. A) Clusters, B) Strategic diagram, C) Evolution map.

Strategic diagrams and an evolution map were used to determine the positions of the thematic clusters in the research field. A strategic diagram is a two-dimensional map with centrality on the x axis and density values on the y axis. The map was obtained by calculating the centrality and density values of the thematic clusters determined over the keywords using co-word analysis and placing them in this coordinate system. The two main measures calculated after identifying thematic clusters within the keyword network are centrality and density. The centrality value indicates the position of a cluster in the research field based on the strength of its communication with other clusters. Conversely, the density value indicates how well the links of keywords in the cluster are developed [28,32]. A cluster with a high centrality value has more network relationships with all other clusters. Density indicates the relational density of keywords within these clusters. A cluster with a high-density value indicates that the keywords within it are used much more frequently with each other.

The centrality value was calculated according to the strength of the links of a given cluster with other clusters, and the density value was calculated based on the strength of the links among the keywords within the cluster [28, 31, 32]. Strategic diagrams were used to determine the positions of thematic clusters within the research field. Strategic diagrams have quadrants that allow clusters to be interpreted differently according to their centrality and density values. An example strategic diagram is shown in Figure 2-b.

Clusters in the upper-right quadrant (Q1) of the strategic diagram have both high-density and high-centrality values. These clusters have intensely developed inner links and strong communications with other clusters. The clusters in the upper left quadrant (Q3) show clusters that are highly developed but relatively isolated. Although these clusters had highly developed inner links between keywords, they had relatively weak communications with other clusters. The clusters in the lower-right quadrant (Q2) show the basic clusters in areas with low and high centrality values. The bottom left quadrant (Q4) had both low and low-density values. The clusters in this quadrant indicate emerging or declining research areas [13,28,32,37].

After analyzing the thematic clusters in the research field, an evolution map was used to show the periodic development and transformation of these thematic clusters (Figure 2-c). The evolution map consisted of columns with thematic clusters. Each column represents the analysis period. The thematic research areas in anesthesiology were monitored, with connections established between thematic clusters over time. These links indicate that the clusters share common keywords. Solid lines indicate that the connected clusters share one of their central keywords, and dotted lines indicate connected cluster shares other than central keywords. In addition, the size of the lines was proportional to the number of common keywords in the two clusters. Monitoring the connections between clusters allows us to understand the periodic conceptual transformation and communication in different clusters.

At all stages of the study, thematic clusters were evaluated using performance values calculated over the number of publications, citations, and Hirsch index (h-index) [38,39]. Two values are used to indicate the number of publications in the performance analysis. The first value is the number of core publications, which represents the number of publications with at least two keywords in the cluster. The values in parentheses indicate all publications with at least one keyword in the cluster. Performance analysis enables quantitative evaluation and comparison of the effectiveness of thematic clusters in the research field.

RESULTS

Bibliometric analyses were performed on 119,842 articles and proceedings papers in the anesthesiology category of the WoS Core Collection. Three strategic diagrams (Figure 3-5) were created using SciMAT to analyze the notable themes in each sub-period. In these representations, the sphere volume was proportional to the number of documents associated with each theme. The effect of these themes in each subperiod is shown in Tables (Table 1-3) containing information on performance values (number of documents, h-index, and number of citations).

First Period (1990-1999)

The results for this period are shown in the strategic diagram in Figure 3, and the performance indicators in Table 1. During Period 1, 22 themes with 29,208 publications were identified. Among the themes with 150 or more publications, which can be considered as the most productive themes according to performance indicators, the motor themes in the first quadrant are "MORPHINE," "BUPIVACAINE," "ANAESTHETICS-LOCAL," "VECURONIUM," "NEUROPATHIC-PAIN," and "EQUIPMENT," and the basic and transversal themes in the second quadrant were "PROPOFOL," "HALOTHANE" and also "INTUBATION" and "CHRONIC-PAIN" themes from the fourth quadrant, which were placed in emerging or declining themes. Considering the performance indicators, the most significant themes in the first period were "NEUROPATHIC-PAIN," "CHRONIC-PAIN," "MORPHINE," "BUPIVACAINE," "PROPOFOL," and "SYMPATHETIC-NERVOUS-SYSTEM."

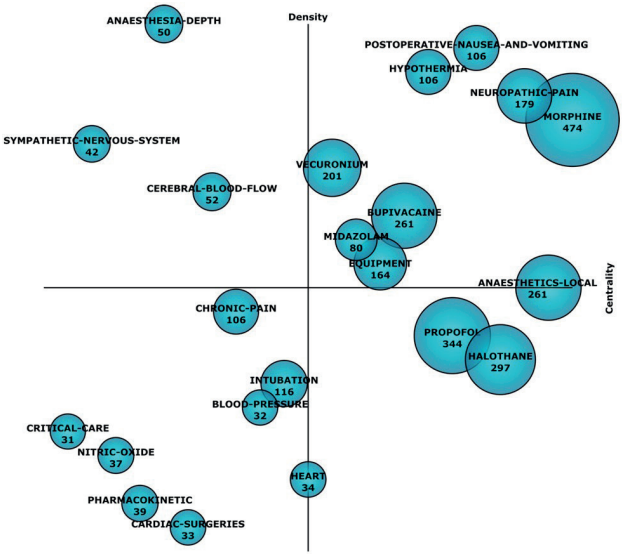


Figure 3. Period 1 (1990-1999).

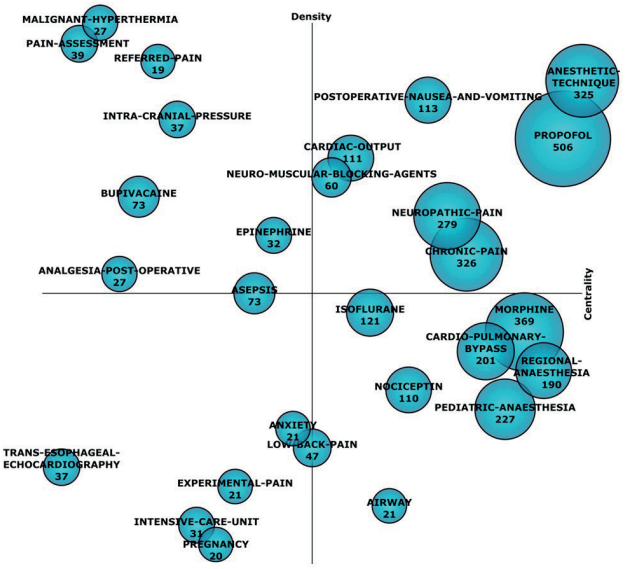


Figure 4. Period 2 (2000-2009).

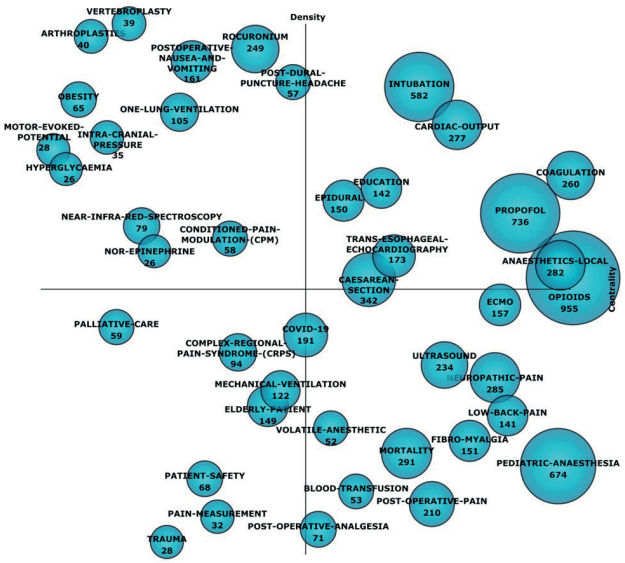


Figure 5. Period 3 (2010-2021).

Table 1. Themes Performance for the 1990-1999 Period

Themes	Quadrant	Core Documents (All Documents)	h-index	Average Citations
Morphine	Q1	474 (1761)	69	42,61
Propofol	Q2	344 (1713)	52	31,94
Halothane	Q2	297 (1703)	48	28,58
Bupivacaine	Q1	261 (1211)	56	40,81
Anesthetics-local	Q1	261 (1267)	42	27,77
Vecuronium	Q1	201 (648)	26	16,07
Neuropathic pain	Q1	179 (695)	74	107,53
Equipment	Q1	164 (1133)	33	22,82
Intubation	Q4	116 (604)	35	32,94
Chronic pain	Q4	106 (473)	57	108,45
Hypothermia	Q1	106 (678)	34	36,88
Postoperative-nausea-and-vomiting	Q1	106 (503)	32	30,75
Midazolam	Q1	80 (546)	25	22,98
Cerebral-blood-flow	Q3	52 (241)	20	23,77
Anesthesia-depth	Q3	50 (280)	24	38,88
Sympathetic-nervous-system	Q3	42 (267)	22	40,95
Pharmacokinetic	Q4	39 (340)	19	31,87
Nitric oxide	Q4	37 (284)	14	19,41
Heart	Q4	34 (283)	14	15,21
Cardiac surgeries	Q4	33 (327)	14	19,18
Blood-pressure	Q4	32 (227)	16	26,03
Critical care	Q4	31 (329)	4	1,71

Q1: Motor themes; Q2: Basic and transversal themes; Q3: Highly developed and isolated themes; Q4: Emerging or declining themes

The motor theme "MORPHINE" and the basic and transversal theme "PROPOFOL" had high publication counts. The theme "MORPHINE" focused on analgesia and pain management in the postoperative period or in cancer patients. The theme "PROPOFOL" was related to intravenous anesthetic techniques and the induction of general anesthesia. The basic and transversal theme "HALOTHANE" with the motor theme "BUPIVACAINE" and "ANESTHETICS-LOCAL" presented similar publication values, considered as highlighted themes. The theme "HALOTHANE" was related to other volatile anesthetics, such as malignant hyperthermia as a complication, and pediatric anesthesia. The theme "BUPIVACAINE" was composed of documents that enhanced the importance of obstetric surgeries under spinal and epidural anesthesia and post-operative pain.

The emerging theme "CHRONIC-PAIN" and motor theme "NEUROPATHIC-PAIN" had moderate publication counts, but the highest number of citations. "CHRONIC PAIN" is related to psychological (anxiety and depression) and socioeconomic (sex and social classes) characteristics. The "NEUROPATHIC-PAIN" theme had the highest h-index in this period related to experimental animal studies and hyperalgesia.

Second Period (2000-2009)

The results for this period are shown in the strategic diagram in Figure 4, and the performance indicators in Table 2. During Period 2, 28 themes were identified among 35,566 publications. The most productive themes according to performance indicators for which the themes had 150 or more core documents, were from the first quadrant "PROPOFOL," "CHRONIC-PAIN," "ANAESTHETIC-TECHNIQUE," and "NEUROPATHIC-PAIN," and from the second quadrant "MORPHINE," "PEDIATRIC-ANAESTHESIA," "CARDIO-PULMONARY-BYPASS." Considering the performance indicators, the most significant themes in the second period were "CHRONIC-PAIN," NEUROPATHIC-PAIN, "MORPHINE," NOCICEPTIN, "PROPOFOL," and "LOW-BACK-PAIN."

The theme "PROPOFOL," which became a motor theme during this period, had the highest number of publications. Studies on propofol have focused on target-controlled anesthesia techniques, anesthesia depth, and the comparison of propofol

Table 2. Themes Performance for the 2000-2009 Period

Themes	Quadrant	Core Documents (All Documents)	h-index	Average Citations
Propofol	Q1	506 (1793)	47	21,96
Morphine	Q2	369 (2363)	66	46,97
Chronic pain	Q1	326 (1511)	90	78,47
Anesthetic-technique	Q1	325 (1765)	48	28,39
Neuropathic pain	Q1	279 (1106)	76	67,49
Pediatric anesthesia	Q2	227 (1950)	42	25,40
Cardio-pulmonary-bypass	Q2	201 (1132)	28	18,36
Regional anesthesia	Q2	190 (1425)	35	24,14
Isoflurane	Q2	121 (851)	26	17,00
Postoperative-nausea-and-vomiting	Q1	113 (734)	24	19,33
Cardiac output	Q1	111 (733)	33	26,52
Nociceptin	Q2	110 (727)	47	55,86
Asepsis	Q3	73 (671)	25	21,53
Bupivacaine	Q3	73 (387)	26	21,85
Neuro-muscular-blocking-agents	Q1	60 (298)	17	15,50
Low-back-pain	Q4	47 (556)	32	81,91
Pain, -assessment	Q3	39 (247)	26	74,77
Trans-esophageal echocardiography	Q4	37 (369)	11	7,30
Intra-cranial pressure	Q3	37 (179)	13	14,70
Epinephrine	Q3	32 (322)	15	18,97
Intensive-care-unit	Q4	31 (404)	11	13,97
Malignant hyperthermia	Q3	27 (137)	7	7,41
Analgesia, -post-operative	Q3	27 (202)	20	33,19
Experimental pain	Q4	21 (252)	18	82,24
Airway	Q2	21 (277)	10	23,33
Anxiety	Q4	21 (228)	19	76,00
Pregnancy	Q4	20 (263)	10	14,50
Referred pain	Q3	19 (146)	15	46,74

Q1: Motor themes; Q2: Basic and transversal themes; Q3: Highly developed and isolated themes; Q4: Emerging or declining themes

with volatile anesthetics. "MORPHINE," which turned into a basic and transversal theme in this period, and the motor themes "ANAESTHETIC-TECHNIQUE" and "CHRONIC-PAIN" were highlighted themes with similar high publication counts. The "MORPHINE" theme was mostly related to postoperative pain and analgesia. However, the most cited articles from morphine-themed publications of this period were about the adverse effects of opioids, such as M Ricardo Buenaventura, M Rajive Adlaka and M Nalini Sehgal [40]. The "ANAESTHETIC-TECHNIQUE" theme focused on complications, central neuraxial blocks, and local anesthetics. The most cited articles on the theme of "ANAESTHETIC-TECHNIQUE" concerned the use of ultrasound in nerve blocks [41,42], complications, and local anesthetics [43,44]. The other highlighted theme in this period, "CHRONIC-PAIN," had the highest h-index. During this period, the "CHRONIC-PAIN" theme was related to the management of opioid treatment and psychological factors of patients. It was remarkable that "LOW-BACK-PAIN" and "EXPERIMENTAL-PAIN" themes, which were in the emerging and declining themes quadrant, had the highest average citation numbers, although the number of publications was low. Another pain-related theme, "NEUROPATHIC-PAIN," had the second highest h-index value during this period. The motor theme "NEUROPATHIC-PAIN" is related to sensory testing, hyperalgesia treatment, and complex regional pain syndrome.

Third Period (2010-2021)

The results for this period are shown in the strategic diagram in Figure 5, and the performance indicators in Table 3. During Period 3, 42 themes in the field, with 54,469 publications, were identified. Among

Table 3. Themes Performance for the 2010-2021 Period

Themes	Quadrant	Core Documents (All Documents)	h-index	Average Citations
Opioids	Q1	955 (5361)	53	13,85
Propofol	Q1	736 (2740)	30	6,93
Pediatric anesthesia	Q2	674 (4471)	33	8,21
Intubation	Q1	581 (1718)	35	9,82
Caesarean-section	Q1	342 (1561)	18	5,09
Mortality	Q2	291 (2104)	25	9,47
Neuropathic pain	Q2	285 (1499)	42	20,82
Anesthetics-local	Q1	282 (1699)	31	12,47
Cardiac output	Q1	277 (1232)	27	9,82
Coagulation	Q1	260 (1344)	26	11,45
Rocuronium	Q3	249 (596)	22	7,82
Ultrasound	Q2	234 (1359)	15	4,67
Post-operative-pain	Q2	210 (1788)	24	9,67
COVID-19	Q4	191 (1599)	17	7,61
Trans-esophageal echocardiography	Q1	173 (1521)	13	3,94
Postoperative-nausea-and-vomiting	Q3	161 (866)	17	7,76
ECMO	Q2	157 (988)	15	5,15
Fibromyalgia	Q2	151 (1343)	28	21,56
Epidural	Q1	150 (875)	19	11,29
Elderly-patient	Q4	149 (1308)	23	12,21
Education	Q1	142 (1563)	14	5,7
Low-back-pain	Q2	141 (976)	25	15,84
Mechanical ventilation	Q4	122 (1074)	13	4,85
One-lung-ventilation	Q3	105 (488)	12	5,17
Complex-regional-pain-syndrome	Q4	94 (865)	26	24,38
Near-infra-red-spectroscopy	Q3	79 (441)	11	8,33
Post-operative-analgesia	Q2	71 (914)	11	7,54
Patient-safety	Q4	68 (765)	11	9,84
Obesity	Q3	65 (308)	12	6,92
Palliative-care	Q4	59 (616)	11	7,98
Conditioned-pain-modulation	Q3	58 (469)	20	23,88
Post-dural-puncture-headache	Q3	57 (458)	10	5,18
Blood-transfusion	Q2	53 (636)	11	15,45
Volatile anesthetic	Q2	52 (537)	15	11,46
Arthroplasties	Q3	40 (119)	12	19,4
Vertebroplasty	Q3	39 (147)	11	8,54
Intra-cranial pressure	Q3	35 (226)	9	6,69
Pain-measurement	Q4	32 (544)	10	15,91
Motor-evoked-potential	Q3	28 (205)	6	2,89
Trauma	Q4	28 (417)	7	5,54
Hyperglycemia	Q3	26 (192)	6	5,27
Nor-epinephrine	Q3	26 (336)	7	5,96

Q1: Motor themes; Q2: Basic and transversal themes; Q3: Highly developed and isolated themes; Q4: Emerging or declining themes

the themes with 150 or more core publications according to the performance indicators, the motor themes in the first quadrant were "OPIOIDS," "PROPOFOL," "INTUBATION," "CAESAREAN-SECTION," "ANAESTHETICS-LOCAL," "CARDIAC-OUTPUT," "COAGULATION," "TRANS-ESOPHAGEAL-ECHOCARDIOGRAPHY," and "EPIDURAL," the second and third quadrants were "PEDIATRIC-ANAESTHESIA," "MORTALITY," "NEUROPATHIC-PAIN," "ROCURONIUM,""ULTRASOUND,""POST-OPERATIVE-PAIN," "POSTOPERATIVE-NAUSEA-AND-VOMITING," "ECMO," and "FIBRO-MYALGIA," and finally, the "COVID-19" theme from the fourth quadrant, which was in the emerging or declining themes. Although the third period's values were lower compared with other periods due to citation lag, significant themes in the third period with h-indexes and average citation values were "OPIOIDS," "NEUROPATHIC-PAIN," "INTUBATION," "PEDIATRIC-ANAESTHESIA," "PROPOFOL," "ANAESTHETICS-LOCAL," and "FIBRO-MYALGIA."

The motor theme "OPIOIDS" had the best performance results, with the highest publication counts and h-indexes. The "OPIOIDS" theme was related to chronic pain, analgesia, and the adverse effects of opioids. The basic and transversal theme "PEDIATRIC-ANAESTHESIA" and motor themes "PROPOFOL" and "INTUBATION" were the highlighted themes after opioids. They had similar h-index and average citation numbers. The theme of "PEDIATRIC ANESTHESIA" has been studied in many different subjects. Some of the most frequently mentioned were general anesthesia, complications, premedication, postoperative delirium, and pain management. The "PROPOFOL" theme was related to anesthesia depth, sedation, and other sedatives such as dexmedetomidine and ketamine. The other motor theme, "INTUBATION," was related to airway management, air management devices (e.g., laryngoscope and video laryngoscope), and difficult intubation. The most cited article on the "INTUBATION" theme after C Frerk, VS Mitchell, AF McNarry, C Mendonca, R Bhagrath, A Patel, EP O'Sullivan, NM Woodall and I Ahmad [45] guideline for the management of unanticipated difficult intubation in adults was the T Cook, K El-Boghdadly, B McGuire, A McNarry, A Patel and A Higgs [46] consensus guideline for airway management in patients with COVID-19.

Although it had a lower publication count than "PROPOFOL" and "PEDIATRIC-ANAESTHESIA" themes, the basic and transversal theme "NEUROPATHIC-PAIN" had the higher number of average citations and h-index. During this period, the "NEUROPATHIC-PAIN" theme was related to testing sensory signs and modeling the mechanism and treatment of pain. The FIBROMYALGIA," "COMPLEX REGIONAL PAIN SYNDROME," and" "CONDITIONED-PAIN-MODULATION" themes, which were related to pain, were also highly cited, although they had a lower number of publications.

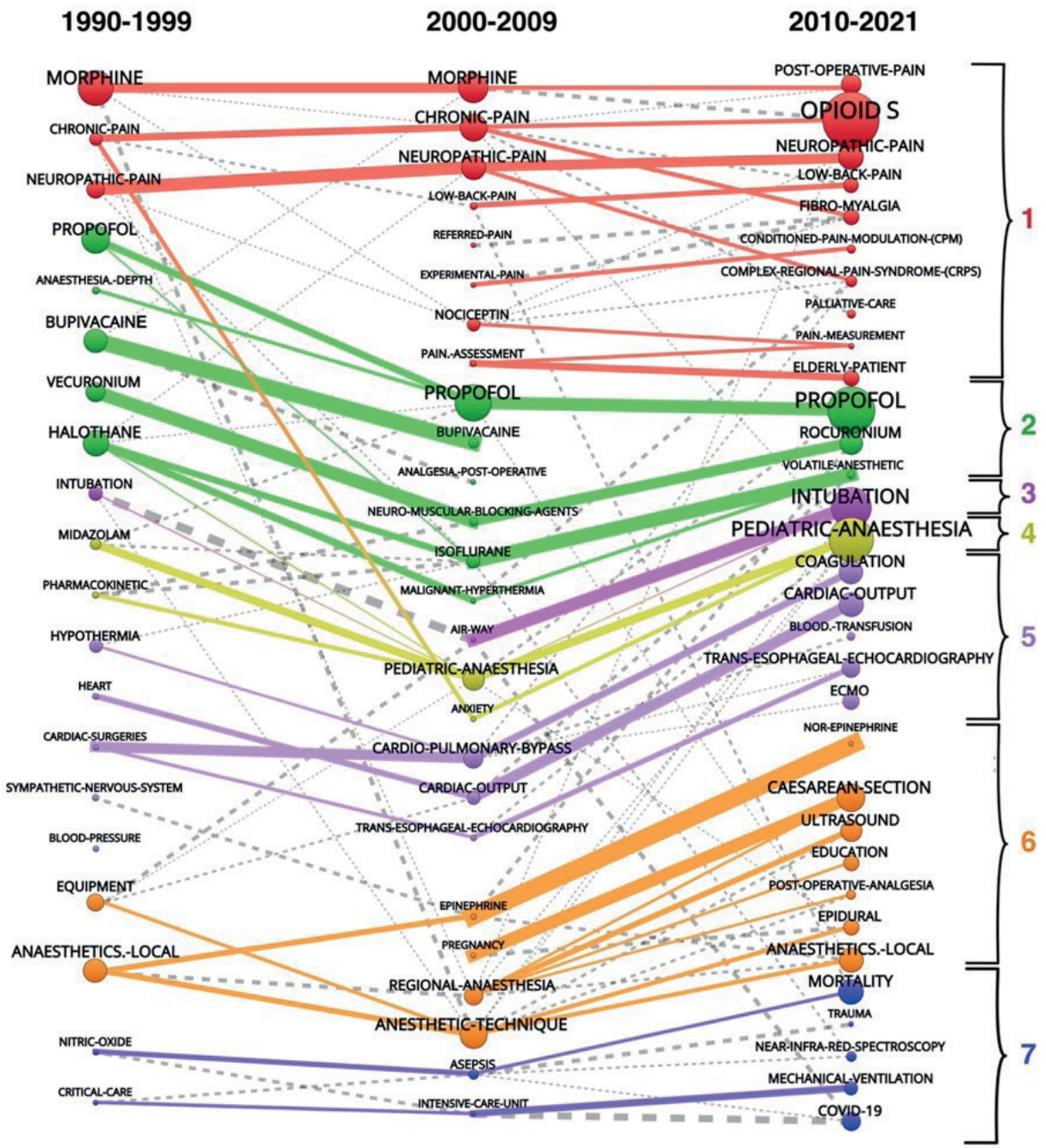
The emerging or declining themes regarding pain in the last 10 years were "PAIN MEASUREMENT" and "POST-OPERATIVE ANALGESIA." Other emerging or declining themes were "PATIENT SAFETY," "ELDERLY PATIENTS," and "PALLIATIVE-CARE.

Finally, the "COVID-19" theme, which has become the most popular topic in recent years, emerged as a basic and transversal theme during the 2010-2021 period. The "COVID-19" theme was above the middle rank in terms of both the number of publications and citations. Some topics related to COVID-19 include airway management, personal protective equipment, intensive care units, and prone positioning. T Cook, K El-Boghdadly, B McGuire, A McNarry, A Patel and A Higgs [46], J Begley, K Lavery, C Nickson and D Brewster [47], M Sorbello, K El-Boghdadly, I Di Giacinto, R Cataldo, C Esposito, S Falcetta, G Merli, G Cortese, R Corso and F Bressan [48] and W Yao, T Wang, B Jiang, F Gao, L Wang, H Zheng, W Xiao, S Yao, W Mei and X Chen [49] were the most cited publications on the "COVID-19" theme.

Thematic Evolution in Thirty Years

Seven thematic areas were identified over three time periods: pain, medications, regional anesthesia, cardiovascular anesthesia, pediatric anesthesia, critical care, and airway management. The thematic areas and conceptual evolutions are shown in Figure 6. The performance measures for these thematic areas are presented in Table 4.

Pain was the largest thematic area and the best performance indicators were obtained. In the last period of anesthesiology literature, the number of core articles and average citations on the topic of pain continued to increase exponentially. Pain had twice



1= Pain, 2= Medications, 3= Airway Management, 4= Pediatric Anesthesia, 5= Cardiovascular Anesthesia, 6= Regional Anesthesia, 7= Critical Care

Figure 6. Evolution map.

Table 4. Thematic Areas in Evolution Map

Thematic areas	Core Documents	Sum Citations	Average Citations
Pain (1)	4103	161363	39,33
Medications (2)	2954	57790	19,56
Airway Management (3)	718	10017	13,95
Pediatric Anesthesia (4)	1041	15976	15,35
Cardiovascular Anesthesia (5)	1566	24467	15,62
Regional Anesthesia (6)	2239	35243	15,74
Critical Care (7)	883	8391	9,5

the average number of citations as a medication, which was the closest thematic field. Regarding the structure and theme composition, the number of themes related to pain increased during the second period. "NEUROPATHIC PAIN," "MORPHINE," and "CHRONIC-PAIN" were the highlighted themes in pain research. "NEUROPATHIC PAIN," which is seen in all periods, became the basic theme from the motor theme in the last period. "MORPHINE," which was a motor theme in the first period, continued as a basic theme in the second period. In the last period, morphine transformed into two themes as a basic theme "POSTOPERATIVE-ANALGESIA," and a motor theme, "OPIOIDS". "CHRONIC PAIN," which was an emerging theme in the first period, turned into a basic theme in the second period. In the last period, "CHRONIC-PAIN" turned into two themes as another motor theme "OPIOIDS," and the basic theme "FIBRO-MYALGIA." Pain was comprised of motor and emerging/declining themes during the first period. Motor and basic themes comprised most themes in the second period. Although basic and emerging/declining themes were the major themes in the last period, the motor theme "OPIOIDS" had the highest core document values in the third period. The pain thematic area covered topics related to pain measurement, opioid medication, and types of pain. In the last period, there was an increase in interest in pain research on the theme of "PALLIATIVE CARE" and "ELDERLY PATIENTS."

Medications, the second-largest thematic area in anesthesiology, is a thematic area of anesthetics (intravenous, inhalers, and some local anesthetics) and neuromuscular blockers. In each period, the themes in this area were scattered as motor, basic, or isolated. "PROPOFOL," the largest theme in this area, became a motor theme from a basic theme after the first period. The motor theme, "VECURONIUM," which is a neuromuscular blocker, transformed into another motor theme "NEURO-MUSCULAR-BLOCKER-AGENTS" in the second period. In the last period, "NEURO-MUSCULAR-BLOCKER-AGENTS" became an isolated theme "ROCURONIUM," also a neuromuscular blocker. "VOLATILE ANESTHETIC" agents were basic themes in each period. "HALOTHANE" transformed into the basic theme "ISOFLURANE" and the isolated theme "MALIGNANT HYPERTHERMIA" in the second period. These two themes then turned into the basic theme "VOLATILE-ANESTHETIC" in the last period.

Regional anesthesia started with a motor theme, "ANESTHETICS-LOCAL." In the second period, the basic themes "REGIONAL ANESTHESIA" and motor theme "ANESTHETIC TECHNIQUE" were highlighted. In the last period, most of the themes related to regional anesthesia were motor themes. The theme of this thematic area has increased during each period. From the second period, "PREGNANCY" and "CESAREAN-SECTION" themes entered "REGIONAL ANESTHESIA." The motor theme, "EDUCATION," and basic theme, "ULTRASOUND," were among the notable themes in the thematic area of regional anesthesia in the last period.

Cardiovascular anesthesia and pediatric anesthesia had similar average citation numbers. However, cardiovascular anesthesia had more core document numbers and themes than pediatric anesthesia. "PEDIATRIC-ANESTHESIA" became a basic theme after the second period and continued as one of the largest themes in the third period.

Critical care was a thematic area with emerging and declining themes until the last period. In the last period, this area had one isolated theme, "NIRS," one basic theme "MORTALITY," and three emerging/declining themes, "TRAUMA," MECHANICAL VENTILATION," and "COVID-19."

Airway management has emerged as an emerging and declining theme. In the second period, it evolved into a basic theme. During the last period, this area continued to have a motor theme. The last period had the largest number of themes. Advanced and difficult airway devices, such as fiber-optic bronchoscopes, video laryngoscopes, and difficult airway algorithms, have been mentioned more frequently.

DISCUSSION

The objective of this study is to comprehend the conceptual framework and thematic progression of anesthesiology over the past three decades, employing comprehensive bibliometric methodologies. Seven thematic areas have been identified in anesthesiology literature over the last 30 years. Among them, pain was the largest thematic area. Most of the pain-related themes were motor themes during the three periods. The morphine theme has been replaced with the opioid theme over the years. In the last period, the

number of articles on the adverse effects of opioids increased. After the second period, the association of pain with the postoperative period, chronic pain, and older age emphasized that pain treatment was an acute and important phenomenon that required continuity. In this context, the theme of pain will maintain its importance in the field of anesthesiology literature in the coming decades.

Medications, the second thematic area, comprised three important daily anesthetic agents: propofol, volatile anesthetics, and neuromuscular blockers. Propofol, together with opioids, was the two most popular theme in each period. Although propofol was the only subgroup of medications that did not change in any of the three periods, volatile anesthetics and neuromuscular blocking drugs were updated over the years.

The third thematic area, regional anesthesia, started with the themes of local anesthetic and anesthesia equipment and increased the diversity of sub-groups over the years. The education and ultrasound subthemes of this thematic area in the last period could be an indication of the popularity and growing trend of regional anesthesia in anesthesia practice.

Cardiovascular and pediatric anesthesia were the fourth and fifth largest thematic areas, respectively. The cardiovascular area has many more subthemes than the pediatric area. In this context, it is possible that pediatric anesthesia is growing.

During the last period under examination, a discernible uptick was observed in the proliferation of subthemes within the critical care domain. This escalation could be attributed to the emergence of sub-themes centered around mortality, mechanical ventilation, and COVID-19. Although the COVID-19 sub-theme materialized only within the final two years, it appears to have assumed a prominent position within the annals of anesthesiology history over the last decade. It is of particular note that the critical care thematic sphere exhibited a lower average citation count compared to its thematic counterparts. The clusters scrutinized within the ambit of critical care generally encompassed a substantial number of recent investigations. Notably, the inclusion of "COVID-19" substantially contributed to the body of work within this cluster. As a consequence, our assessment posits that the relatively diminished average citation count within the critical care thematic purview may be attributed to the necessity for a certain temporal window for citations to accumulate.

CONCLUSION

In conclusion, this study has provided a comprehensive and insightful exploration of the evolving landscape of anesthesiology through the lens of bibliometric analysis. By investigating the thematic trajectory and conceptual shifts within the field over the past three decades, this research contributes valuable insights to the existing body of knowledge.

The findings of this study underscore the dynamic nature of anesthesiology research, as evidenced by the shifting thematic domains and emerging subthemes. The identification of key thematic areas such as pain, medications, regional anesthesia, cardiovascular and pediatric anesthesia, and critical care serves as a foundation for researchers, practitioners, and policymakers to navigate the diverse landscape of anesthesiology research.

Moreover, the study's inclusion of the COVID-19 sub-theme within the critical care domain demonstrates the relevance and adaptability of bibliometric analysis in capturing contemporary issues and their impact on research trends.

While this study offers a comprehensive overview, it is important to acknowledge its limitations, including potential database biases, subjectivity in data preprocessing, and the constraints of citation-based metrics. However, these limitations open avenues for future research to refine methodologies and expand the scope of analysis.

In sum, this study's multidimensional approach sheds light on the past, present, and potential future directions of anesthesiology research. It enriches our understanding of the thematic evolution within the field, presents opportunities for targeted investigations, and invites scholars to explore novel methodologies that can enhance our comprehension of this evolving domain. As the field of anesthesiology continues to evolve, this study serves as a steppingstone for further inquiry, collaboration, and advancement in the pursuit of enhanced patient care and medical knowledge.

Author contribution

Study conception and design: MT, TÖ, and HY; data collection: MT, TÖ, and Mİ; analysis and interpretation of results: MT and TÖ; draft manuscript preparation: MT, TÖ. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

Due to the nature of our study as a literature review, ethical approval has not been sought.

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Conflict of interest

The authors declare that there is no conflict of interest.

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acta medica

ORIGINAL ARTICLE

The cytologic analysis of aqueous humor in eyes with uveitis

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ABSTRACT

Purpose: The purpose of this study was to evaluate the cytologic analysis of aqueous humor (AH) of the eyes with Fuchs uveitis (FU) and idiopathic anterior uveitis (IAU).

Methods: AH of 25 uveitic eyes (12 FU and 13 IAU) were evaluated cytologically. The samples of the anterior chamber were taken before cataract surgery. Aqueous fluid samples were evaluated by applying a Giemsa stain.

Results: The 12 patients with FU and 13 patients with IAU were included in this study. In AH, the number of lymphocytes was similar in eyes with FU and IAU (the number of lymphocytes: min:1 and max:7). The acellular amorphous structures were detected in eyes with FU, and the same finding was not observed in the eyes with IAU. In addition, any pigmented cells or accumulation were not detected in any of the eyes.

Conclusion: The acellular amorphous structures were the remarkable finding of this study. It has been considered that amorphous structures in the AH of the eyes with FU could be related to chronic, low-grade anterior chamber inflammation and responsible for several complications of uveitis. FU is a uveitic entity with mysterious findings that await clarifications in its etiopathogenesis.

Keywords: Cytology, Fuchs uveitis, anterior uveitis.

INTRODUCTION

The cytologic analysis of aqueous humor (AH) in uveitic eyes has numerous significances from clinical evaluation to elucidating the etiology of uveitis. In eyes with uveitis, cellular and non-cellular constituents have been detected in the AH as a reflection of the inflammatory process [1-4].

Fuchs uveitis (FU) and idiopathic anterior uveitis (IAU) are the most common causes of anterior uveitis. Although anatomically classified as anterior uveitis, they have completely different clinical findings. FU is characterized by distinctive anterior uveitis findings. It has low-grade, predominantly unilateral, and chronic inflammatory disease with widely scattered small/medium keratic precipitates (KPs), and a variable degree of iris atrophy [5]. In contrast, IAU is characterized by either acute or chronic inflammation without iris atrophy, and its KPs are spread predominantly in the inferior half of the corneal endothelium [6].

In the past years, cytocentrifuge, and flow cytometric analyses of the AH have been reported in different uveitis entities. A great number of leukocytes have been displayed in the AH during active uveitis and multiple different leukocyte forms have been described in eyes with uveitis [1-3]. FU has a different significance in all aspects from other anterior uveitic entities and several theories have been included in the etiopathogenesis of FU, recently. In light of these findings, we aimed to evaluate and compare the results of cytologic analysis of aqueous humor (AH) in eyes with FU and IAU in this study.

MATERIAL AND METHODS

This clinical prospective comparative study was performed at University of Health Science, Ulucanlar Eye Training and Research Hospital. The study protocol was approved by the Ethics Committee of of Ankara Education and Researach Hospital (Protocol number: 355; date:04/2018), and the study was carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from all of the patients.

Aqueous samples were obtained from uveitic eyes that were followed up at our Uvea department and undergoing cataract surgery. The diagnostic criteria of FU included diffusely spread medium and/or stellate KPs, chronic low-grade anterior chamber reaction, iris atrophy accompanied or not with heterochromia, varying degrees of the vitreous cell, and fibril-like vitreous appearance, absence of acute uveitis symptoms. IAU was described as acute anterior uveitis without any systemic disease, and detailed clinical and laboratory investigations were made for systemic evaluation.

In all eyes, findings consistent with the clinically inactive uveitic period were detected for 3 months before cataract surgery. A detailed medical history was obtained in all cases, including previous diagnoses, therapies, and if any surgical interventions. All patients had no systemic steroid usage. The topical dexamethasone drops (16 x 1/ day) were started one week before surgery.

Anterior chamber paracentesis was performed using a 30-gauge needle at the beginning of the surgery. AH (100-200 µl) was aspirated and immediately transferred into Eppendorf tubes. Aqueous fluid samples were centrifuged for 5 minutes at 800 rpm by cytospin and transferred into cytopsin microscope slides. Giemsa stain was applied to the prepared slides. The examination was reported by taking notes of the number of cells, and repetitive acellular amorphous structures and photographing them.

Statistical analyses

Descriptive analyses were performed using IBM SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA). Demographic characteristics and clinical data were expressed as mean, standard deviation, frequency, or percentage.

RESULTS

This study included 25 eyes of 25 uveitic patients; 12 were FU and the remaining 13 were diagnosed as IAU. The patients with FU consisted of 5 men and 8 women with a mean age of 32.6±9.6 years old (range, 28-39), while the IAU included 3 men and 10 women with a mean age of 51.8±11.2 years old (range, 32-59).

Bilateral involvement was determined in 3 eyes (25 %) with FU. There were diffusely spread non-pigmented KPs [(medium-sized in 9 (75 %) eyes, stellate-medium in 3 (25 %) eyes)], Koeppe nodules

in 4 (33.3 %) eyes, and Busacca nodules in 2 (16.6 %) eyes with FU. The color of all eyes with FU was brown and there were varying degrees of iris atrophy in these eyes. There were no KPs and iris atrophy in the eyes with IAU. 12 (48%) eyes had posterior subcapsular cataracts, 10 (40 %) eyes had cortical cataracts, and there was a mature cataract in 3 (12 %) eyes with FU. The participants' characteristics and clinical findings are shown in Table 1.

The number of lymphocytes was similar in eyes with FU and IAU. Leukocytes were detected in the aqueous humor in a total of 4 patients; 2 with FU and 2 with IAU. 3 leukocytes and 7 leukocytes were detected in FU patients respectively. 1 leukocyte was detected in both 2 IAU patients. The striking finding in eyes with FU has detected the appearance of dense acellular sediment (uncellular amorphous structures) (Figures 1, 2, and 3). These uncellular structures were not observed in eyes with IAU. In addition, no pigment-containing cell or accumulation was observed in any eye.

DISCUSSION

The cytological analysis of AHs of eyes with uveitis has been conducted to elucidate the etiopathology of uveitis. It has been demonstrated that mixed leucocytes infiltrate composed of lymphocytes, neutrophils, and macrophages accumulated when there was an active inflammation in the AHs. The lymphocyte predominance has been reported

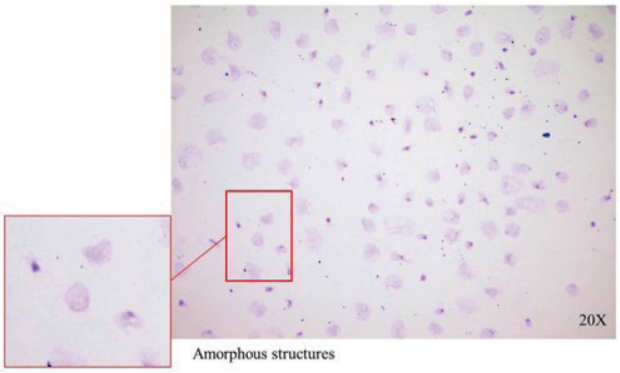

Figure 2. The amorphous structures in aqueous humor sample of eyes with Fuchs uveitis (Giemsa stain).

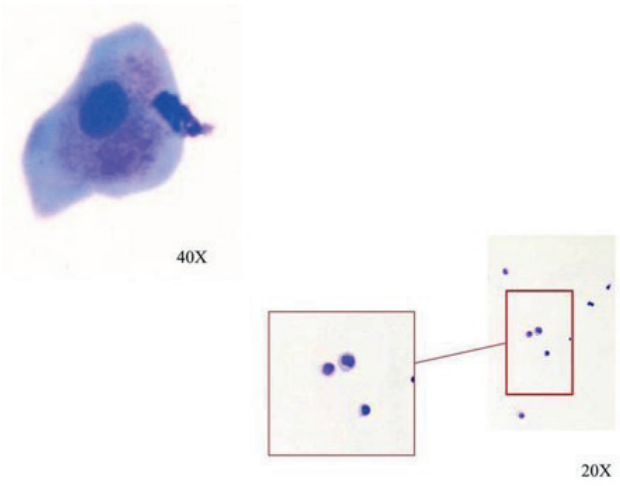

Figure 1. The lymphocytes in an aqueous humor sample of eyes with idiopathic anterior uveitis (Giemsa stain).

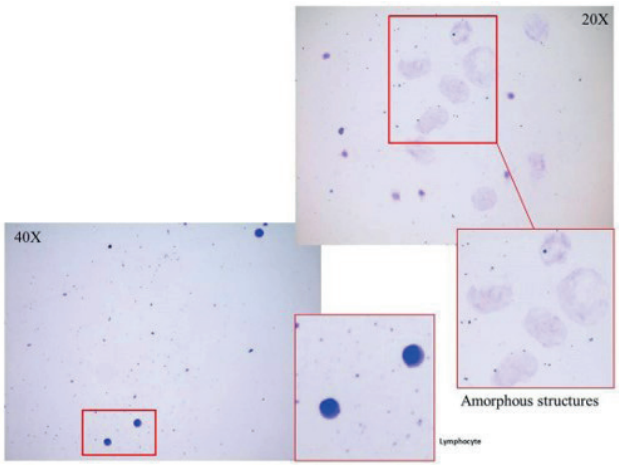

Figure 3. The lymphocytes and amorphous structures in aqueous humor sample of eyes with Fuchs uveitis (Giemsa stain).

Table 1. Demographic and clinical characteristics of the participants

	Age (years) Mean±SD (min-max)	Sex, n (%)	Keratic Precipitates n (%)	Koeppe Nodules n (%)	Busacca Nodules n (%)	Cataract Type n (%) Posterior Subcapsular Cortical Mature
Fuchs uveitis (n=12)	32.6±9.6 (28-39)	10 M (83.3) 2 F (16.7)	Medium-sized 9 (75) Stellate-medium 3 (25)	4 (33.3)	2 (16.6)	9 (75) 0 3 (25)
Idiopathic Anterior Uveitis (n=13)	51.8±11.2 (32-59)	5 M (38.5) 8 F (61.5)	No	No	No	3 (23.1) 10 (76.9) 0

in AH of the majority of idiopathic or HLA-B27 anterior uveitis. Examinations of herpes-viral uveitis or FU, which do not have any neutrophils, have shown the most severe cases of a deviation in distributions of leukocytes [1-3]. The comparison of the cellular phenotypes and the cytokine profile between patients with FU and IAU has been done by the research of Muhaya et al. [7]. According to this, while CD4+ T cells were more in IAU, CD8 + T cells were more dominant in FU [7].

FU is mainly unilateral, low-grade, chronic anterior uveitis, bilateral involvement has been reported in 5–10% of patients with FU [5,8]. Approximately 2-11% of all uveitis cases include patients with FU and the rate of FU in Turkish patients with uveitis has been reported as 6.3% [5,9,10]. Although the etiology of FU is not completely known, immunologic and infectious etiologies are among the most important reasons in recent studies. In addition, ocular fluid analysis findings in patients with FU were reported to indicate a predominance of CD8 + T cells supporting chronic low-grade inflammation [11].

FU and IAU have differences from each other in both clinical appearance and the steroid response. Thus, we evaluated the cytological analysis of the AHs of two different uveitis entities. The number of lymphocytes was similar in eyes with FU and IAU and these findings were consistent with the clinically inactive uveitic period before cataract surgery. The most remarkable feature of this study was the uncellular amorphous structures in eyes with FU. These structures were not detected in eyes with IAU.

The proteins of human AH are low in concentration (0.1–0.5 mg/ml) [12]. Richardson et al. [13] utilized an enhanced proteomic viewpoint to identify the AH proteome by depleting abundant (albumin) proteins and employing multidimensional protein identification technology. To the best of our knowledge, uncellular amorphous structures were detected in eyes with FU which were not reported before. They are not artifacts since the samples were collected and stained at different times. We are considering that these accumulations can be "proteinous amorphous structures". It can be interpreted that these accumulations can develop as a result of low-degree and chronic anterior chamber inflammations. It has been known that the low degree of inflammation of the eyes with FU was not regressed in response to steroids, even though the anterior chamber inflammations in other uveitis entities mostly can be managed with local and/or systemic steroids. On the other hand, patients with FU have frequently and unnecessarily used these steroids. Based on this, we can assume that these accumulations' "proteinous amorphous structures" may be a revealing cause of steroid nonresponsiveness in eyes with FU.

The atrophy of iris stroma was classically the first pathologic finding of the eyes with FU and it can be clinically observed as varying degrees of iris depigmentation and/or heterochromia. Atrophy of the iris stroma and ciliary body has not occurred equally in each eye with FU [5,14,15]. Hence, it indicates that the pathologic involvement of uveal pigment is at different levels in these eyes. Even though pigmented cells have been observed in AHs or on corneal endothelium in various uveitic entities such as herpetic uveitis or chronic uveitis [6], pigmented KPs are occasionally observed in patients with FU [15], and also, our electron microscopic investigation showed no pigment accumulation on the anterior capsule of the lens [16]. In our study, we did not identify any pigmented cells in AH. The absence of any pigment formation in eyes with FU was another striking result of this study.

In studies, the cataract development rate has been reported as 50%-70% in many uveitis entities [17-19]. Posterior subcapsular cataract (PSCC) is the most common cataract type in uveitic eyes [20,21]. Tugal-Tutkun et al. [22] reported that 8-year the risk of cataract development was 56% in 181 eyes with FU without using steroids. The development of mature cataracts has not frequently occurred in eyes with other uveitis entities except in the end-stage phase of uveitis complication, but the mature cataract is a clinical finding encountered in eyes with FU [17,18,22-24]. In the study of Tugal-Tutkun et al. [22] mature cataract was reported in 4 of 181 eyes (2.2%) and PSCC in 108 of 181 eyes (9.7 %) with FU. Also, Zareiet al. [23] reported that the rates of mature cataracts and PSCC were 7% and 80% in 89 eyes with FU, respectively. In our previous study, mature cataracts and PSCC were shown in 8 of 281 eyes (2.8%) and 121 of 281 eyes (43.1 %) with FU,

respectively [24]. It can be interpreted that this proteinous material, identified in this study, can be accumulated in the lens tissue and can be one of the causes of cataract development. Therefore, we think that this finding should be supported by a more detailed evaluation of lens contents in eyes with uveitis cataracts. It has been demonstrated that proteineous amorph materials have been detected in the human body and have a role in the etiopathology of some diseases. Accumulated proteinous material is named amyloid protein. Research about this accumulation has been ongoing in diseases such as multiple sclerosis, Alzheimer and Parkinson [25,26]. The blood-aqueous barrier of the eye provides a limited selective diffusion of paracellular transport. Dysfunction of the barrier contributes to the pathophysiology of ocular inflammations through the vascular leakage of inflammatory cells and blood-borne molecules into the anterior chamber [27,28]. In the literature, a mild alteration of the blood-aqueous barrier has been detected in Fuchs uveitis [29]. Therefore, there could be a relationship between the number of lymphocytes in the aqueous and the amorphous material due to the breakdown of this barrier and the ease of passage. Also, it can be argued that the amorph materials in the eyes with FU could accumulate in the bodies of patients. Thus, there is a need for more comprehensive studies on this subject.

The intraocular pressure (IOP) elevation in FU patients is generally intermittent. Elevated IOP is typically not seen at admission, it has been estimated approximately 50% of the patients during the follow-up period. Many reasons have been proposed as the cause of IOP elevations such as the use of topical corticosteroids, recurrent hypema, angle neovascularization, and trabeculitis [5,30,31]. As far as we know, there is no study in the literature on the accumulation of amorphous material at the anterior chamber angle. The presented study may lead to future studies on amorphous material accumulation that may cause increased intraocular pressure in Fuchs patients. Besides, we showed hyperreflective spots in the vitreolenticular interface in Fuchs patients in our last study [32]. The hyperreflective points we visualized in Berger's space may be the amorphous materials we detected in this histological study. This material may be accumulating both in the angle and the Berger area and it could be one of the reasons triggering the development of glaucoma.

The present study showed that clinically inactive uveitic periods were confirmed with AH analysis in eyes with uveitis. The acellular amorphous structures and no pigment formation in eyes with FU were the remarkable findings of this study. The limitation of our study is the limited number of patients. Furthermore, other staining or examination techniques were not used. More comprehensive studies and examinations are needed.

To conclude, the cytological analysis will lead to the understanding of complex mechanisms in eyes with uveitis. FU is a uveitic entity with mysterious findings that await clarifications in its etiopathogenesis.

Author contribution

Study conception and design: YÖE, BK, BS, RK; data collection: YÖE, BK, PK, BS, GB, RK, PÖ; analysis and interpretation of results: YÖE, BK, BS,RK, PÖ; draft manuscript preparation: YÖE, BK, PK, BS, GB, RK, PÖ. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Ethics Committee of Ankara Education and Research Hospital (Protocol no. 355).

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Conflict of interest

The authors declare that there is no conflict of interest.

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acta medica

ORIGINAL ARTICLE

The determination of heart failure with preserved systolic function by using Galectin-3 and other cardiac markers is affected by vitamin D status*

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ABSTRACT

Objective: Vitamin D affects heart muscle contraction through its direct and indirect effects on calcium and phosphorus metabolism. We aimed to investigate N-terminal pro-brain natriuretic peptide, high-sensitive troponin-I, galectin-3, fibroblast growth factor-23, suppression of tumorigenesis-2, and parathormone levels concerning Vitamin-D status in patients with heart failure with preserved systolic function.

Materials and Methods: Seventy-one patients diagnosed with heart failure with preserved ejection fraction and 61 healthy individuals as a control group were enrolled in the study. The relation between vitamin-D level and heart failure with preserved ejection and the determination of cut-off levels of all parameters was evaluated using ROC and logistic regression analyses.

Results: While vitamin-D levels in the patient group were lower than the controls (median 14.5 vs. 21.11 ng/ml, P=0.002), N-terminal pro-brain natriuretic peptide, galectin-3, suppression of tumorigenesis-2 and parathormone levels in the patient group were significantly higher. The area under the curve values of these four elevated markers in the ROC analysis was significant for the diagnosis of heart failure with preserved ejection fraction. In the logistic regression analysis, a one-unit decrease in vitamin-D increased the risk of having heart failure with preserved systolic function 1,084 times, while a 10-unit categorical decrease in vitamin-D increased the risk 2,27 times. In the patient group, the area under the curves for N-terminal pro-brain natriuretic peptide, galectin-3, suppression of tumorigenesis-2 and parathormone were higher when vitamin-D was >20 ng/ml compared to the group with vitamin-D < 20 ng/ml.

Conclusions: N-terminal pro-brain natriuretic peptide, galectin-3, suppression of tumorigenesis-2 and parathormone were affected by the vitamin-D status in determining heart failure with preserved systolic function, with increased detection capability when vitamin-D is >20 ng/ml.

Keywords: vitamin D, heart failure.

INTRODUCTION

Vitamin D (Vit D) influences cardiac muscle contraction through its various effects on calcium-phosphorus metabolism. Many studies have been conducted on this subject [1,2], and the relationship between systolic heart failure (HF) with vitamin D deficiency has been investigated. These studies did not find a significant link between vitamin D deficiency and heart failure and concluded that vitamin D supplementation does not provide a significant improvement in heart failure. Systematic reviews and meta-analyses have also shown that vitamin D supplementation has no positive effect on cardiovascular outcomes, including myocardial infarction and stroke. Meta-analyses also showed that vitamin D supplementation had no significant effect on cardiovascular risk factors (lipids, glucose, blood pressure) [3,4]. On the other hand, the effect of vitamin D on diastolic dysfunction of the heart has not been adequately studied.

Heart failure with preserved ejection fraction (HFpEF) describes patients who have a left ventricular ejection fraction (LVEF) greater than 50% and HF symptoms [5]. Various criteria are used to define HFpEF syndrome: (1) Clinical signs or symptoms of HF, (2) Evidence of preserved or normal LVEF, (3) Evidence of abnormal LV diastolic dysfunction as detectable by Doppler echocardiography or cardiac catheterization [6]. Diastolic dysfunction also seen in many people without HFpEF, is an important part of natural human aging. However, the presence of diastolic dysfunction is a risk factor for the development of HFpEF [7].

While echocardiography is used as the imaging method in the diagnosis and follow-up of HF, various plasma biomarkers are proven helpful in determining the diagnosis and prognosis of HF [8-10]. In recent years, many studies have investigated new HF biomarkers that may be useful in prognosis or grading. Although many biomarkers have been investigated, their practical application has been largely unsuccessful [11-15]. Although cardiac-specific biomarkers, including natriuretic peptides (Atrial and Brain Natriuretic Peptides) and High-Sensitive Troponin, are widely used in clinical practice, the benefits of other biomarkers have yet to be proven. Because they are influenced by general pathological processes such as cell death, inflammation and fibrosis these biomarkers are not specific to the heart or HF. To a large extent, these biomarkers cannot be linked to a single disease [15]. The levels of these markers are likely affected by vitamin D status in HFpEF because of the effects of vitamin D on myocardial function [1,2].

In this study, we investigated, for the first time, the relationship between the levels of HF markers and vitamin D status in patients with HFpEF. To this end, we measured N-terminal pro-brain natriuretic peptide (NT-ProBNP), high-sensitive troponin-I(HsTpn-I), galectin-3(Gal-3), fibroblast growth factor-23(FGF-23), suppression of tumorigenesis-2 (ST2) levels and vitamin D level in these patients in comparison with healthy controls. Since parathyroid hormone (PTH) and vitamin D levels are correlated with each other [16], hyperparathyroidism has various cardiac effects such as LV hypertrophy, hypertension, and diastolic dysfunction [17]; PTH was also included as a covariate in the analyses. The effect of vitamin D status on HF detection levels of these biomarkers in HFpEF was investigated by appropriate statistical methods.

MATERIALS AND METHODS

Patient selection and data collection

Our research was approved by Hacettepe University Ethics Committee in January 2021. The research was carried out in Hacettepe University Faculty of Medicine, Department of Internal Medicine (Endocrinology Division), and Department of Cardiology. Seventy-oneconsecutive patients (New York Heart Association (NYHA) class I, II [18]) attending inpatientand outpatient clinics with HFpEF and 61 healthy individuals without a diagnosis of HF formed the patient and control groups, respectively. The control group was selected from the hospital staff through face-to-face interviews. Vitamin D, HsTpn, NTproBNP, FGF-23, PTH, Gal-3, and ST2 serum levels were measured in both groups.

When selecting the participants, the patients with LV systolic dysfunction (EF < 50%) and those with various diseases that could cause cell death and inflammatory conditions such as infection, malignancy, and autoimmunity were excluded. Participants with kidney and liver failure, which

could potentially affect the marker level, were also excluded from the study.

The research was carried out between January 2021 and August 2021. The blood samples taken from the patients were centrifuged at 4000 rpm for 5 minutes, and serum samples were stored at -80°C until they were studied. After the samples were thawed in … University Biochemistry Department Laboratory, they were run with the Enzyme-Linked Immuno Sorbent Assay (ELISA) method(Diasource ® KAP1971-F1 Human 25 OH Vitamin D Total ELISA Kit, Cloud Clone-USCNK- ® SEA746Hu ELISA Kit for Human FGF23, Cloud Clone-USCNK- ® CEA866Hu Human PTH ELISA Kit, Cloud Clone-USCNK-® SEA485Hu Human N-Terminal Pro-Brain Natriuretic Peptide ELISA KIT, Cloud Clone-USCNK- ® HEA478Hu Human Cardiac Troponin I High Sensitive ELISA Kit, Cloud Clone-USCNK-® SEA303Hu Human Galectin-3 ELISA Kit, Cloud Clone-USCNK-® SEH820Hu Human ST2 (IL-33R, IL1RL1) Human ELISA Kit) (Human 25 OH Vitamin D, DIAsourceImmunoAssays SA, Belgium; Human, FGF23, Cloud-Clone Corp.,USA; Human PTH Cloud-Clone Corp.,USA; Human N-Terminal Pro-Brain Natriuretic Peptide, Cloud-Clone Corp.,USA; Human Cardiac Troponin I High Sensitive Cloud Clone Corp.,USA; Human Galectin-3 Cloud-Clone Corp.,USA; Human ST2 (IL-33R, IL1RL1) Cloud-Clone Corp.,USA).

Transthoracic echocardiographies of the participants, whose consent was obtained, were performed simultaneously (same day) with the collection of blood samples.

Statistical analysis

Frequencies and percentages for qualitative variables, mean ± standard deviation, or median (minimum-maximum) values for numerical variables were given as descriptive statistics. The means of normally distributed numerical variables in the groups with and without HFpEF were compared with the "student t test", and the distributions of the variables that did not show normal distribution were compared using the Mann-Whitney U test. Classification performances of biomarkers that may help to diagnose HFpEF were examined by receiver operating characteristic (ROC) analysis. At the end of this evaluation, the best cut-off points for diastolic heart failure markers were determined according to the Youden index criterion, and the sensitivity and specificity values, which are the basic test performance measures, were calculated for these points. The effect of biomarkers on heart failure was investigated with a multiple Logistic Regression Analysis along with vitamin D levels. Adjusting the effect of vitamin D level, the independent effect of biomarkers was evaluated with the odds ratio. The logistic regression model was created using the Backward Wald method.

Analyzes were reperformed for two subgroups, as vitamin D < 20 ng/ml and vitamin D > 20 ng/ml, according to vitamin D levels. Changes in the performance of biomarkers in each subgroup were examined by the area under the ROC curve. A p-value <0.05 was considered statistically significant in the study. Data were analyzed by using SPSS software (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp)

RESULTS

The distribution in terms of age, gender and body mass index in the patient and control groups is presented in Table 1.

The most common complaint in the patient group was dyspnea, followed by symptoms such as chest pain, edema, and palpitations. The patients in our patient group consisted of stage I and stage II HF patients according to the congestive heart failure classification of the NYHA. The control group was selected from asymptomatic healthy individuals.

Drug take in the control and patient groups is shown in table 2. As expected, the patient group takes more drugs in all drug groups.

The EF in the transthoracic echocardiograms of the controls was greater than 55% (mean 60.57%), with no evidence of diastolic dysfunction. The EF in the patient group was greater than 55 (mean 60.58%), and diastolic dysfunction was noted(When mitral flow indices were evaluated, the peak early [E]/late diastolic velocity [A] ratio was greater than 1 [19]).

Vitamin D, NTproBNP, Gal-3, FGF-23, PTH, HsTpn-I, and ST2 levels were evaluated between the two groups using the Mann-Whitney U test due to the non-normal distribution of the data. Vitamin D, NTproBNP, Gal-3, PTH, and ST2 levels were statistically different between the two groups (Table 3).

Table 1. Distribution of control and patient groups in terms of gender, age, and body mass index (BMI)

	Controls (n=61)		Patients (n=71)	
Features	N	%	n	%
Male	29	47.6	36	50.7
Female	32	52.4	35	49.3
Age				
<40	22	36.2	4	5.6
40-49	24	39.3	18	25.4
50-60	15	24.5	26	36.6
>60			23	32.4
BMI (kg/m^2)				
Weak (<18,5)	1	1.6	0	0
Normal (18,5-24,9)	20	32.8	8	11.3
Overweight (25-29,9)	27	44.3	35	49.3
Obese (>30)	13	21.3	28	39.4

Table 2. Drug take in control and patient groups

	CONTROL (n=61)	%	PATİENT (n=71)	%
Angiotensin-converting enzyme inhibitörü	3	4,9	16	22,5
Angiotensin receptor blockers	3	4,9	19	26,8
Acetylsalicylic acid	5	8,2	21	29,6
STATIN	4	6,4	21	29,6
Beta Blocker	4	6,4	20	28,1
THIAZID	2	3,2	15	21,1
METFORMIN	2	3,2	15	21,1
Proton pompa inhibitörü	2	3,2	7	9,9
INSULIN	1	1,6	7	9,9
Sodium-glucose Cotransporter 2 Inhibitors	0	0	3	4,2
DPP-IV inhibitors	2	3,2	3	4,2
GLICLAZID	1	1,6	3	4,2
calcium channel blocker	1	1,6	11	15,5
Clopidogrel	2	3,2	5	7

NTproBNP, Gal-3, FGF-23, PTH, HsTpn-I, and ST2 were evaluated by ROC analysis to determine whether they could be diagnostic in HFpEF and the cutoff values that provided the highest sensitivity for those that could (Figure 1).

FGF-23 and HsTpn-I levels between the two groups did not show a significant difference, and the area under the curve (AUC) values on the ROC curve were low. These two markers were not included in the subsequent calculations, and it was accepted that there was no significant difference between the HFpEF and control groups.

In ROC analysis, the best cut-off points were determined in terms of sensitivity and specificity for four markers (NTproBNP, Gal-3, ST2, PTH) according to the Youden index. As to this index, >2.92 value for Gal-3 (sensitivity 71% specificity: 68%) >27.87 value for NT-pro BNP (sensitivity 53%, specificity 75%), >9.22 value for ST2 (sensitivity % 53, specificity 73%) and a value >19.71 for PTH (sensitivity 50%, specificity 80%) were the most appropriate values for sensitivity and specificity. At these values, the highest sensitivity was in Gal-3, and the highest specificity was in PTH.

Four markers (the ones which are statistically different between the two groups) and vitamin D were evaluated by logistic regression analysis. Only Vitamin D, Gal-3, and NTproBNP were statistically significant.

The patient and control groups were divided into two groups according to their Vitamin D level as <20 ng/ml and >20 ng/ml. Twenty-seven patients in the control group and 50 patients in the patient group had a Vitamin D level of <20 ng/ml. Vitamin D level was >20 ng/ml in 34 patients in the control group and 21 in the patient group. In total, 41.7%

Table 3. Comparison of Vitamin D, NTproBNP, Gal-3, ST2, FGF-23, PTH, HsTpn-I, and ST2 results between control and patient groups

	Control(n=61) Median(Min-Max)	Patient(n=71) Median(Min-Max)	p-value
25-OH Vitamin D (ng/ml)	21.11 (5.97-57.39)	14.5 (6.73-34.62)	0.002
NTproBNP (pg/ml)	16.64 (0.35-319.76)	30.16 (1.8-1730)	0.007
Galectin-3 (ng/ml)	2.7 (1.15-7.13)	3.27 (1.53-27.86)	0.000
ST2(ng/ml)	6.22 (0.58-45.96)	9.91 (0.62-38.81)	0.015
PTH (pg/ml)	15.48 (4.09-78.69)	19.99 (4.13-49.68)	0.013
FGF-23 (pg/ml)	35.87 (25.27-306.19)	63.8 (33.83-265.03)	0.661
High Sensitive Troponin I (pg/ml)	8.15 (0.5-2800)	0.46(0.46-3055)	0.535

of the participants had a Vitamin D level >20 ng/ml, and 59.3% had a vitamin D level <20 ng/ml. Then, ROC analyses were performed for NTproBNP, Gal-3, ST2, and PTH in both groups to determine whether these markers were diagnostic in HFpEF. The AUC values were greater in the vitamin D-sufficient group for all four markers, but only Gal-3 AUC values were statistically significant in both groups (Table 4, Figure 2, 3).

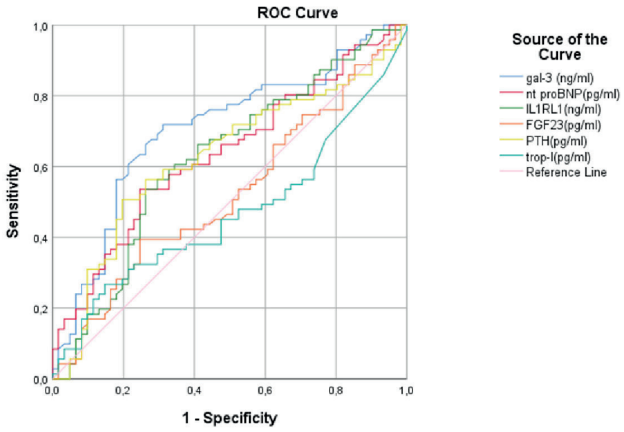

Figure 1. ROC curve results for NTproBNP, Gal-3, FGF-23, PTH, HsTpn-I and ST2.

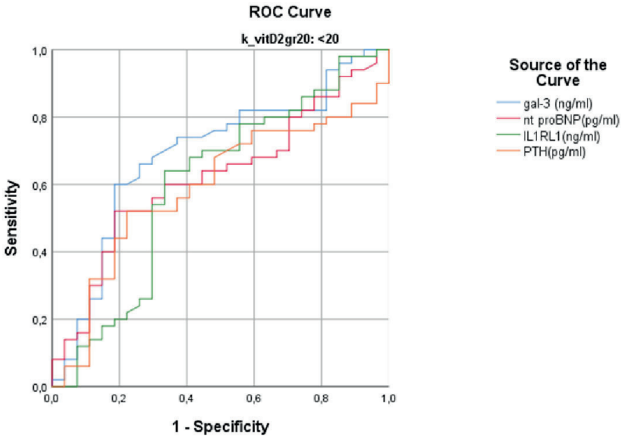

Figure 2. Evaluation of NTproBNP, Gal-3, ST2, and PTH levels by ROC curve in patients with vitamin D levels <20 ng/ml.

DISCUSSION

Our study is the first one in the literature investigating the effect of vitamin D on the detection levels of NTproBNP, Gal-3, ST2, FGF-23, PTH, and HsTpn-I for HFpEF. In our study, NT-ProBNP, Gal-3, ST2, and PTH were statistically significantly higher in the HFpEF group than in the control group. NTproBNP, Gal-3, ST2, FGF-23, PTH, and HsTpn-I were evaluated by ROC analysis to determine whether they could be diagnostic in HFpEF and the cut-off point values that gave the highest sensitivity for those that could be diagnostic. The areas under the curve(AUC) of Troponin-I and FGF-23 in the ROC curve were low and not statistically significant. Among NTproBNP, Galectin 3, ST2, FGF-23, and PTH, when evaluated without considering vitamin D levels, the highest AUC value was in Gal-3 and then NTproBNP. Vitamin D was significantly lower in the HFpEF group than in the control group. According to our logistic regression analysis results, having tenunits lower Vitamin D increases the risk of having HFpEF by 2.27 times. AUC values of NT ProBNP, Gal-3, ST2, and PTH

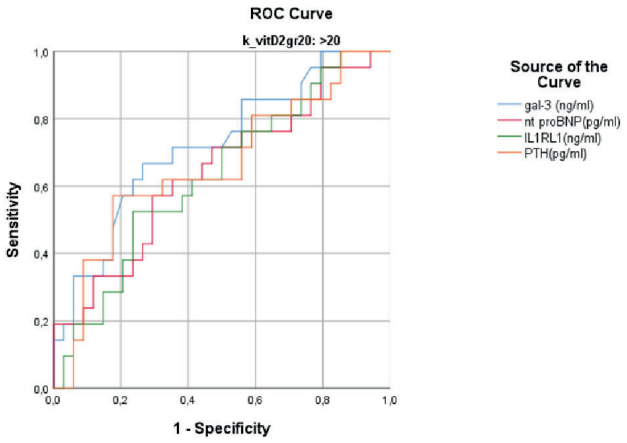

Figure 3. Evaluation of NTproBNP, Gal-3, ST2, and PTH levels by ROC curve in patients with Vitamin D levels >20 ng/ml.

Table 4. Evaluation of the areas under the curve according to the ROC curve when the patient and control groups were divided into two groups according to their vitamin D levels

Vitamin D level	Markers	AUC	p-value	95% Confidence Interval
<20 ng/m	gal-3 (ng/ml)	0.691	0.006	0.565-0.818
	ntproBNP (pg/ml)	0.623	0.077	0.495-0.750
	ST2 (ng/ml)	0.603	0.139	0.462-0.743
	PTH (pg/ml)	0.590	0.196	0.458-0.721
<20 ng/m	gal-3 (ng/ml)	0.721	0.006	0.582-0.861
	ntproBNP (pg/ml)	0.641	0.080	0.488-0.795
	ST2 (ng/ml)	0.632	0.103	0.481-0.782
	PTH (pg/ml)	0.658	0.050	0.505-0.812

in different vitamin D groups vary in ROC analysis for HFpEF diagnosis. For all four markers, the AUC values for the diagnosis of HFpEF were higher in the vitamin D>20 ng/ml group than in the vitamin D <20 ng/ml group. When Vitamin D was >20 ng/ml, NTproBNP, Gal-3, ST2, and PTH had an increased chance of success in diagnosing HFpEF. NTproBNP, Gal-3, ST2, and PTH were associated with Vitamin D status in determining ejection fraction preserved heart failure.

Markers that can be used in HF are affected by general pathological processes such as inflammation, fibrosis and cell death; therefore, they are not specific to the heart or HF. To a large extent, these biomarkers cannot be associated with a single disease [15]. In our study, we did not include those with various diseases that may cause cell death and inflammatory conditions such as infection, malignancy, autoimmunity, and those with kidney and liver failure, which could potentially be effective at the marker level.

In the PARAGON-HF Study published in 2018, the most common complaints in patients with HFpEF were dyspnea and fatigue [20]. The most common complaint was dyspnea in our patient group, as well.

Vitamin D deficiency may impair cardiac functions indirectly as well through PTH elevation. None of the participants included in our study (control group and HFpEF in both groups) had PTH elevations above normal. In addition, PTH was investigated in conjunction with other markers to elucidate whether it could be used as a marker in HFpEF. Interestingly, the specificity of PTH was higher than Gal-3 and NTproBNP when the optimal values for sensitivity and specificity were determined according to the Youden index for four markers that could be diagnostic in HFpEF (NTproBNP, Gal-3, ST2, PTH). Low PTH values may be more critical than NTproBNP and Gal-3 in excluding HFpEF. Lower PTH can be used to exclude the diagnosis of HFpEF in the future, but more clinical studies are needed on this subject.

In a study by Borbély et al., the cardiomyocytes of patients with HFpEF were harder than the control group [21] In our study, markers that could potentially reflect cardiac fibrosis, such as Gal-3 and ST2 [15], were higher in the HFpEF group. Vitamin D level is influential in determining heart failure with preserved ejection fraction may be explained by the fact that vitamin D deficiency is effective in cardiac diastolic dysfunction through fibrosis. On the other hand, Scragg et al. [22] showed that monthly high-dose vitamin D supplementation did not prevent cardiovascular diseases in 5000 patients. We believe that our study has indicated a possible relationship between vitamin D and fibrosis. However, more clinical studies are needed on this subject.

Plasma levels of natriuretic peptides are widely used in diagnosing patients with suspected HF and help evaluate patients with both systolic HF and HFpEF [23]. Normal natriuretic peptide levels largely exclude the presence of HF, particularly useful in acute situations to rule out HF [23,24]. Among the markers we investigated (NTproBNP, Gal-3, FGF-23, HsTpn, ST2), NTproBNP is the only marker used in clinical practice in HF. In our study, NTproBNP was significantly higher in the HFpEF group compared to the control group.

In a study by Tschöpe et al. [25] the median value of NTproBNP in the HFpEF group was 189.54 pg/ml, and the median value of NTproBNP in the control group was 51.89 pg/ml.The AUC was 0.83 in the ROC analysis performed for NTproBNP to diagnose HFpEF. At a cut-off value of 110 pg/mL, NT-proBNP showed 72% sensitivity and 90% specificity. Compared to our study, the median values were higher in both HFpEF and control groups. According to our study, AUC, sensitivity, and specificity values are higher. The differences in the results may be due to the wider differences between the minimum and maximum NTproBNP values in our study, which may be since we used ELISA as the measurement method. In the study of Polat et al. from Turkey on heart failure patients with preserved ejection fraction [26], NTproBNP was 617.75 pg/ml in the patient group while it was 66.35 pg/ml in the control group. In the ROC analysis, the AUC was 1, and the sensitivity and specificity for the 234 pg/ml value were 100%.Compared to our study, the differences may be since our study included more participants in both the patient and control groups, and our patients were in group I-II compared to the NYHA group, whereas the patients of Polat et al. [26] were NYHA II-III.

Many studies have shown that plasma levels of Gal-3 are associated with cardiac function [27]. In the review of Boer et al. [28], it was stated that Gal-3 is associated with various aspects of the pathophysiology of heart failure, particularly myocardial fibrosis, a transition from compensated to decompensated heart failure, and comorbidities such as kidney disease and diabetes. In a study by Kimmenade et al. [29], Gal-3 level was significantly higher in acute heart failure compared to the control group. Also, in that study, a high Gal-3 level effectively affected mortality. In our study, Gal-3 was higher in the HFpEF group than in the control group. The median Gal-3 value in our study was lower in both the patient and control groups compared to the value of Kimmenade et al. This result may be explained by the fact that the patient group in their study has presented with acute heart failure. In addition, both of our groups consisted of younger individuals compared to the study of Kimmenade et al. In the study by Polat et al. [26] the median serum Gal-3 level in patients was significantly higher than that of controls (5.35 vs. 0.51 ng/ml). A value of 1.79 ng/mL for galectin-3 had a sensitivity of 86.4%, a specificity of 100%, and an AUC of 0.98 in detecting HFpEF. Sensitivity, specificity, and AUC values are higher compared to our study. The differences between the two studies may be due to the strict exclusion criteria we have used. In addition, in the study of Polat et al., the patient and control groups were older than the ones in our study.

Our knowledge so far about the meaning of ST2 in HF is quite limited. Various studies have shown that the sST2(soluble ST2) isoform may play a role in cardiac fibrosis and remodeling [30]. On the other hand, the ST2L isoform has also been shown to be potentially cardioprotective by interaction with IL-33 [31]. We found higher ST2 levels in the HFpEF group than in the control group. In a study by Januzzi et al. [32] ST2 levels were measured in 593 patients with dyspnea who wereattending to the emergency department with and without acute destabilized heart failure. ST2 concentrations were higher in patients with acute heart failure compared to those without (0.50 vs. 0.15 ng/ml) [32]. In our study, ST2 levels were higher in both HFpEF and control groups. This difference may be since we worked with the group with preserved ejection fraction. In the study of Januzzi et al., no informationabout the patients' ejection fraction has been quoted.

The FGF-23 was found to be associated with left ventricular hypertrophy in a study by Faul et al. [33] Left ventricular hypertrophy has an important place in the etiopathogenesis of diastolic dysfunction. Roy et al. [34] found that mean FGF-23 was significantly higher in HFpEF patients than in the control group. In addition, high FGF-23 was associated with mortality and hospitalization at the end of the first year. In our study, FGF-23 was not significantly increased in the HFpEF group compared to the control group.

Limitations of The Study

We had only six patients with vitamin D above 30 ng/ml in a total of 132 participants evaluated (i.e., Vitamin D>30 ng/ml (proficiency) was not formed as a distinct group). The patients in our HFpEF group were predominantly NYHA class I and II. However, studies in the literature have been mainly conducted on decompensated heart failure patients. However, we think that the fact that we worked with a mildly symptomatic group also added a unique value to our study. Transthoracic echocardiography is a non-invasive, low-cost, and easily applicable method to evaluate the heart's systolic and diastolic functions. However, it may vary depending on the person's experience who performs it(We worked with an experienced cardiologist for echocardiography).In addition, the echocardiographic parameters to be interpreted for the diagnosis of HFpEF are complex, further complicating its evaluation.

In conclusion, NTproBNP, Gal-3, ST2, and PTH were affected by the vitamin D status in determining HFpEF, with increased detection capability when vitamin D is >20 ng/ml. Preserved ejection heart failure (HFpEF) is often difficult to diagnose, and using biomarkers to aid diagnosis is helpful in clinical practice. These biomarkers may reflect different aspects of HFpEF. Given the mixed pathophysiology of HFpEF, single biomarker may not be viable. We believe that it would be more accurate to use multi-marker approaches as in evaluating acute coronary syndrome.Prevention and treatment of vitamin D deficiency may be protective for HFpEF. However, extensive prospective clinical studies are needed to clarify this.

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Author contribution

The study was designed by AG and ME. Transthoracic echocardiographies of the patients were performed by SZ with the support and supervision of BE. Data collection was done by ME, IL, BGI, and SHO. Analysis and interpretation of the results were made by JK, AG and ME. The study was compiled into an article by AG and ME.

Ethical approval

The study was approved by Hacettepe University Non-Interventional Clinical Research Ethics Committee (Protocol no: 2021-02-02 /date 19.01.2021).

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Conflict of interest

The authors declare that there is no conflict of interest.

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acta medica

ORIGINAL ARTICLE

Retrospective analysis of healthcare workers admitted to emergency department due to side effects of the inactivated COVID-19 vaccine*

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ABSTRACT

Objective: This study aimed to evaluate the clinical characteristics and outcomes of healthcare workers who presented to the emergency department with vaccine-related reactions following the first dose of the CoronaVac vaccine.

Materials and Methods: A retrospective analysis was conducted in Ankara Bilkent City Hospital's Emergency Department between 13.01.2021 and 13.02.2021, encompassing healthcare workers aged 18 and above who experienced Vaccine Adverse Effects after CoronaVac vaccination. Data regarding demographics, medical history, symptoms, treatments, and outcomes were extracted from the hospital management system and analyzed using SPSS Version 24.

Results: Of the 43 healthcare workers presenting with Vaccine Adverse Effects, 76% were female. Known history of anaphylaxis/urticaria was recorded in 32.6% of the patients, while 11.6% had a prior history of COVID-19. Common presenting symptoms included chest pain (16.3%) and headache (14%). A majority (58.1%) required no treatment, though two patients received adrenaline for anaphylaxis, with one requiring hospitalization. The median time from vaccination to symptom onset was approximately 16 hours.

Conclusion: The study demonstrates a higher incidence of Vaccine Adverse Effects among female healthcare workers and those with a history of anaphylaxis/urticaria, although most reactions were mild and did not necessitate treatment. This analysis sheds light on the CoronaVac vaccine's safety profile among healthcare workers and provides a foundation for addressing vaccine-related concerns in preparation for future mass vaccination endeavors against emerging COVID-19 variants or new pandemics.

Keywords: COVID-19, COVID-19 vaccines, adverse effects, emergency department.

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INTRODUCTION

The COVID-19 pandemic, stemming from the first reported case in Wuhan province, China, in 2019, has emerged as one of the most pivotal events of the 21st century, spreading rapidly worldwide. Initial high mortality rates persisted until the development of initial treatments and preventive measures. As per current World Health Organization data, it has led to approximately 7 million deaths [1].

Vaccination stands out as a paramount defense against viral infections [2]. Various vaccines, including mRNA, inactivated, and adenovirus vector-based, have been devised to combat COVID-19 [3]. One such vaccine, CoronaVac, an inactivated SARS-CoV-2 vaccine developed by Sinovac/Biotech, China, obtained global emergency use approval [4]. Owing to limited production and the urgent need in vaccination programs, priority has been rightly accorded to vaccinating high-risk individuals globally, with healthcare workers prominently featured among them.

Commencing on January 13, 2021, healthcare workers and individuals above the age of 65 began mass vaccinations with CoronaVac, the inaugural COVID-19 vaccine receiving emergency use approval in Turkey [5]. In Turkey, hospitals have been designated as mass vaccination centers, facilitating the provision for vaccinated individuals to seek assistance from the emergency department in case of adverse reactions. Side effects linked to inactivated vaccines typically manifest as local symptoms or exhibit mild to moderate severity, encompassing fever, fatigue, headache, and pain/redness at the injection site [6]. In our study, we undertook an evaluation of the clinical characteristics and outcomes of healthcare workers who presented to the emergency department following vaccine-related reactions subsequent to the administration of the first dose of the CoronaVac vaccine.

METHODS

Our study follows a prospective, observational design and was conducted within the Emergency Department of Ankara Bilkent City Hospital. Ethical approval was obtained from the Ethics Committee for Clinical Research, chaired by the Institutional Review Board of Ankara City Hospital, specifically from Research Ethics Committee No. 2. Located in Ankara, the capital of Turkey, Ankara Bilkent City Hospital served as a crucial center during the pandemic, functioning both as a pandemic center hospital and a vaccination center in the city. The Emergency Department of Ankara Bilkent City Hospital attends to approximately 360,000 patients annually. The study period spanned from January 13, 2021, to February 13, 2021, corresponding to the administration of the first dose of the vaccination.

The study cohort comprised healthcare workers exhibiting complaints subsequent to receiving the CoronaVac vaccine and seeking care in the emergency department. Vaccine Adverse Effect (VAE) was defined as "any adverse medical event occurring after vaccination and thought to be related to a vaccine (including known or unknown vaccine effects)." Inclusion criteria involved healthcare workers aged 18 and above, providing informed consent for participation, and presenting to the emergency department with VAE. Key data, including gender, age, chronic diseases, COVID-19 history, anaphylaxis/urticaria history, vaccine-symptom interval, presenting symptoms, emergency department treatments, and outcomes, were systematically recorded.

Statistical analysis employed the SPSS statistical package program. Descriptive findings for categorical variables were presented as numbers and percentages, while continuous variables were expressed as mean ± standard deviation for normally distributed data and median (minimum, maximum) for non-normally distributed data. The normal distribution of continuous variables was assessed through visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Pairwise comparisons utilized Mann-Whitney U and Chi-square tests, with statistical significance set at $P < 0.05$.

RESULTS

A total of 43 healthcare workers sought admission to the emergency department with Vaccine Adverse Effect (VAE). Among them, 76% (n=33)

were female. Notably, 32.6% (n=14) had a known history of anaphylaxis/urticaria, while 11.6% (n=5) reported a previous episode of COVID-19. The distribution of patients concerning medical history and gender is detailed in Table 1.

Table 2 provides insights into the age distribution of patients and the time elapsed after vaccination until the manifestation of symptoms.

The most prevalent presenting symptoms were chest pain, reported by 16.3% (n=7), and headache, reported by 14% (n=6). A significant portion of patients (58.1%) successfully completed the follow-up period without necessitating treatment. Only two patients received adrenaline, with one of them requiring hospitalization for further observation, as outlined in Table 3.

DISCUSSION

In the context of pandemics, safeguarding healthcare workers, who are at high risk due to increased healthcare demands and heightened exposure to the infectious agent, is of paramount importance. Vaccination stands out as the most effective measure for their protection against pandemics. Despite being healthcare workers, concerns about vaccine side effects persist [4-7-8-9].

Our study revealed a notable predominance of females among patients seeking admission to the emergency department with Vaccine Adverse Effect (VAE). This observation aligns with existing literature trends [10-12]. Considering that the majority of admissions were linked to immune response-related reasons, a gender-based influence might be at play [13]. Additionally, the presentation habits of women could contribute to this phenomenon [14].

Approximately 33% of patients had a history of anaphylaxis, surpassing figures reported in the literature [15,16]. Notably, patients with a history of anaphylaxis and allergy exhibited a higher incidence of side effects [12].

Common reactions to inactivated vaccines, such as VAE, typically involve local pain, rash, headache, fever, and malaise [9]. Our study corroborates the high incidence of headache. Notably, there were no admissions related to pain, redness, or fever, possibly attributed to patients with such

Table 1. Gender distribution and medical history of patients

		(n)	(%)
Sex	Male	10	23,3%
	Female	33	76,7%
Chronic Disease	Hypertension	6	14,0%
	Hypothriodism	6	14,0%
	Asthma	2	4,7%
	Rheumatological disease	2	4,7%
Anaphylaxis/ Urticaria History	Yes	14	32,6%
	No	29	67,4%
COVID 19 History	Yes	5	11,6%
	No	38	88,4%

Table 2. Analysis of patients' age and time from vaccination to symptom onset

	Mean	SD	Median	Percentile 25	Percentile 75
Age (years)	35	11	35	26	41
Vaccine symptom interval (hours)	16,4	15,5	23,0	1,0	24,0

Table 3. Clinical features and outcomes of patients

		(n)	(%)
Symptoms	Headache	6	14,0%
	Dizziness	2	4,7%
	Nausea	2	4,7%
	Palpitation	2	4,7%
	Urticaria	4	9,3%
	Joint pain	3	7,0%
	Chest pain	7	16,3%
	Fatigue	3	7,0%
	Diarrhea	2	4,7%
	Pruritus	5	11,6%
	Dyspnea	4	9,3%
	Paresthesia	1	2,3%
	Finger swelling	1	2,3%
	Syncope	1	2,3%
Treatment	Nontreatment	25	58,1%
	Antihistamines +steroid	9	20,9%
	Analgesic (NSAİD)	7	16,3%
	Adrenaline	2	4,7%
Hospitalized	Hospital admission	1	2,3%
	Discharged	42	97,7%

complaints not seeking emergency department care for relatively simple side effects.

The median time from vaccination to emergency department presentation was approximately 16 hours, with 25 patients undergoing observation without requiring treatment. This aligns with the prevailing understanding that the majority of COVID VAE occurs within the initial 48 hours [3-14].

Of significance, only 2 patients received adrenaline for anaphylaxis, and one necessitated hospitalization. Both patients, females, presented with dyspnea, were promptly attended by the code team from the vaccination center within the hospital, and received intramuscular adrenaline. Symptoms regressed without further intervention (intravenous adrenaline, vasopressors, etc.). Despite the incidence of anaphylaxis after routine vaccination being reported as approximately 1 in a million [10], our study indicates a higher rate compared to routine vaccination. This heightened risk is consistent with existing literature on COVID-19 vaccines [17,18]. However, considering the protective benefits and the low incidence of severe side effects, applying COVID-19 vaccination is deemed logical [9-14].

CONCLUSION

Our investigation reveals a higher incidence of Vaccine Adverse Events (VAE) associated with CoronaVac in females and individuals with a history of allergies. Importantly, the majority of these cases were uncomplicated and did not necessitate follow-up. As new variants of COVID-19 with the potential to evade existing vaccines and unforeseen pandemics may emerge in the future, preparations for novel mass vaccination strategies will be crucial. We anticipate that the insights from our study will serve as a valuable guide in shaping these preparations.

Limitations

Our study is not without limitations. First, we lack follow-up data for patients discharged from the emergency department, preventing an assessment of the duration of their symptoms and their resolution over time. Additionally, information about the specific units or departments where the healthcare workers, participants in our study, were employed is not available. These limitations warrant consideration when interpreting the results and may provide avenues for further investigation in future research endeavors.

Author contribution

Study conception and design: ÇY, Nİİ; data collection: ÇY, Nİİ; analysis and interpretation of results: ÇY, Nİİ; draft manuscript preparation: ÇY, Nİİ. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Ethics Committee No. 2 of Ankara City Hospital (Protocol no. E2-21-120/10.02.2021).

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Conflict of interest

The authors declare that there is no conflict of interest.

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acta medica

ORIGINAL ARTICLE

Comparison of blood gas parameters with hematological and biochemical parameters in critically III intensive care patients connected to mechanical ventilation

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ABSTRACT

Objective: The primary aim of this study was to compare blood gas parameters with laboratory parameters in critically ill intensive care patients who were connected to mechanical ventilation. Our secondary aim was to evaluate the effects of blood gas and laboratory parameters on mortality.

Materials and Method: Critical patients over the age of 18 who were admitted to intensive care unit from emergency room or clinics and monitored with mechanical ventilation between January 1, 2021, and December 30, 2021, were included in this study. Analyses were done with the R 4.3.1 (R Core Team) program. Statistical significance was defined by $p < 0.05$.

Results: A total of 150 patients were included in this study, and 42.6% of them were male. 62% of the men and 50% of the women in this study died. We studied the trend accuracy of laboratory hemoglobin, hematocrit, sodium, potassium, glucose and blood gas hemoglobin, hematocrit, sodium, potassium, and glucose values using a modified Bland–Altman analysis. When the analyses from the first day were examined, mean bias (LOA g/dl) for hemoglobin was 0.001 (–0.15 to 0.14) and mean bias (LOA mEq/l) for potassium was –0.19 (–0.23 to –0.16). When the third day analyses were examined, mean bias (LOA g/dl) for hemoglobin was 0.29 (–0.05 to 0.63) and mean bias (LOA mEq/l) for potassium was –0.25 (–0.29 to –0.21).

Conclusion: Hemoglobin and potassium, which are blood gas parameters, can be used instead of laboratory parameters in clinical decision-making in critically ill intensive care patients on mechanical ventilation.

Keywords: Blood gas parameters, hemoglobin, intensive care unit, mechanical ventilation, potassium.

INTRODUCTION

Blood gas analysis is performed in many clinical departments, such as emergency services, nephrology, pulmonology, general surgery, and pediatrics, as well as widely used for patient monitoring in critical intensive care units. It helps clinicians working in critical intensive care units to evaluate patients' vital functions, lung capacity, dialysis needs, and organ failure. However, it has been noted that the rate of unnecessary blood gas requests sometimes reaches up to 50% in intensive care units [1,2]. Although controversial, one study found that approximately 80% of blood gas analyses were requested without any indications [3]. The indications for blood gas analysis may also be only acid-base balance disorders, especially pCo2, hCO3, and lactate evaluation. The glucose, hematocrit, hemoglobin, potassium, and sodium values found in blood gas analyses should be used in clinical approaches instead of the values in hematology and biochemical tests [4].

While blood gas analyses use direct ion selectors, laboratory analyses use indirect ion selectors. Potassium measurements are affected by hemolysis in blood gas and laboratory analyses [5]. Studies have been conducted to compare blood gas analyses with hematological and biochemical tests and evaluate their effects on mortality [6–8]. There have also been studies in which similar comparisons were made for intensive care patients [9–11].

The primary aim of this study was to compare blood gas parameters with laboratory parameters in critically ill intensive care patients who were connected to mechanical ventilation. Our secondary aim was to evaluate the effects of blood gas and laboratory parameters on mortality.

MATERIALS AND METHOD

Study design

The retrospective study was conducted with the approval of the University of Karamanoglu Mehmetbey, Faculty of Medicine Ethics Committee (Date: 19/02/2023, Decision No: 2022-KAEK-154/19). This study was prepared following the directions in the Declaration of Helsinki. Critical patients over the age of 18 who were admitted to the intensive care unit from emergency room or clinics and monitored with mechanical ventilation between January 1, 2021, and December 30, 2021, were included in the present study. Patients who were not monitored with mechanical ventilation, received non-invasive mechanical ventilation, received mechanical ventilator treatment for less than three days, had laboratory test results that were found to be incomplete according to the parameters of this study, had a tracheostomy and received mechanical ventilator treatment for a certain period of time, were monitored due to trauma, and were under the age of 18 were excluded from the study. Patients who developed cardiac arrest and underwent cardiopulmonary resuscitation during follow-up were also excluded. The PCR test was performed on all patients included in the study. Patients with a positive PCR test were excluded in the study.

Data collection

Blood gas samples were obtained from patients accepted to the critical care unit using heparinized syringes (PICO50 Arterial Blood Sampler, Radiometer Medical ApS,Brønshøj,Denmark). The samples were studied with the blood gas analyzers used by the Karaman Education and Research Hospital (Siemens RAPIDPoint 500, Blood Gas Analyzer Siemens Healthcare Diagnostics Inc, Norwood, USA). These blood gas analyzers were calibrated six times each day. A hematology analyzer was used for the hematological samples (Mindray BC-6800 Auto Hematology Analyzer, Shenzhen, P. R. China). The ion-selective diluted technique was used for biochemical data(AU680 Clinical Chemistry Analyzer, Holliston, USA).

The demographic characteristics of these patients, how long it took them to be connected to mechanical ventilation, the biochemistry values taken on the first and third days of mechanical ventilation, and values for glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, calcium, creatinine, potassium, sodium, c-reactive protein, hemogram (hemoglobin, hematocrit), and venous blood gas (PH, PCO2, PO2, HCO3, glucose, sodium, potassium, hematocrit and hemoglobin) were simultaneously recorded. Biochemistry, hemogram, blood gas laboratory findings, and 30-day mortality information were obtained using the hospital data system. According

to Turkey's national death notification system, the patients were separated into two groups: those who survived (Group S) and those who non-survived (group D). The relationship between the parameters obtained on the first and third days of mechanical ventilation and mortality was evaluated. On the first day of mechanical ventilation, the glucose, potassium, sodium, hematocrit, and hemoglobin values obtained from the biochemistry laboratory and the glucose, potassium, sodium, hematocrit, and hemoglobin values obtained from blood gas were compared with mortality. The biochemistry and blood gas parameters on the third day of mechanical ventilation were also compared in terms of their relationship with mortality.

Statistical analysis

The descriptive statistics of the qualitative data were shown as percentage and frequency, and the quantitative data of the study were shown as median (Q1–Q3), mean, and standard deviation values. The suitability of quantitative variables for normal distribution was determined using the Shapiro–Wilk test. The Mann–Whitney U test was utilized to compare two independent groups of variables that were not normally distributed. Mixed-effects models were used in the analysis of numerical variables. Logistic regression analysis methods were used to find variables associated with mortality. For the logistic regression, univariable and multivariable logistic regression analyses were conducted. Bland-Altman charts and limits of agreement (LOA) values were used to match laboratory and blood gas values, and intraclass correlation coefficients (ICC) were calculated. Bland–Altman plots were utilized to evaluate bias and 95% LOA between the two measurement methods. A paired t-test was utilized to quantify the mean difference between the associated 95% confidence interval (CI) and the two measurement methods. The association between the number of tests per person per day and the variables of interest was evaluated utilizing univariable negative binomial regression models. These associations were noted as incident rate ratios (IRR) with a 95% CI. Analyses were done with the R 4.3.1 (R Core Team) program. $P < 0.05$ was described as the statistical significance level.

RESULTS

A total of 150 patients were included in this study, and 42.6% of them were male. 62% of the men and 50% of the women in this study died. Based on the data we obtained on the first day of being connected to a mechanical ventilator, a statistically significant association was detected between a high laboratory urea value and mortality ($p = 0.025$). There was a statistically significant association between a high APACHE score on the first day and a low Glasgow coma scale and mortality ($p < 0.001$, $p = 0.009$, respectively) (Table 1). The blood gas and laboratory results determined on the first day are shown in Table 2.

There was a statistically significant relationship between low pH and high lactate levels, which were the blood gas parameters taken on the third day of mechanical ventilation, and mortality ($p = 0.045$, $p = 0.009$, respectively). When the laboratory parameters taken on the third day were examined, there was a statistically significant relationship between low calcium and high c-reactive protein levels and mortality ($p = 0.016$, $p = 0.016$, respectively). The blood gas and laboratory results taken on the third day are shown in Table 3.

Table 1. Demograhic data

Variable	Group D, N = 77	Group S, N = 73	Total	P value
Age (mean, ±)	78.90±11.51	78.05±11.54	78.49±11.49	
Gender (n, %)				
Male	48 (62%)	38 (52%)	86 (57%)	
Female	29 (38%)	35 (48%)	64 (43%)	
Time until it is intubated (day)	2.99±6.60	2.18±4.53	2.59±5.68	
APACHE score (mean, ±)	28.11±7.47	24.04±6.40	26.13±7.24	*
Mortality (mean, ±)	60.44±18.60	49.32±18.20	55.03±19.17	*
Glaskow coma scale (mean, ±)	6.95±2.64	8.05±2.50	7.49±2.63	**0.009**

(BG: blood gas, ALT; alanine aminotransferase, AST; aspartate aminotransferase; APACHE: Acute Physiology and Chronic Health Evaluation) * $p < 0.05$ was described as the statistical significance level.

Table 2. 1st day blood gas analyzes and 1st day laboratory analyzes and 30-day mortality of patients

	Group D, N = 77	Group S, N = 73	Total	P value
1st day blood gas analyzes (mean, ±)				
PH	7.40±0.11	7.41±0.10	7.41±0.10	
pCO2	41.91±11.63	41.87±10.18	41.89±10.91	
pO2	48.97±13.70	47.00±11.01	48.01±12.46	
HCO3	25.69±6.68	26.81±5.60	26.24±6.19	
Lactate	1.91±1.48	1.62±1.23	1.77±1.36	
Ionised Calcium (mg/dl)	1.05±0.09	1.07±0.12	1.06±0.10	
Carboxyhemoglobin (%)	0.82±0.50	0.85±0.47	0.83±0.49	
BG Hemoglobin (g/dl)	10.91±2.53	10.75±2.28	10.83±2.40	
BG Hematocrit (%)	32.56±7.39	32.04±6.55	32.31±6.97	
BG Sodium (mEq/l)	138.62±5.98	137.35±7.07	138.01±6.54	
BG Potassium (mEq/l)	3.79±0.71	3.71±0.70	3.75±0.70	
BG Glucose (mmol/l)	153.08±51.96	143.88±44.83	148.60±48.68	
1st day laboratory analyzes (mean, ±)				
Blood urea nitrogen (mg/dL)	94.61±59.69	75.46±43.26	85.29±53.05	**0.025***
Creatinine (mg/dL)	1.66±1.25	1.53±1.04	1.60±1.15	
ALT (IU/L)	18.00(11.00-36.00)	22.00(11.00-31.00)	30.00(18.00-47.75)	
AST (IU/L)	32.00(18.00-51.00)	26.00(18.00-41.00)	19.50(11.00-35.00)	
Calcium(mg/dL)	7.85±0.66	8.05±0.68	7.95±0.68	
Clor(mg/L)	104.79±6.91	103.89±7.02	104.35±6.95	
C-reactive proteine (mg/L)	120.60(57.50-174.90)	95.20(29.80-147.30)	106.50(40.70-164.90)	
Hemoglobin (g/dl)	10.99±2.33	10.68±2.20	10.84±2.26	
Hematocrit (%)	33.47±7.97	32.64±6.85	33.07±7.43	
Sodium (mEq/l)	141.65±6.59	140.71±6.56	141.19±6.57	
Potassium (mEq/l)	3.98±0.69	3.91±0.65	3.95±0.67	
Glucose (mmol/l)	160.00±52.60	149.65±45.98	154.96±49.60	

(BG: blood gas, ALT; alanine aminotransferase, AST; aspartate aminotransferase)
* $p < 0.05$ was described as the statistical significance level.

In the regression analysis in which Glasgow coma score (GCS), APACHE scores, laboratory urea, and calcium values taken on the first day were included, the APACHE score was the determining factor (Odds Ratio (OR)= 1.06, 95% CI = 1.00–1.13, p = 0.045). In the regression analysis, which included GCS, Apache scores, blood gas pH taken on the third day, lactate, laboratory c-reactive protein, and laboratory calcium values, it was determined that blood gas lactate values were predictive of mortality (OR = 1.55, 95% CI = 1.02–2.65, p = 0.037). (Table 4).

While the blood gas hematocrit value decreased on the third day in all patients, the decrease in deaths was statistically significant (p = 0.006). The blood gas hemoglobin value also decreased on the third day but was statistically insignificant (p = 0.522, p = 0.881, respectively). While there was an increase in blood gas sodium values in Group D and Group S on the third day, it was statistically insignificant (p = 0.271, p = 0.273, respectively). A statistically significant decrease was observed in blood gas potassium values in both groups on the third day (p = 0.027, p = 0.008, respectively). While a decrease was observed in laboratory hemoglobin and hematocrit values on the third day in both groups, the hemoglobin and hematocrit decrease in Group D were statistically significant (p < 0.001, p = 0.004, respectively). There was an increase in laboratory sodium values on the third day in both groups, with the sodium increase in Group S being statistically significant (p = 0.108, p = 0.038, respectively). There was an increase in the laboratory potassium value in all patients on the third day, and the increase in potassium in Group S was statistically significant (p = 0.087, p = 0.050, respectively). While there was an increase in laboratory glucose levels in both groups on the third day, it was statistically insignificant (p = 0.537, p = 0.703, respectively).

Table 3. Third-day blood gas analyzes, 3rd day laboratory analyzes, and 30-day mortality of patients

Variable	Group D, N = 77	Group S, N = 73	Total, N=150	P value
3rd day blood gas analyzes				
Ph	7.43±0.09	7.46±0.07	7.44±0.08	**0.045**
pCO2	41.19±10.08	40.22±9.80	40.72±9.93	
pO2	46.86±11.91	46.27±10.95	46.57±11.42	
HCO3	27.16±7.34	28.27±6.59	27.70±6.99	
Lactate	1.99±1.78	1.42±0.57	1.71±1.36	**0.009**
Ionised Calcium (mg/dL)	1.07±0.10	1.08±0.09	1.07±0.10	
Carboxyhemoglobin (%)	0.81±0.46	0.78±0.42	0.80±0.44	
BG Hemoglobin(g/dl)	10.72±3.76	10.70±2.08	10.71±3.05	
BG Hematocrit (%)	30.90±7.24	31.99±6.33	31.43±6.81	
BG Sodium(mEq/l)	139.49±6.57	138.25±5.90	138.89±6.26	
BG Potassium(mEq/l)	3.60±0.70	3.48±0.73	3.54±0.71	
BG Glucose (mmol/l)	159.22±52.42	146.26±49.48	152.91±51.25	
3rd day laboratory analyzes				
Blood urea nitrogen (mg/dL)	98.84±61.22	83.37±55.93	91.31±59.02	
Creatinine (mg/dL)	1.62±1.29	1.49±1.24	1.56±1.27	
ALT (IU/L)	23.0(12.0-46.0)	21.00(10.0-42.0)	21.00(11.0-43.0)	
AST (IU/L)	32.0(17.00-59.0)	26.00(17.0-54.0)	30.50(17.0-57.75)	
Calcium(mg/dL)	7.75±0.58	7.98±0.57	7.86±0.59	**0.016**
Klor(mg/L)	106.21±7.33	105.00±6.48	105.62±6.93	
C-reactive proteine (mg/L)	104.10(57.7-186.5)	92.50(31.0-132.4)	95.00(44.93-141.98)	**0.016**
Hemoglobin(g/dl)	10.33±2.27	10.51±2.06	10.42±2.16	
Hematocrit (%)	31.84±7.20	31.96±6.25	31.90±6.73	
Sodium(mEq/l)	142.80±6.65	142.24±5.76	142.53±6.22	
Potassium(mEq/l)	3.84±0.68	3.74±0.66	3.79±0.67	
Glucose (mmol/l)	163.90±54.58	152.12±52.53	158.17±53.74	

(BG: blood gas, ALT; alanine aminotransferase, AST; aspartate aminotransferase)

Table 4. Multivariate regression analysis of Glasgow coma scale, APACHE score, 1st day blood urea nitrogen, calcium and 3rd day PH, lactate, c-reactive protein, calcium

Variable	OR	95% CI	P value
1st day Glasgow coma scale	0.91	0.78-1.05	0.2
1st day APACHE score	1,06	1.00-1.13	0.045
1st day blood urea nitrogen (mg/dL)	1.00	1.00-1.01	0.3
1st day calcium (mg/dL)	0.65	0.37-1.09	0.10
Variable	OR	95% CI	P value
1st day Glasgow coma scale	0.87	0.74-1.02	0.082
1st day APACHE score	1,05	0.98-1.12	0.14
3rd day PH	0.01	0.00-1.29	0.064
3rd day lactate	1,55	1.02-2.65	0.037
3rd day c-reactive protein (mg/L)	1.00	1.00-1.01	0.2
3rd day calcium (mg/dl)	0.52	0.26-1.01	0.052

(APACHE: Acute Physiology and Chronic Health Evaluation)

We studied the trend accuracy of laboratory hemoglobin, hematocrit, sodium, potassium, glucose, and blood gas hemoglobin, hematocrit, sodium, potassium, and glucose values using a modified Bland–Altman analysis. When the analyses from the first day were examined, the mean bias (LOA g/dl) for hemoglobin was 0.001 (–0.15 to 0.14) (Figure 1), and the mean bias (LOA mEq/l) for potassium was –0.19 (–0.23 to –0.16) (Figure 2). When the third-day analyses were examined, the mean bias (LOA g/dl) for hemoglobin was 0.29 (–0.05 to 0.63) and the mean bias (LOA mEq/l) for potassium was –0.25 (–0.29 to –0.21) (Table 5).

DISCUSSION

In this study, we compared blood gas and laboratory parameters taken on the first and third days in critically ill intensive care patients who were connected to mechanical ventilation. We found that the hemoglobin and potassium values taken on the first day were compatible with the blood gas and laboratory results. However, when we looked at the blood gas and laboratory values taken on the third day, a higher agreement was achieved in the blood gas and laboratory values of hemoglobin and potassium compared to hematocrit, sodium, and glucose.

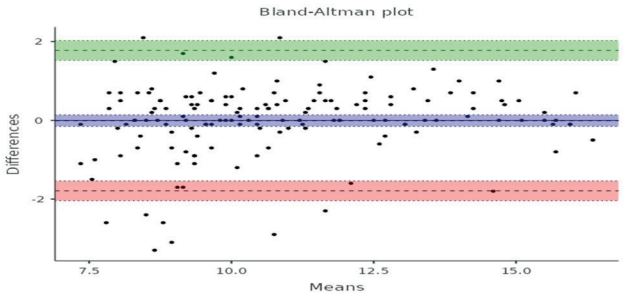

Figure 1. Bland-Altman Scatter plots for the means and differences of laboratory analyzer and blood gas analyzer first-day hemoglobin values.

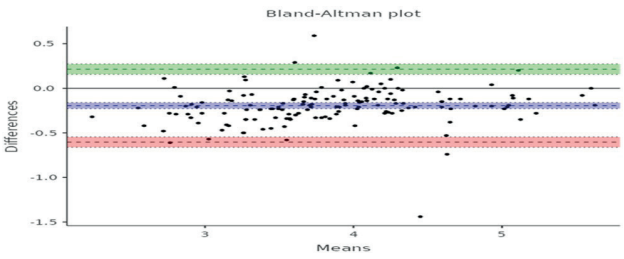

Figure 2. Bland-Altman Scatter plots for the means and differences of laboratory analyzer and blood gas analyzer first-day potassium levels.

We know that blood gas evaluation is important for clinicians to manage early treatment, especially in critically ill intensive care patients who are on ventilators. A similar situation applies to emergency services. However, various results were acquired in the literature in studies comparing blood gas and laboratory parameters [6,8].

When glucose, hematocrit, hemoglobin, potassium, and sodium values were examined in a study conducted in an emergency room, it was observed that there was no correlation between blood gas and laboratory parameters in any of these values [6]. In another emergency room study on glucose, hematocrit, hemoglobin, sodium, and potassium values, a correlation was detected between laboratory and blood gas analyses [8]. In a study, which was planned to be multicentric, of 584 intensive care patients, a high correlation was detected between blood gas CO_2 and serum CO_2 values [9]. There are different results in the literature when comparing sodium values with blood gas and laboratory analyses [5,10,11]. In another study conducted on critically ill intensive care patients, no statistically significant relationship was detected between blood gas sodium and potassium values and sodium and potassium values in hypernatremic patients [5]. On the other hand, another study observed that the sodium and potassium values obtained in blood gas analyses were correlated with sodium and potassium in laboratory parameters [10]. In a study conducted on patients with heart and lung diseases, no statistically significant difference was observed

Table 5. Bland–Altman analysis of blood gas and laboratory parameters

Variables	Bias	95% Confidence Interval	
		Lower	Upper
1st day values			
Hemoglobin(g/dl)	-0.01	-0.15	0.14
Hematocrit (%)	-0.76	-1.32	-0.19
Sodium(mEq/l)	-3.19	-3.76	-2.61
Potassium(mEq/l)	-0.19	-0.23	-0.16
Glucose (mmol/l)	-6.36	-8.70	-4.03
3rd day values			
Hemoglobin(g/dl)	0.29	-0.05	0.63
Hematocrit (%)	-0.47	-0.97	0.03
Sodium(mEq/l)	-3.64	-4.02	-3.25
Potassium(mEq/l)	-0.25	-0.29	-0.21
Glucose (mmol/l)	-5.26	-6.95	-3.56

between arterial blood gas and laboratory sodium values in critical intensive care unit patients with lung disease; blood gas sodium values in patients with heart disease were statistically significantly higher than laboratory sodium values [11]. In a prospective observational study conducted on 219 intensive care patients, there was no statistically significant difference between blood gas and laboratory values in terms of hemoglobin, as in our study, but, unlike in our study, potassium and sodium values were statistically significantly lower in blood gas analyses than in laboratory analyses, and the average bias was small for all three values [12]. In a multicentrically planned study, it was shown that the sodium and potassium values obtained from blood gases could be used in clinical decision-making instead of laboratory parameters [13]. In a study involving 200 patients, statistical significance was detected with an acceptable bias between the blood gas parameters of potassium and sodium and laboratory parameters; no statistically significant relationship could be detected between blood gas and laboratory hemoglobin values [14].

While this study, unlike other studies, showed that the blood gas parameter values of hematocrit, sodium, and glucose were not compatible with laboratory parameters, the association between blood gas and laboratory parameters and mortality was also evaluated. We found that the values on the first and third days of hemoglobin, hematocrit, sodium, potassium, and glucose did not have a statistically significant association with mortality. Alternatively, we observed that the decrease in blood gas hematocrit, laboratory hematocrit, blood gas potassium, laboratory potassium, and laboratory haemoglobin values was statistically significant on the third day in deceased patients compared to on the first day. Although there was a decrease in blood gas hemoglobin values in those who died, it was not statistically significant.

Limitations

In this study, we did not correlate the duration of hospital stays with the parameters. While we tried to reduce the risk of errors by including only patients connected to mechanical ventilation, the possibility of missing clinical conditions, such as multiple organ failure, and treatments, such as fluid and blood transfusions, were among the factors that affected this study. Although we included patients over the age of 18 and there were also patients under the age of 65, the mean age was over 65 years of age in our study.

In conclusion, hemoglobin and potassium, which are blood gas parameters, can be used instead of laboratory parameters in clinical decision-making in critically ill intensive care patients on mechanical ventilation. Laboratory parameters must be used for sodium, hematocrit, and glucose values.

Author contribution

Study conception and design: AB and HŞA; data collection: AB; analysis and interpretation of results: HŞA; draft manuscript preparation: AB and HŞA. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the University of Karamanoglu Mehmetbey, Faculty of Medicine Ethics Committee (Date: 19/02/2023, Decision No: 2022-KAEK- 154/19).

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Conflict of interest

The authors declare that there is no conflict of interest.

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ORIGINAL ARTICLE

How difficult is it to diagnose and report an occupational disease in a developing country? A modified delphi study

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ABSTRACT

Aim: The roles of occupational medicine specialists are very important in the diagnosis and notification of occupational diseases. In this regard, the opinions of the parties are needed to identify the problems and propose solutions.

Methods: This is a Modified Delphi exercise. It was conducted through an online survey in 2 rounds in Turkey between June 4, 2021 and August 31, 2021. The population of the research consists of occupational medicine specialists and sub-branch students in Turkey and some physicians working in the central organizational units of the Ministry of Health and the Ministry of Labor and Social Security regarding the diagnosis, registration and notification of occupational diseases. In both rounds, e-mails were sent to 127 people. The survey was sent via , and the response frequency was 27.5% and 18.1% in rounds 1 and 2, respectively. A survey form was created for the second round by evaluating the open-ended information obtained as a result of the first round using the thematic analysis method and making suggestions. This form was sent to participants via e-mail and questions were asked about participation in the proposals in the second round. Proposals with 70% or more participation were accepted unanimously.

Results: The most important difficulties regarding the diagnosis and details of occupational diseases are; The lack of a national occupational disease surveillance system, the fact that the current occupational disease diagnosis and summary system is focused on pricing, and the difficulties people experience in diagnosis were determined.

Conclusion: In order to increase the reporting of occupational diseases, a surveillance-oriented system should be switched instead of a compensation-based system. In addition, the deficiencies in manpower, financing, technical infrastructure and legislation in the occupational disease reporting system need to be rapidly revised.

Keywords: Occupational disease, diagnosis, recording, reporting, notification.

INTRODUCTION

Occupational disease, as defined by the World Health Organization (WHO), is the common name of diseases that are related to exposure to factors in the workplace environment [1]. The International Labor Organization (ILO); defines occupational diseases as diseases contracted as a result of workplace exposures [2]. According to the ILO, 2 million people die every year in the world due to working conditions. About 6300 workers die from work-related causes every day, of which 5300 are due to work-related diseases. ILO also estimates that there are 160 million non-fatal work-related diseases [2].

It is obligatory to record and report occupational diseases in Turkey. According to the laws, every physician who suspects an occupational disease is obliged to refer the patient to an authorized hospital. These hospitals are; occupational disease hospitals, university hospitals and training and research hospitals. When these hospitals diagnose a patient with an occupational disease, they are authorized and obliged to notify the Social Security Institution (SSI), which is the only institution that receives these notifications. SSI evaluates an occupational disease decision, regulates the compensation and explains the number of indemnified cases per year. Neither workplace physicians nor other physicians working in primary or secondary care have the authority to notify SSI directly [3,4].

The number of occupational diseases is expected to be between 0.4 and 1.2 percent of employment, globally [5]. According to SSI data in our country, it is seen that occupational accidents are at significant levels, and the number of occupational diseases is much lower than expected [6]. According to the estimations, the number of occupational diseases should be between 64,040 and 192,120 in 2019, however, it was announced as 1088 [7]. There are also loss of data that are not reflected in the SSI statistics and occur as a result of work accidents and occupational diseases that are not covered and are not notified. In addition, it is known that the data on occupational diseases are only from the cases that have been decided to be compansated. The number of medical diagnoses of occupational diseases have not yet been collected and announced in Turkey. These statistics show that are problems in the detection and notification of occupational diseases, and that result-oriented preventive work should be done in this direction.

Opinions and roles of the occupational health professionals working in the field of diagnosis and notification of occupational diseases are very important. In this respect, the opinions of the parties are necessary in order to determine the problems in the diagnosis and notification of occupational diseases and to propose solutions. In this research, we aimed to evaluate the opinions of the professionals working in this field in Turkey and to reach a consensus on the solutions on this issue. Therefore, we aim to understand the problems and possible solutions about underreporting of occupational diseases.

METHODS

This study is a modified Delphi type qualitative research. It was conducted in 2 rounds between 4 June 2021 and 31 August 2021 in Turkey, via online survey. The researchers who carried out the study are academicians and specialists in the occupational medicine specialty training program. As Delphi construction experts, the researchers carried out the study by forming the questions, determining the research group, evaluating the open-ended questions' answers and creating a questionnaire according to the themes.

The population of the research is occupational medicine training program trainers, specialists and subspecialty students in Turkey and some physicians working in the organizational units of the Ministry of Health and Ministry of Labor and Social Security related to the diagnosis, recording and notification of occupational diseases. No sample selection was made. The invitation to participate was sent to the entire universe via e-mail. The levels of reaching the universe are presented in Table 1 together with the socio-demographic information of the participants at each stage of the research. Questionnaires were sent to 127 people in both rounds, with a response frequency of 27.5% and 18.1%, respectively, in the 1st and 2nd rounds.

In the first round of the research, the researchers held a meeting in terms of basic content via online panel.

Open-ended questions were formed on the topics that are recommended to be included as priority in Turkey on the subject of diagnosis and notification of occupational diseases. The questionnaires were sent to the participants electronically (by sending 2 reminders per week) and a response was expected within 2 weeks. A questionnaire form was created for the second round by evaluating and classifying the open-ended information obtained as a result of the first round with thematic analysis method and forming propositions.

This form was sent to the participants via an online questionnaire and they were asked about their participation in the propositions in the 2nd round. Response was expected within 2 weeks (by sending 2 reminders per week) after the 2nd round questionnaire was sent. Propositions with 70% or more participation were accepted as consensus (8).

Ethics committee approval was received from Hacettepe University Non-Interventional Clinical Research Ethics Committee (04.05.2021, GO 21/483). Informed consent was obtained from the participants before the survey.

RESULTS

The sociodemographic characteristics of the participants and their working status are summarized in Table 1.

Compensation-oriented system by the SSI (Consensus level (CL): 89.58%), hiding the occupational diseases due to dismissal, fear stigmatization, loss of income and similar concerns of the employee diagnosed with an occupational disease (CL: 86.46%), lack of occupational disease surveillance of the Ministry of Health (MoH) and not being active in the diagnosis and notification processes (CL: 84.38%), insufficient knowledge and experience of physicians working in primary and secondary healthcare on occupational diseases (CL: 82.29%), and inability to diagnose occupational diseases (CL: 80.21%), lack of an institutional strategy of Ministry of Health regarding the employment of occupational medicine specialists (CL: 81.25%), the lack of recognition of the occupational medicine specialists in the field (CL: 80.21%), the low number of occupational medicine specialists (CL: 76.04%), employees hesitancy to apply for the diagnosis of occupational disease due to the long and difficult diagnosis and notification processes (A CL L: 75.0%), lack of qualified manpower and testing utilities for the diagnosis of occupational disease in authorized health institutions (CL: 75.0%), not performing standard and appropriate periodic examination follow-ups in the workplace (CL: 73.96%), and insufficient training on occupational diseases in the medical education processes (CL: 69.8%) are obstacles and weaknesses in our country, regarding diagnosis and notification of occupational diseases. Although it is thought that there are difficulties in diagnosis due to the necessity of a health committee

Table 1. The sociodemographic characteristics of the participants and their working status

		1st Round (n=35)	2nd Round (n=23)
Gender	Female	%45,7 (n=16)	%43,4 (n=10)
	Male	%54,3 (n=19)	%56,6 (n=13)
Age	(Mean±SD)	48.8 ± 10.6	49.5±10.7
Education	Faculty of Medicine	%20,0 (n=7)	%30,4 (n=7)
	Specialty in Medicine	%42,8 (n=15)	%56,5 (n=13)
	Subspecialty in Medicine	%22,8 (n=8)	%4,3 (n=1)
Specialty Area	Pulmonary Medicine	%40,0 (n=5)	%53,8 (n=7)
	Public Health	%46,6 (n=7)	%30,8 (n=4)
	Internal Medicine	%13,4 (n=1)	%15,4 (n=2)
Institution	University	%51,4 (n=18)	%52,1 (n=12)
	Ministry of Health	%22,8 (n=10)	%13,0 (n=3)
	Ministry of Labor	%5,7 (n=3)	%4,3 (n=2)
Position	Program coordinator and lecturer	%45,7(n=16)	%39,1 (n=9)
	specialty in Medicine	%14,2 (n=5)	%8,6 (n=2)
	Subspecialty Fellow	%17,1 (n=6)	%21,7 (n=5)

SD: Standard Deviation

report for the diagnosis of an occupational disease, therefore it is among the obstacles and weaknesses of diagnosis and notification of occupational diseases, a sufficient level of consensus on this issue has not been achieved (CL: 66.7%).

The development of occupational medicine specialty training programs and the increase in the number of competent occupational medicine specialists (CL: 78.13%), the employment of occupational physicians and other health personnel in workplaces (CL: 73.96%), the fact that occupational diseases are an open field for scientific research (CL: 72.92%) are strengths and opportunities regarding diagnosis and notification of occupational diseases in our country.

The approval of the ILO conventions and agreements on occupational health and safety and occupational disease by Turkey and structuring the legislation (Law No. 6331) (CL: 68.75%), the presence of hospitals authorized to diagnose occupational diseases (occupational hospital, training and research hospitals, universities, etc.) (CL: 65.63%), the obligation of recording and notification of occupational diseases in the workplaces (CL: 62.50%), the presence of the Association of Occupational Medicine Specialists (CL: 60.42%), contribution of the related institutions and organizations (unions, universities, associations, non-governmental organizations) to the diagnosis, recording and notification of occupational diseases (CL: 59.38%), emerging of occupational diseases as a current issue during pandemic (CL: 59.38%), increasing awareness of physicians in this area (CL: 56.25%), increase in occupational hygiene measurement opportunities (CL: 55.21%), having a good information technology infrastructure in the field of occupational health and safety (CL: 50.00%), having an open list in the diagnosis of occupational disease (CL: 46.88%) are considered as strengths and opportunities regarding diagnosis and notification of occupational diseases, however, there is no consensus among the participants on these issues.

About the aspects that need to be developed in our country regarding diagnosis and notification of occupational diseases, it has been suggested by the participants that different systems should be developed for diagnosing occupational diseases for informal sectors (CL: 83.33%), an occupational health institute should be established (CL: 79.17%), and the association of occupational medicine specialists should work more actively in professional organizations (CL: 76.04%).

Solution proposals agreed by the participants regarding the legislation (scope, definition, etc.) in the diagnosis and notification of occupational diseases are; standardizing the definition of occupational disease for the public and private sectors (CL: 88.54%), developing an algorithm for the diagnosis of occupational diseases and updating the occupational diseases list (CL: 86.46%), increasing the authority of occupational medicine specialists to diagnose occupational diseases (CL: 83.33%), removing the disability condition for the diagnosis of occupational disease (CL: 80.21%), developing a mandatory quota system to increase the employment of patients diagnosed with an occupational disease (CL: 76.04%), and giving incentives to those who employ workers diagnosed with an occupational disease (CL: 72.92%), the determination of the health institutions authorized to diagnose occupational diseases according to the needs in their regions (CL: 84.38%) and incentives to the workplace physicians who make occupational disease referrals and those who prepare patient files (CL: 76.04%). Increasing the awareness and knowledge level of employers on work accidents and occupational diseases and developing approaches to prevent them from refraining from reporting work accidents and occupational diseases (CL: 88.54%) are the solutions for the roles of employers in the diagnosis and notification of occupational diseases, according to the participants.

Solutions for the roles of OHS professionals in the diagnosis and notification of occupational diseases are; occupational health and safety (OHS) professionals' awareness to perform their duties in accordance with national and international standards (CL: 88.54%), the support of scientific research in workplaces (CL: 87.50%), and working with different institutions to promote the field (Ministry of Labor and Social Security, The Ministry of Health, universities, etc.), and making efforts together (CL: 84.38%), ensuring professional independence (CL: 82.29%), and paying their wages through a public audit fund to be established by employers (CL: 76.04%).

Suggestions for solutions agreed by the participants regarding the roles of employees and unions in the diagnosis and notification of occupational

diseases are; increasing the awareness and level of knowledge of employees on sector-specific occupational diseases (AL: 89.58%) and ensuring a good union organization in terms of occupational safety and health (CL: 87.50%).

According to the participants, problems related to employment conditions and macroeconomic conditions in the diagnosis and notification of occupational diseases are; the workers diagnosed with occupational diseases are not preferred for employment due to the increase in unemployment (CL: 81.25%) and increase in informal work due to the increase in vulnerable groups (immigrants, etc.) (CL: 80.21%). A consensus was reached on the establishment of vocational rehabilitation programs (CL: 87.50%) as a solution proposal.

Solution suggestions regarding the SSI system and its applications in diagnosis and notification of occupational diseases are; facilitating bureaucratic practices (CL: 89.58%), taking a more active role in vocational rehabilitation and return to work (CL: 81.25%), and to have another decision-making authority other than compensation at the stage of diagnosing occupational diseases (CL: 81.25%).

Suggestions for workplace inspections in diagnosis and notification of occupational diseases are; to increase the number of labor inspectors originating from health personnel (CL: 90.63%), compliance inspection of workplace health surveillance (CL: 88.54%) and occupational hygiene measurements (CL: 87,50%), regular inspections in workplaces where no notification of work accidents and occupational diseases are made (CL: 86.46%) and improvement of income and working conditions of labor inspectors (CL: 76.04%).

Suggestions for health and workplace surveillance in diagnosis and notification of occupational diseases are; establishment of a follow-up system for occupational safety and health monitoring and workplace surveillance (CL: 90.63%), increasing and disseminating occupational hygiene laboratory facilities (CL: 87.50%), providing training to OSH professionals on this subject (CL: 87.50%) and standardization of workplace measurements and health surveillance by developing guidelines (CL: 86.46%).

Solution proposals for manpower in the field of OSH in diagnosis and notification of occupational diseases are; increasing the number of qualified OSH professionals (CL: 86.46%), training of occupational health nurses (CL: 86.46%) and technicians (CL: 83.33%).

In general, there are several deficiencies and insufficiencies in each authorized institution regarding the adequacy and accessibility of subjects such as manpower, laboratory and technical equipment required for the preliminary diagnosis or diagnosis of occupational diseases (CL: 77.08%).

All propositions and consensus levels are given in Appendix.

DISCUSSION

In this study, the absence of a national occupational disease surveillance system and the compensation-oriented process of the current occupational disease diagnosis and notification system, the difficulties experienced by the employees in the diagnosis-notification process of the occupational diseases (preparation of a health board report) and their concerns (dismissal, stigma, loss of income, etc.) have been identified as the most important difficulties regarding to diagnosis and notification of occupational diseases. Problems and obstacles in the occupational disease diagnosis-notification system are schematized and presented in Figure 1.

Diagnosis and notification of occupational diseases

Many European Union countries have national registry systems where occupational diseases are registered and reported. In addition, some countries have established additional surveillance systems [8,9]. In most European Union countries, national systems are linked to social insurance systems established for compensation for occupational diseases [10]. Therefore, the progress of diagnosis and notification of occupational diseases through the social insurance system is not unique to our country. However, in this situation, where the experts participating in our research have reached the highest level of consensus, the compensation-oriented data collection systems are insufficient to provide the data required for the preventive approach for occupational diseases. Therefore, many international occupational health experts recommend using additional and complementary

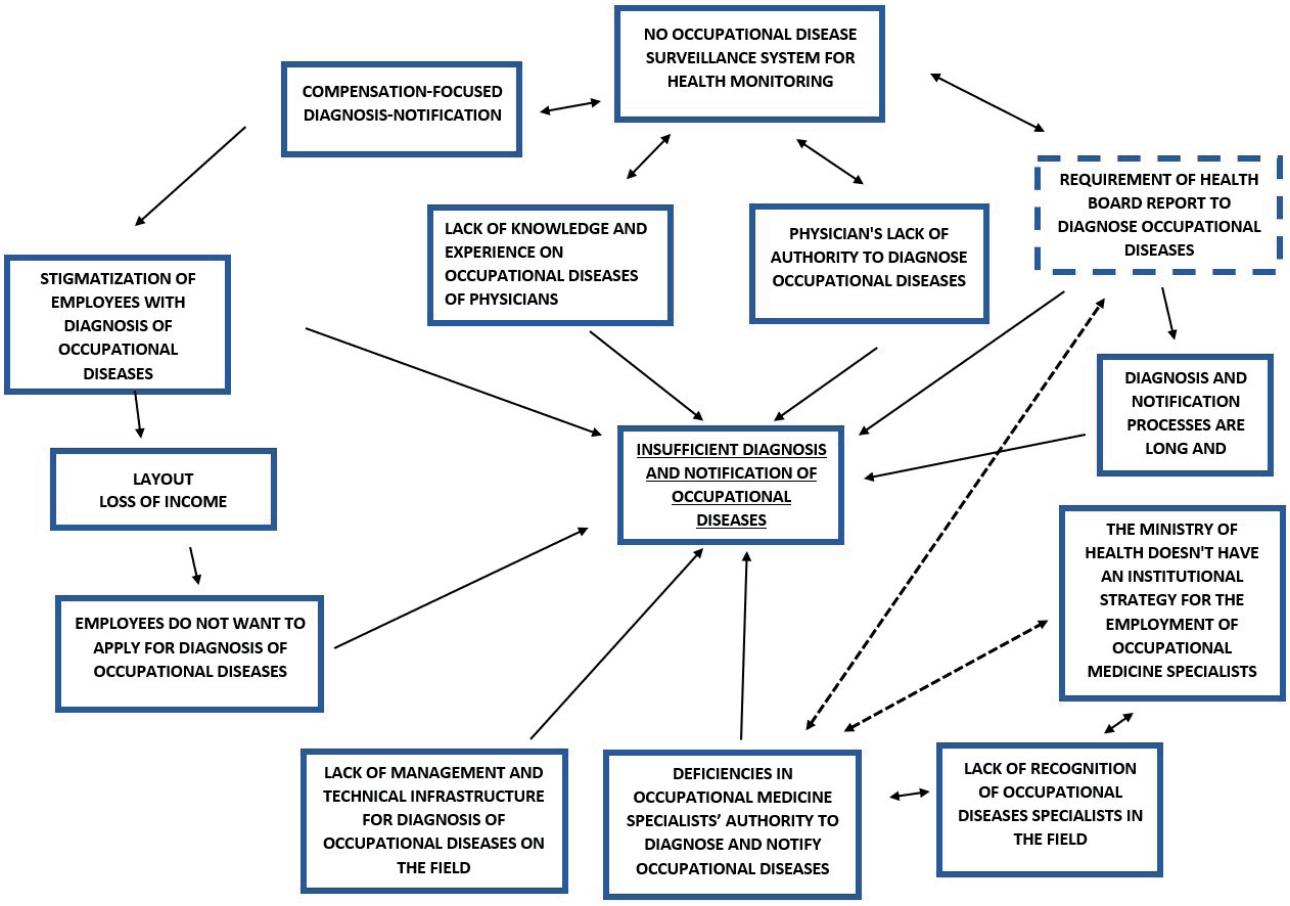

Figure 1. Problems in the diagnosis and notification system

surveillance systems that will provide the necessary data to evaluate occupational health conditions and understand health effects, necessary to prevent occupational diseases [10-12]. For example, reporting systems designed for a scientific purpose, such as Finland and the UK, were found to be more successful in monitoring occupational diseases when compared to countries using compensation-based systems [13]. In addition, compensation-oriented surveillance systems make cross-country comparison impossible [9]. *The Ministry of Health's lack of occupational disease surveillance system and insufficient involvement in the process* statement draws attention here, in which a consensus was reached between the participants under obstacles and weaknesses section, in our research. Indeed, it is important for the Ministry of Health to develop a prevention-oriented surveillance system, in cooperation with the relevant parties, in order to solve the problem. For example, the MALPROF surveillance system implemented in Italy is not focused on compensation, but aims to detect and prevent occupational diseases and work-related diseases early. In this system, all physicians can make a preliminary diagnosis of occupational disease directly, and the insurance institution re-evaluates those in need of compensation. For this, centers have been established throughout the country and necessary technical and manpower infrastructure has been provided [14]. It is important to facilitate the diagnosis and notification processes of occupational diseases, to prepare the technical infrastructure and to train qualified physicians who are capable of diagnosing occupational diseases in order to establish similar surveillance systems.

Training of physicians on occupational diseases

The inability of physicians to diagnose occupational diseases and their insufficient knowledge and experience on this subject are among the important findings of our study. This situation shows that the training of physicians is very important for the reporting of occupational diseases, as emphasized in other studies [15,16]. In a study conducted in European Union countries, it was observed that there were deficiencies in the involvement of physicians in the diagnosis and notification processes. However, it has been observed that there is a need to improve the training of these notifying physicians [13]. In another study, it

was recommended to conduct a more intensive communication process in order to ensure the participation of these physicians and to increase the compliance of the reporting physicians within the system. To achieve this, it has been proposed to provide training, financial incentives and practical support [10]. In a campaign to increase the awareness of physicians, trainings were provided and notification forms were introduced to familiarize physicians with notification procedures. Interestingly, although these trainings provide benefits in the acute period, they lose their effects in the long term [17].

In this context, in our research, the development of occupational medicine specialty training programs and the spread of physicians with this expertise is emphasized and a consensus has been reached. This situation is similar in many developed countries, but occupational medicine training is required for physicians in other specialties [10]. Because the participation of all physicians in the occupational disease notification process has been deemed necessary for a good notification system structurally [9,18]. In a study, it was determined that there is a high rate of non-reporting among workplace physicians [19]. Another study, when examining the diagnosis and notification processes of occupational physicians, showed that these practices can be improved with information support, training and practical tools [10]. To this end, developing evidence-based guidelines for the use of physicians in notification processes and educating physicians to use them will increase the quality of occupational disease reporting [10]. Such guidelines will also serve to prevent the aggravation of occupational diseases [20].

Considering all these points, it is important that occupational medicine specialists receive a good training and become widespread in health services. This situation creates an opportunity to increase the awareness of other physicians working in tertiary health services. However, among the topics with a high level of consensus "*the lack of an institutional strategy of the Ministry of Health regarding the employment of occupational and occupational diseases specialists*" and perhaps as a result of this "*Lack of recognition of the field of occupational and occupational diseases expertise in the field*" are important problems. Occupational medicine specialization is a fragile field and it is in danger of losing its effectiveness unless their employment and personal rights are supported [21,22].

In addition to all these difficulties, employees may not want to be diagnosed with an occupational disease because they are faced with many problems such as dismissal, stigma, and inability to maintain employment after being diagnosed with an occupational disease [23]. For this reason, it is necessary to take some public measures to prevent employees from being disadvantaged after being diagnosed with an occupational disease.

Roles of the government, employer and employee in the diagnosis of Occupational Diseases

In the section of solution proposals for the roles of the employer, a high level of consensus was achieved on the propositions: "*Awareness and knowledge levels of employers should be increased on work accidents and occupational diseases" and "Solutions should be found to prevent their hesitation from reporting work accidents and occupational diseases*". In a study on this situation, the importance of examining and detecting the behaviors of employers and employees that will prevent recording and notification, as well as studies on the behaviors of physicians related to occupational diseases, was emphasized [13]. Because the preference of the employee/patient is as important as the awareness of the physician in the notification of occupational disease [24]. As one of the employer-related situations, it has been shown that the employer avoids reporting due to fear of punishment [25].

One of the issues in which a significant level of consensus was reached in our research is the proposition that "*an employee diagnosed with an occupational disease may hide his/her occupational diseases due to dismissal, stigma, loss of income and similar concerns*". This was particularly emphasized in a review that highlighted the barriers to reporting occupational diseases [23]. In several studies investigating the lack of reporting in occupational skin diseases, it has been shown that stigma and fear of losing one's job after diagnosis prevented occupational disease reporting [25,26].

One of the important issues, on which a consensus was reached in our research, was solution proposals related to the legislation (scope, definition, etc.)

in the diagnosis and notification of occupational diseases. A high level of consensus has been achieved in almost all propositions under this heading. This shows that there is indeed a need for improvement in this regard. However, it should be noted that strict rules and regulations do not result in an improvement in the reporting of diseases unless good communication is provided. The low number of occupational diseases is an indicator of this situation [27]. For example, in many countries where notification of occupational diseases is legally required, notification of occupational diseases is very insufficient [28]. Moreover, systems that require a health board report during the notification and compensation processes place the burden of proof on the patient, creating a new obstacle in reporting [28]. However, it is certain that eliminating the deficiencies and contradictions in the legislation regarding scope and notification will contribute positively to the notification of occupational diseases.

In our research, there were 3 main topics on which a high level of consensus was reached; the Roles of Occupational Health and Safety Professionals, Workplace Inspections and Workplace Health and Ambient Surveillance. A high level of consensus on all propositions on these topics reveals the importance of health and safety services, practices and supervision in the workplace. In another study, inadequacy of workplace ambient measurements, inadequacy of workplace inspections and insufficient periodic examinations in the workplace were determined as important conditions that prevent the reporting of occupational diseases [29].

Vocational rehabilitation, which is one of the areas on which a consensus has been reached, is of great importance in terms of indirectly contributing to the diagnosis. Vocational rehabilitation includes important steps such as restoring working capacity and returning to work after the diagnosis of an occupational disease [30]. In the studies carried out, it is seen that especially in cases where the understanding of the social state is not sufficient, employees lose their jobs, only because they are diagnosed with an occupational disease [31]. For this reason, rehabilitating the employees after the diagnosis of occupational diseases and bringing them back to working life is more important than the treatment approach [32]. It is necessary to protect the employees from unemployment that arises directly or indirectly due to the diagnosis of occupational disease with adequate legal regulation. It can be predicted that after vocational rehabilitation, when both the working conditions of the employees are improved and there are no problems with unemployment or being out of employment with protective legislation, the concerns of the employees about being diagnosed with an occupational disease will disappear, so they will not hesitate to apply for an occupational disease diagnosis. Thus, in the diagnosis and notification of occupational diseases, physicians will be able to refer more patients and a system will be established for the benefit of the employee.

CONCLUSION

Occupational diseases are expected to be more common in developing or underdeveloped countries due to the shifting of risky jobs during the globalization process. Access to occupational health services may also be more restricted. This study presents the difficulties of diagnosing occupational diseases in a developing country, which is already difficult in many countries. The results of this study emphasize the importance of diagnosing occupational diseases in the and the necessity of establishing a system in terms of providing preventive occupational health services. Special efforts are needed to support occupational disease diagnosis systems in developing and underdeveloped countries. In order to increase the reporting of occupational disease diagnosis, instead of a compensation-based system, a surveillance-oriented system should be adopted. In this way, abstinence of employers and employees from receiving an occupational diagnosis can be prevented. In addition, the deficiencies in the manpower, financing, technical infrastructure and legislation in the occupational disease notification system should be revised quickly.

The fact that our study is a qualitative study, taking the opinions of the participants and analyzing them in detail offers a new perspective on this subject. In our study, only taking the opinions of those working in this field created a limitation. A larger study can be conducted with the participation of employees in different fields of expertise.

Author contribution

Study conception and design: İK, MEA, CŞ, and ANY; data collection: İK, CŞ and ANY; analysis and interpretation of results: İK, MEA, CŞ ; draft manuscript preparation: İK, MEA and CŞ. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Non-Interventional Clinical Research Ethics Committee of Hacettepe University (GO 21/483, 04.05.2021).

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Conflict of interest

The authors declare that there is no conflict of interest.

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