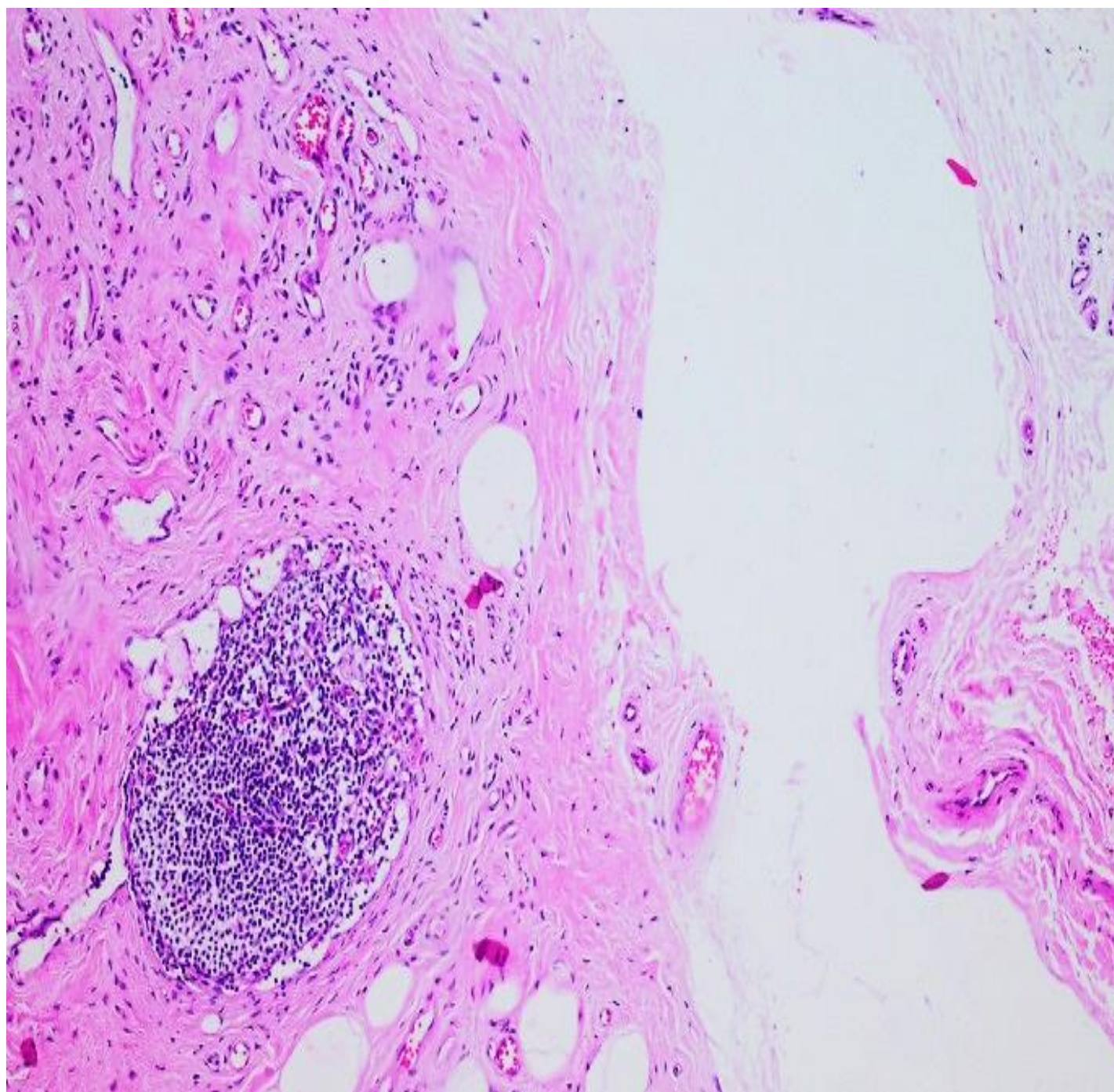


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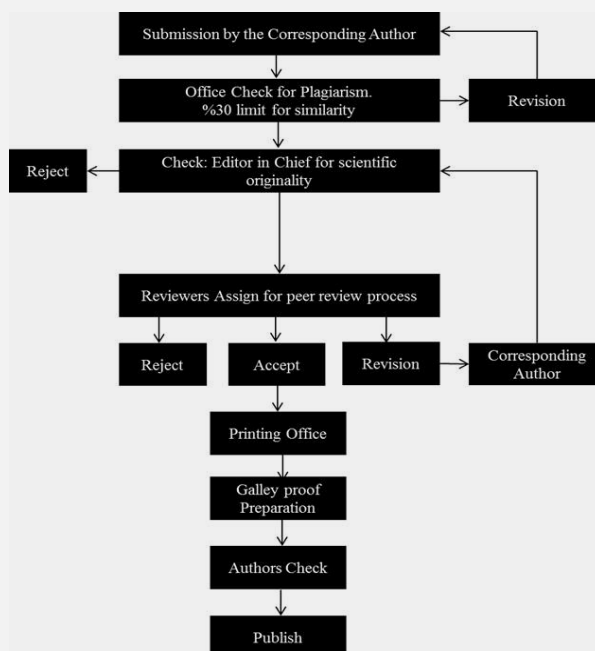
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## Coronavirus disease 2019 Pandemic as a threat for bone health

Irini Kotsalou<sup>1\*</sup>, Athanasios Zafeirakis<sup>1</sup>, Evaggelia Kotsalou<sup>2</sup>

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### ABSTRACT

**Objective:** The worldwide predominance of coronavirus disease 2019 (COVID-19) pandemic will have tremendous consequences on bone health of the general population and specifically to the bone mineral density of both young and elderly adolescents, due to sedentary lifestyle resulting from the prolonged and repeated lockdown. Scientific articles argue about the short and long term consequences on bone health resulting from the social isolation and the subsequent sedentary behaviors, whereas experts focus their scientific interest at guidelines for diagnosis, management and prevention of osteoporosis in the era of the COVID-19 pandemic.

**Keywords:** pandemic, sedentary lifestyle, lockdown, osteoporosis, bone health, COVID-19

### INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which was first reported in the end of 2019 in Wuhan, China. This newly diagnosed disease is characterized by fever, respiratory symptoms as well as fatigue, myalgia, dizziness and delirium, and its rapid spread worldwide caused a pandemic (1). Most countries, under the guidance of World Health Organization (WHO), in order to control the spread of this fatal disease, applied different measures such as quarantine, social distancing, isolation of infected individuals, closure of schools and all leisure and cultural facilities (2-4). All these massive public health interventions which have been implemented to control the outbreak, widely named as “lockdown”, had tremendous effects on peoples mental and physical health, caused stress, social isolation and degraded the quality of their lives resulting in a range of chronic health conditions (5).

During the COVID-19 pandemic several studies from different countries have investigated the characteristics and impact of sedentary lifestyle during the lockdown. It was unanimous that, during this pandemic, young adults demonstrated low physical activity (PA), high sedentary behavior (SB) (including leisure screen time), and long sleep duration, on discordance to the World Health Organization (WHO) guidelines (2-4). As WHO classified physical inactivity as the fourth leading risk factor accounting for 6% of global mortality (after hypertension - 13%, smoking - 9% and diabetes - 6%), this pattern of sedentary lifestyle during the COVID-19 pandemic assists the development of population specific health education and behavior interventions by the public health practitioners (6, 2-4).

### COVID-19 and OSTEOPOROSIS

Osteoporosis is a chronic metabolic disease characterized by low bone mass density which reflects to enhanced bone fragility and associated increased risk for fractures.

While physical activity is one of the most important factors to maintain mass, on the other hand, physical inactivity is a predisposing factor for accelerated bone loss and future automatic fractures (7-8).

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The worldwide predomination of COVID-19 pandemic will have tremendous consequences on bone health of the general population and specifically to the mineral density of both young and elderly adolescents, due to sedentary lifestyle resulting from the prolonged and repeated lockdown. It is expected that it might increase the relevant risk for osteoporotic fragility fractures and the subsequent healthcare costs, morbidity and mortality. Thus, reduced physical activity due to the COVID-19 stay-at-home order may also affect the bone health of patients with known osteoporosis, either by imposing the sedentary lifestyle, or by setting burdens in the provision of health care (7-8). For the moment only a few preliminary studies have proved laboratory or radiographically the effects of immobilization during the lockdown on bone mineral density, indicating that significantly lower serum bone alkaline phosphatase (BAP) concentrations were found in patients receiving anti-osteoporotic treatment after short term lockdown, whereas the bone mineral density (BMD) remained unchangeable (7).

Many scientific articles have stressed the need of social intervention politics on prevention of the potential bone loss, during the COVID-19 pandemic, on patients with osteoporosis. Experimental studies investigated the percentage of decrease on human mobility during the lockdown, which was found diminished by around 50% and estimated the subsequent reduction in bone density scanning and the respective increase on fracture risk. Although past studies including patients with long-term rest reported the reduction of BMD and early increase of bone metabolic markers after 60 days of immobilization, recent studies during the COVID-19 era showed that short period of sedentary life (e.g. 39 days of lockdown) had no significant effect on bone metabolic markers especially in the elderly individuals with osteoporosis which continued their medication. Furthermore, it is remarkable that short-term sedentary lifestyle affects predominantly the young people (23±3 years), than the older men, increasing their bone resorption markers (7, 8). While preliminary studies showed that bone health is retained during a short period of immobilization, thus prolonged or repeated social isolation periods can harm, even those who continue their therapy. Patients with osteoporosis are encouraged to follow low-energy aerobic exercise programs individually at home in order to maintain their muscle mass and strength, balance, posture, to reduce the risk of falls and improve their quality of life (9-11).

### **Covid-19 and Falls – fractures**

COVID-19 disease, and its associated symptoms of fatigue, dizziness and delirium, could contribute to falls and fractures among older people, but so far this hypothesis lacks research data. On the other hand, it remains yet unclear whether the restriction by the lockdown may affect the incidence of osteoporotic fragility fractures, due to reducing the number of outdoor falls and subsequent fractures among older adults (1, 12-14). For the moment, the effect of restriction of outdoor activities is more pronounced on the decrease of non-hip fragility fractures (including the forearm, upper arm, ankle, foot and others), compared with hip fractures, as more frequently the non-hip fractures occur outdoors whereas usually, the hip fractures occur indoors and in older people (12-13). A large observational study, during the lockdown, proved that fewer outpatients attended the Fracture Clinic, for

non-hip fractures, whereas there was no change in admissions for hip fractures (2). This finding could be attributed to fewer outdoors falls, during lockdown especially on winter months, when the incidence of both types of fractures was higher (8, 15).

### **Covid-19 and osteoporosis management**

Bone mineral density (BMD) imaging examinations in specialized diagnostic centers during lockdown might temporarily be postponed and fracture risk stratification for non-diagnosed individuals can instead be performed using the Fracture Risk Assessment Tool (FRAX) (16).

In a newly diagnosed osteoporotic patient therapy (especially oral regimens) can be initiated via teleconference especially in individuals with high-risk scores, like patients with recent osteoporotic fragility fracture or patients taking chronic high-dose glucocorticoids. Those patients should perform a few pretreatment laboratory studies, e.g serum calcium, creatinine, and/or 25-hydroxyvitamin D, before the first prescription of potent antiresorptive agents {intravenous (iv) bisphosphonates and denosumab} to avoid hypocalcemia. However, several studies suggest a temporary subscription of oral bisphosphonate for patients with osteoporosis because intravenous administration of these medications requires clinical intervention and self-presence (17).

In the era of COVID-19 pandemic the appropriate anti-osteoporotic medication should start during the hospitalization of healing fractures, because after discharge from the hospital the approach of health services for osteoporosis follow-up appointment might be difficult (18). It should be acknowledged, however, that > 50% of individuals after an intravenous bisphosphonate infusion face a flu-like reaction (fever, myalgias), which might complicate the diagnosis and be misunderstood as COVID-19 disease especially in hospitalized patients (18-19). Furthermore, the pandemic of COVID-19 might pose obstacles on follow-up management of osteoporosis, so patients who already receive bisphosphonates should be encouraged to continue their current treatment, even nonsufficient, because of the significantly greater risk of new fractures in case of drug intermission (20-21).

Although there is no evidence that therapy against osteoporosis predisposes to COVID-19 illness, or increases the risk or its severity or alters the COVID-19 disease course, however in case of early signals of this pandemic disease, the anti-osteoporotic hormonal medication should be temporarily discontinued, due to moderately increased risk for hypercoagulable complications (22-23). On the contrary, other experts suggest to continue denosumab and all other antiosteoporotic treatments during COVID-19 pandemic and consequent lockdown, despite the higher incidence of side effects from the first one regarding ear, nose, and throat infections, in order to prevent the risk of fractures and the subsequent comorbidities which can finally predispose to COVID-19 disease (24 -29).

### **COVID-19 and osteoporosis prevention**

During 2020, the WHO released accurate guidelines which highlight the importance of regularly undertaking both aerobic and muscle-strengthening activities for all age groups

and suggest the reduce of the sedentary lifestyle. Those guidelines are intended for people in self-quarantine without any symptoms or diagnosis of acute respiratory illness, containing practical advices on relevant activities for how to spend the lockdown indoors and stay fit. WHO recommends that all adults should undertake 150–300min of moderate-intensity (MPA), or 75–150min of vigorous-intensity physical activity (VPA), or combination of moderate-intensity and vigorous-intensity aerobic physical activity (MVPA) per week at home, with no special equipment and with limited space (26, 30-31).

## CONCLUSION

The worldwide predominance of COVID-19 pandemic will have tremendous consequences on bone health of the general population and specifically to bone mineral density of both young and elderly adolescents, due to sedentary lifestyle resulting from the prolonged and repeated lockdown. This short review concluded that current scientific articles argue about the short and long term consequences on bone health due to social isolation and the subsequent sedentary behaviors. Another hot topic for the scientific community is the management and prevention of osteoporosis during the COVID-19 era..

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## COVID-19 infection in patients receiving hemodialysis in Athens: Findings, experience, and outcome from a single Dialysis Unit

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### ABSTRACT

**Objective:** Our Nephrology Department during the spring period on the first wave of COVID-19 was the referral Dialysis Unit for Covid-19 positive HD patients in the district area of Athens, Greece. This study aims to report the characteristics, rates, and outcomes of all patients affected by infection with SARS-CoV-2 undergoing HD and treated under our care. 22 Covid-19 positive HD patients were treated under the care of our facility during the period 8 April 2020-17 June 2020. 16 patients were symptomatic at admission and 13 patients were admitted with or developed during their stay pleural effusions. 12 patients (8 male) of our group died during their hospitalization. The mean age of our patients was 74.5 years. It has to be pointed out that 13 patients were over 75 years old. Mean age was higher in those who died compared with those who were discharged with double negative Covid-19 tests (79 vs 74,5 years old respectively). It seems that despite the fact that the immune response of this population has not been clearly clarified, age comorbidities and above all end-stage renal disease by its self is a significant and unpredictable risk factor for clinical outcome of HD patients with COVID-19 infection.

**Keywords:** Haemodialysis, COVID-19, nephrology, outcome, SARS-CoV-2

### INTRODUCTION

In December 2019 a new strain of coronavirus, officially named severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2), was first isolated from three patients with coronavirus disease 2019 (covid-19) by the Chinese Center for Disease Control and Prevention connected to the cluster of acute respiratory illness cases from Wuhan, China (1, 2). On 30 January 2020, the World Health Organization declared that the outbreak of SARS-Cov-2 constituted a public health emergency of international concern.

End-Stage-Renal Disease patients under hemodialysis (HD) are at increased risk for coronavirus disease 2019 (COVID-19) and its complications, owing to the presence of multiple comorbid conditions. Patients receiving kidney replacement therapy are a vulnerable population as those receiving dialysis are usually older with significant comorbidity and have also impaired immune responses (3) and require regular attendance at a healthcare facility. The logistical aspects within a dialysis facility further increase the risk of disease transmission (4). There are data in the literature that suggesting a more severe disease course in patients with chronic kidney disease (CKD) (5). Still outcomes in End-Stage-Renal Disease patients under HD are unclear, with small case series suggesting a milder course (6). On the other hand, HD patients are usually old and affected by several comorbidities such as diabetes mellitus and hypertension that are known to be associated with a high risk of poor outcomes in patients with coronavirus disease 2019 (COVID-19).

The above notifications clearly imply that although there have been a number of published recommendations on how to mitigate the spread of SARS-CoV-2 in dialysis units (7,8,9,10,11) at the same time there is a lack of data regarding clinical course and outcome in patients receiving HD following infection with SARS-CoV-2.

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Our Nephrology Department during the spring period on the first wave of COVID-19 was the referral Dialysis Unit for Covid-19 positive HD patients in the district area of Athens, Greece. This study aims to report the characteristics, rates, and outcomes of all patients affected by infection with SARS-CoV-2 undergoing HD and treated under our care

## MATERIAL and METHODS

This is an observational study. Our Dialysis Unit has been assigned as a referral unit for Covid-19 positive HD patients. We registered all the data regarding the clinical course of our patients' population. Age, the primary cause of end-stage renal disease, weight, clinical presentation, HD history, outcome, days of hospitalization.

## RESULTS

The 22 Covid-19 positive HD patients were treated under the care of our facility during the period 8 April 2020-17 June 2020. 16 patients were symptomatic at admission and 13 patients were admitted with or developed during their stay pleural effusions. 12 patients (8 male) of our group died during their hospitalization. 3 out of 12 were admitted to the Intensive Care Unit (ICU). 6 patients were septic, 4 had respiratory failure, and 2 developed cardiovascular events. 14.5 days were the mean hospitalization days (range: 1-38 days) for the deceased ones.

The 2 out of 3 patients that were admitted to ICU had quick deterioration, incubated, and stayed in ICU for 48 hours. The third one with severe comorbidities (multiple myeloma, cancer of bladder) developed respiratory failure after 8 days of hospitalization, incubated, became septic, and died after 20 days in ICU.

The mean age of our patients was 74.5 years. It has to be pointed out that 13 patients were over 75 years old. Mean age was higher in those who died compared with those who were discharged with double negative Covid-19 tests (79 vs 74.5 years old respectively). The median dialysis vintage for our patients was 63 months and for the deceased ones was 89 months. The average weight of our patients was 69 kg. The weight of deceased patients was 63 kg. 11 out of 22 patients and 5 out of 12 deceased patients were diabetic. 14 patients were hypertensive and 16 had an official cardiovascular background. 10 out of total 22 patients under our care discharged after 43 days of hospitalization (range: 35-56 days).

## DISCUSSION

There is currently a lack of data on how SARS-CoV-2 affects patients receiving HD. Our morbidity rates are higher than the average number from the literature. In Spain and Italy, mortality rates were 30.5% and 23.6% respectively (12, 13). 23.6% was also recorded in the Alsace region of France, where the vast majority of deceased patients died 2 weeks after the onset of the symptoms (14). Furthermore, in Northern Italy the mortality rates of COVID-19 were calculated as 24.6 % for HD patients (15). Similarly, a study of 59 consecutive patients testing positive from Columbia University in the United States found a mortality rate of 31% (18 patients) (16).

The mean age of our study population is quite high, with significant comorbidities that co-exist, a fact which explains the outcome. A report from the COVID-19 registry of the Spanish Society of Nephrology reported a mortality of 23% in 868 patients with SARS-CoV-2 infection. Mortality was also associated with increasing age in this cohort (17). A study from a single urban center in the United Kingdom showed much higher rates of transmission with over 20% of their HD patients affected within 6 weeks of detection of their first case. Similar mortality rates were demonstrated in this series with 20.3% of patients dying during follow-up, again with increasing age being associated with a higher likelihood of mortality (18).

In the general population, several comorbid conditions such as diabetes, hypertension, and obesity have consistently been linked to increased risk of death with COVID-19 (19). The majority of our patients were old, diabetic with hypertension and cardiovascular background. It is crucial to point out that for patients requiring HD, the risk attributable to the requirement for HD is such a strong risk factor for death that it is challenging to identify subgroups of dialysis recipients at the highest risk. At the same time, the immune response of this group of patients is not clearly understood and known especially in conjunction with the behavior of Covid-19 infection.

Our data confirm reports from recent literature that the mortality in HD patients is much higher than in the general population, 13% vs 4% respectively (4% is the official mortality in the general COVID-19 population reported in China). Nevertheless, although the infection may be fatal, COVID-19 symptoms in dialysis patients in many cases seem to be less aggressive, a fact that we also noticed in some of our patients. It has been postulated that HD patients may be relatively protected from violent cytokine storms by their impaired immune systems (20).

The number of patients in our cohort who required intensive care is perhaps lower than might be expected. However, all 3 patients that were admitted to ICU died. They had severe comorbidities, but one of them remained 20 days in ICU before passed away.

The question of whether a patient dies by COVID-19 or with COVID-19 is misleading especially in HD population and perhaps it is going to remain unanswered. However HD patients are such complex and fragile patients and a violent infection such as COVID-19, that mainly targets lungs and heart, already very compromised for many years of dialysis, brings a very high risk of death, even if the infection itself is not the final cause (21, 22).

Another issue, that is not referred to our patients, is dialysis adequacy for COVID-19 positive HD patients. Despite the heavy schedule of our medical-nursing personnel, all of our patients received HD sessions 3 times a week (no exceptions) for 3 to 4 hours per session. In 2 cases where tunneled lines were not performing well, they were replaced. This is the reason that we did not have any death from hyperkalemia. A question has raised in patients where dialysis frequency was reduced to minimize infection risk: how many victims of other diseases will be indirectly related to the coronavirus pandemic? (20).

## CONCLUSION

As a conclusion, our data provide clues regarding our experience of caring for HD patients with COVID-19. Mortality was high. It seems that despite the fact that the immune response of this population has not been clarified, age, comorbidities and above all end-stage renal disease by its self is a significant and unpredictable risk factor for the clinical outcome of HD patients with COVID-19 infection.

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# Cranial hyperthermia resulting from decreased parotid gland salivation induced by ischemic degeneration of glossopharyngeal network in subarachnoid hemorrhage

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## ABSTRACT

**Objective:** We speculated that subarachnoid hemorrhage (SAH) induces ischemic lesions in the cranial parasympathetic nerves, which may decrease saliva secretion and lead to hyperthermia. We tested this hypothesis by examining histologic features of parotid glands and glossopharyngeal nerves (GPNs) in a rabbit model of SAH.

**Material and Methods:** Rabbits (n = 25) were divided into control (n = 5), sham (n = 5), and SAH (n = 15) groups. Animals in the sham and SAH groups were examined over a 3-week period before sacrifice. Salivation score (SC) was determined by measuring the mean wetted area of an orally inserted cotton ball. Sections of parotid glands and intracranial and intraparotid branches of the GPNs were stained with hematoxylin and eosin and SAH-induced damage was analyzed by terminal deoxynucleotidyl transferase dUTP nick end labeling of apoptotic cells. Specimens were stereologically examined to determine saliva-filled total vesicle volume (TVV) per cubic meter; vasospasm index (VSI) based on wall/lumen ratio of parotid glands arteries, and degenerated neuron density (DND) of glossopharyngeal ganglia.

**Results:** The mean oral temperature was 36.9°C. In the control group, mean values were as follows: SC, 46±8 mm<sup>2</sup>; DND, 19±4/mm<sup>3</sup>; VSI, 1.065±0.049; and TVV, (780±1187) × 106/μm<sup>3</sup>. In the sham group, mean values were as follows: SC, 31±6 mm<sup>2</sup>; DND, 98±23/mm<sup>3</sup>; VSI, 1.67±0.32; and TVV, (617±110) × 106/μm<sup>3</sup>. In the low hyperthermia SAH group, mean values were as follows: SC, 16±5 mm<sup>2</sup>; DND, 1520±261/mm<sup>3</sup>; VSI, 2.12±0.21, and TVV, (314±98) × 106/μm<sup>3</sup>. In the high hypothermia SAH group, mean values were as follows: SC, 9±2 mm<sup>2</sup>; DND, 3210±912/mm<sup>3</sup>; VSI, 3.18±0.30; and TVV, (432±99) × 106/μm<sup>3</sup>.

**Conclusions:** Decreased salivary secretion due to secretory gland atrophy originated from ischemia-induced GPN network degeneration at the brainstem, which may be responsible for cranial hyperthermia following SAH.

**Keywords:** Hyperthermia, subarachnoid hemorrhage, Glossopharyngeal Nerve, Parotid Gland

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## INTRODUCTION

Fever is a common clinical complication in patients with subarachnoid hemorrhage (SAH) and is usually related to poor prognosis in early stages (1, 2) fever following SAH is linked to increased mortality (3). The parotid gland is the largest salivary gland and is an important secretory and digestive organ (4).



The glossopharyngeal nerves (GPNs) provide parasympathetic innervation of this gland (5), controlling both secretion and vasodilation (6). Parasympathetic innervation controls the secretion of large volumes of watery saliva, while sympathetic innervation contributes to the production of dense, low-volume, but enzyme-rich saliva (7). Saliva is essential for taste perception; the initiation of salivation is regulated by brainstem gustatory-salivary reflex networks (8).

The present study investigated whether salivary gland dysfunction induced by SAH causes hyperthermia. This is the first study to demonstrate decreased salivation of the parotid and other glands as causing the development of hyperthermia following SAH.

## MATERIAL and METHODS

### Animal selection and experimental groups

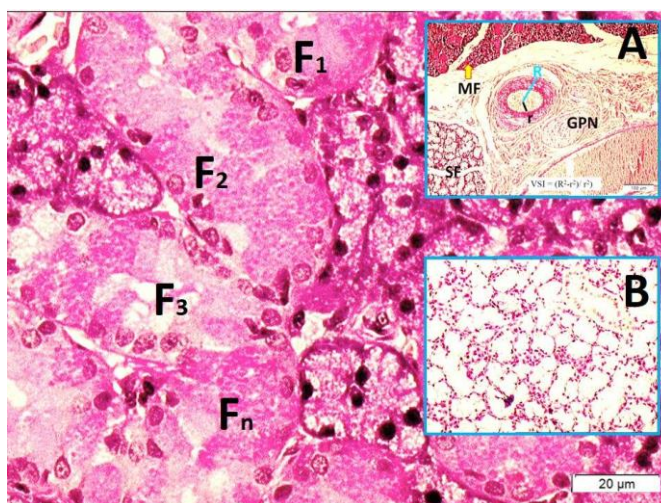
A total of 25 rabbits were randomly assigned to one of three groups (Group I, control,  $n = 5$ ; Group II, sham,  $n = 5$ ; and Group III, SAH,  $n = 15$ ). Animals were maintained in individual metal cages at room temperature and 50% relative humidity on a 12:12-h light/dark cycle and were under veterinary supervision. The animals had free access to a standard laboratory diet and water. The study protocol was reviewed and approved by the Ethics Committee for Animal Experiments of the Faculty of Medicine, Ataturk University, Turkey. Animal care and experimentation were according to guidelines set forth by the Ethics Committee. Anesthesia was first induced with isoflurane administered via a face mask, followed by subcutaneous injection (0.2 ml/kg; total volume, 1 ml) of a cocktail consisting of ketamine HCl, 150 mg/1.5 ml; xylazine HCl, 30 mg/1.5 ml; and distilled water before surgery. Blood (1 ml) was collected from the auricular artery of each animal. While the head of the animal was held in a hyperflexed position, the posterior notch of the foramen magnum was identified, the cisterna magna penetrated with a needle, and cerebrospinal fluid was aspirated. A 1-ml volume of blood (SAH group) or saline (sham group) was injected using a 22-gauge needle into the cisterna magna over a period of about 1 min. Control animals were not injected.

All rabbits were monitored for 10 min twice daily for 3 weeks. Oral temperature was measured using a standard hospital thermometer; the mean oral temperature was measured as 36.8°C; 37.8°C and 38.7°C were taken as low and high hyperthermia, respectively. Oral salivary secretion was assessed as follows. A rolled cotton ball (0.5 cm in diameter) was inserted into the oral cavity of rabbits for 30 s, then placed on a paper and compressed by with a 250-g iron weight with a 3-cm<sup>2</sup> base surface area for 20 s. The wetted area on the paper was measured and used for salivation score (SC). After 3 weeks, animals were decapitated under general anesthesia and their bodies were immersed in 10% formalin solutions for 7 days. The parotid glands, GPNs, and their ganglia along with other brainstem nerves were dissected, dehydrated through a graded alcohol series, and embedded in liquid paraffin.

### Histopathological analysis

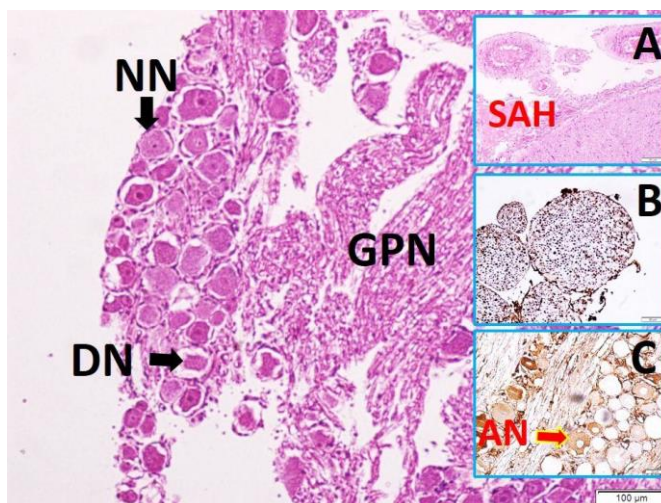
The parotid glands were sectioned at a thickness of 5  $\mu$ m separated by a distance of 30  $\mu$ m. Every 30th and 31st section

was used for saliva follicle counts. The total number of saliva follicles in the parotid glands was estimated by the fractionator method. Tissue sections from each block were collected on glass slides for histopathological examination and stained with hematoxylin and eosin (H&E) (Fig. 1).



**Figure 1.** Histopathological analysis of parotid gland. **A:** Serous follicles (SF), mucous follicles (MF), glossopharyngeal nerve (GPN) axons, and parotid artery are shown along with formula for calculation of vasospasm index (VSI) (LM, H&E, 10 $\times$ ). **B:** Secretory material-filled follicles (LM, TUNEL, 10 $\times$ ) and follicles almost completely filled with saliva (Fn) (LM, PAS, 10 $\times$ ).

For the analysis of GPN lesions, brain specimens were sectioned parallel to the long axis for the examination of both axons and ganglia. To determine neuronal density in the GPN ganglion (GPNG), the whole tissue along with the extensions was embedded in paraffin blocks in a longitudinal orientation to observe all the roots during the histopathological examination. Sections were cut and stained with H&E and analyzed by the TUNEL assay, and examined under a light microscope. Images were acquired at 10 $\times$  and 100 $\times$  magnification (Fig. 2). The Cavalieri method was used to evaluate GPNG density.

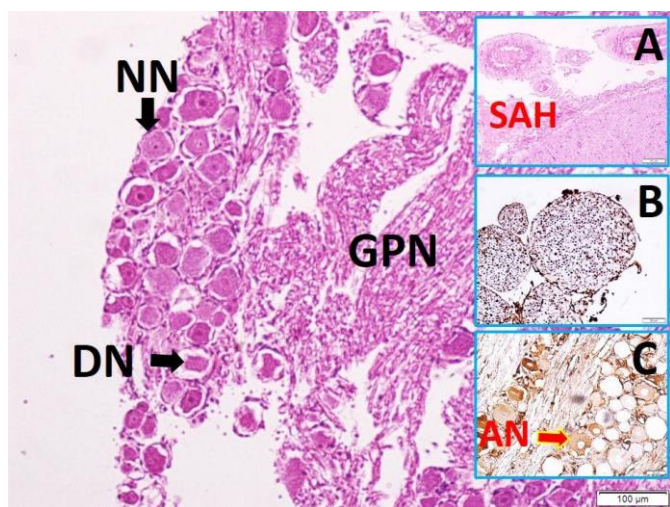


**Figure 2.** A–C: Histopathological analysis of Sylvian subarachnoid hemorrhage (SAH) and spastic middle cerebral arteries (LM, H&E, 10 $\times$ ) (A), normal (dark) and degenerated axons of parotid branches of glossopharyngeal nerves (GPN; LM, S-100, 10 $\times$ ) (B), normal and apoptotic neurons (AN) of petrosal ganglion (LM, TUNEL, 10 $\times$ ) (C), and GPN axons (GPN) and normal and degenerated neurons (NN and DN, respectively) at the base (LM, H&E, 10 $\times$ ).

## Stereological analysis

Stereological methods are tools that enable the estimation of bulk parameters, such as the total volume of a structure and the number and size of cells. The first sampled pair of sections were randomly selected from a starting point within the first 20-section interval. Thereafter, every 20th section and its neighbor were sampled. Therefore, the section sampling fraction ( $f_1$ ) was  $f_1 = 1/30$ . Section pairs not containing the parotid glands and ganglia were discarded. The sampling fraction yielded on average 10 to 11 section pairs. The area of the sampling fraction,  $f_2$ , was  $1/1$ . We used the physical dissector method to evaluate the number of saliva follicles in the parotids since it is simple; free from assumptions about particle shape, size, and orientation; and unaffected by overestimation errors and truncation. Two consecutive sections (dissector pairs) obtained from tissue samples with a named reference were mounted on each slide. The order of reference and look-up sections were reversed to double the number of dissector pairs without having to cut new sections. The number of counted follicles was designated  $\Sigma Q^-$ . The total number of saliva-filled follicles ( $N$ ) in the parotid glands was estimated from the equation  $N = \Sigma Q^- \times 1/f_1 \times 1/f_2$ . In all groups, the number of follicles did not show a normal distribution according to Kolmogorov–Smirnov, and Shapiro–Wilk tests ( $P < 0.05$ ). Before scores were separately compared using the post-hoc Mann–Whitney U test for pairwise comparisons of independent samples, followed by the Kruskal–Wallis test. The P-value used for multiple comparisons (0.05, 95% confidence interval) was divided by 6 with a Bonferroni correction (i.e., P values were considered statistically significant at  $\leq 0.0083$ ).

Vasospasm index (VSI) was calculated as follows:  $2R$  and  $2r$  are external and internal diameters, respectively, of parotid arteries. VSI was determined as a proportion of the external surface to lumen ratio [ $\pi R^2 - \pi r^2 / \pi r^2 = (R^2 - r^2 / r^2)$ ] (Fig. 1-3 A). After counting, total vesicle volume (TVV) was estimated by summation of VV. Each vesicle was considered as a sphere with a volume of  $V_n = 4/3\pi r_n^3$ . TVV was estimated as:  $TVV = N = 1N = NN \times V_n$



**Figure 3.** A: Histopathological analysis of parotid gland with follicles (SF) and spastic parotid artery, with the formula for calculating vasospasm index (VSI) (LM, H&E, 10 $\times$ ). B: The number of secretory material-filled small follicles and saliva-filled small follicles (Fn) (LM, PAS, 10 $\times$ ) with neuronal apoptosis and nerve degeneration (LM, TUNEL, 10 $\times$ ).

The physical dissector method was used to evaluate the numbers of living and degenerated GPNG neurons. Two consecutive sections (dissector pairs) obtained from reference tissue samples were mounted on each slide. The order of paired reference sections was reversed to double the number of dissector pairs without having to cut new sections. The mean GPNG neuronal density ( $N_v/G_v$ ) per mm<sup>3</sup> was estimated with the following formula:

$$N_v/G_v = \Sigma Q^- N / \Sigma A \times d$$

where  $\Sigma Q^- N$  is the total number of counted neurons appearing only in reference sections,  $d$  is section thickness, and  $A$  is the area of the counting frame. The most effective way of estimating  $\Sigma A$  for the set of dissectors is by  $\Sigma A = \Sigma P \times a$ , where  $\Sigma P$  is the total number of counting set frame points and  $a$  is a constant area-associated set point.  $A$  and  $B$  are the areas of the counting frames. The Cavalieri volume estimation method was used to determine the total number of neurons in each specimen, which was calculated by multiplying the volume (mm<sup>3</sup>) and numerical density of neurons in each GPNG. Differences between salivary follicle number and degenerated neuron density in the GPNG were compared statistically. Neuronal angulation, cytoplasmic condensation, nuclear shrinkage, and apoptotic changes were used as criteria for neuronal degeneration. Data were analyzed using SPSS for Windows v.12.0 (SPSS Inc., Chicago, IL, USA). Data were analyzed with the Kruskal–Wallis and Mann–Whitney U tests. Differences were considered significant at  $P < 0.05$ .

## RESULTS

Four animals in the study group and one in the sham group died within 7 days of surgery. Neck stiffness, unconsciousness, convulsive attacks, fever, apnea, cardiac arrhythmia, and breathing disturbance were observed in all hyperthermic animals. In control animals, heart rate was  $280 \pm 15$ /min, respiratory rate was  $35 \pm 9$ /min, and blood oxygen concentration was  $95\% \pm 7\%$ . Soon after inducing SAH, heart rate decreased to  $150 \pm 30$ /min, respiratory rate to  $18 \pm 5$ /min, and blood oxygen concentration to  $78\% \pm 10\%$ . Electrocardiographic changes were also observed, including ventricular extrasystole, ST depression, QRS separation, bi- or trigeminal extrasystole, and fibrillation. However, in the late phase of fatal SAH, heart rate increased to  $350 \pm 40$ /min. A decrease in respiration frequency (bradypnea) ( $14 \pm 3$ ) and increase in respiration amplitude were observed in the first few hours following SAH. However, after longer intervals, an increase in respiration frequency (tachypnea) and a decrease in respiration amplitude were observed, resulting in shortened inspiration and longer expiration times, apnea-tachypnea attacks, diaphragmatic breath, and respiratory arrest. Massive subarachnoid hemorrhage was observed in the basal cisterns of animals in Group III, which showed meningeal irritation signs and cardiorespiratory dysrhythmia. The oral temperature was measured as  $36.80C \pm 0.3$ ,  $37.80C \pm 0.4$ ,  $38.70C \pm 0.2$ ,  $38.70C \pm 0.3$  were considered as low and high hyperthermia, respectively. The mean GPNG neuronal density was estimated as  $12.230 \pm 1065$ /mm<sup>3</sup> in normal animals (Group I),  $11.606 \pm 975$ /mm<sup>3</sup> in the sham group (Group II), and  $9.940 \pm 732$ /mm<sup>3</sup> and  $7.500 \pm 650$ /mm<sup>3</sup> in the low and high hyperthermia groups, respectively (i.e., Group IIIA and IIIB, respectively).



**Table 1.** TVV was decreased while DND and VSI were increased in hyper thermic animals after SAH

	Group I (Control)	Group II (Sham)	Group IIIA (low hyperthermia)	Group IIIB (high hyperthermia)	P value
SC/mm <sup>2</sup>	46±8	31±6	16±5	9±2	< 0.05
DND/mm <sup>3</sup>	19±4	98±23	1520±261	3210±912	< 0.05
VSI	1.065±0.049	1.67±0.32	2.12±0.21	3.18±0.30	< 0.05
TVV (× 10 <sup>6</sup> )/μm <sup>3</sup>	780±1187	617±110	314±98	432±99	< 0.05
Temp. V.	36.8 <sup>0</sup> C±0,3	37.8 <sup>0</sup> C±0,4	38.7 <sup>0</sup> C±0,2	38.7 <sup>0</sup> C±0,3	

Data were analyzed with the Kruskal–Wallis and Mann–Whitney U tests ( $P < 0.05$ ).

DND, degenerated neuron density; SC, salivation score; TVV, total vesicle volume; VSI, vasospasm index, Temp. V; Temperature Value.

In Group I, mean values were as follows: SC, 46±8 mm<sup>2</sup>; DND, 19±4/mm<sup>3</sup>; VSI, 1.065±0.049, and TVV, (780±1187) × 106/μm<sup>3</sup>. In Group II, mean values were as follows: SC, 31±6 mm<sup>2</sup>; DND, 98±23/mm<sup>3</sup>; VSI, 1.67±0.32; and TVV, (617±110) × 106/μm<sup>3</sup>. In Group IIIA, mean values were as follows: SC, 16±5 mm<sup>2</sup>; DND, 1520±261/mm<sup>3</sup>; VSI, 2.12±0.21; and TVV, (314±98) × 106/μm<sup>3</sup>. In Group IIIB, mean values were as follows: SC, 9±2 mm<sup>2</sup>; DND, 3210±912/mm<sup>3</sup>; VSI, 3.18±0.30; and TVV, (432±99) × 106/μm<sup>3</sup>. TVV decreased markedly with increased DND and VSI in hyperthermic animals ( $P < 0.05$ ). The total volume of saliva-filled vesicles per cubic meter was reduced in these animals, especially in Group IIIA ( $P < 0.05$ ) (Table 1). TVV also decreased with increased DND and VSI in Group IIIB ( $P < 0.005$ ); this trend was moderately significant between Groups II and IIIB ( $P < 0.0005$ ); highly significant between Groups II and IIIA ( $P < 0.005$ ); and most significant between Groups I and IIIB ( $P < 0.0001$ ). VSI of parotid arteries and follicles volumes were correlated in Group I vs. III ( $P < 0.0001$ ) and Group II vs. III= ( $P < 0.0001$ ).

## DISCUSSION

Fever is common in neurocritical care patients and has a negative impact on neurological outcome (9); it is also linked to increased in-hospital mortality after SAH (1, 3). Fever is associated with worse outcomes and late recovery in high- and low-grade patients, respectively (10). Functional improvement after SAH is associated with cumulative fever burden, admission neurological grade, aneurysm obliteration procedure, admission computed tomography score, vasospasm, and external ventricular drainage (11). Hyperthermia is a potential risk factor for vasospasm (12).

In our study, brain edema, stiffness, leptomeningeal thickness, brain swelling, and increased brain weight were observed in all animals that developed hyperthermia. Basal cisterns and occasionally fourth and lateral ventricles were filled with blood, while arachnoidal membranes in the lower cranial parts adhered to lower cranial nerve roots.

Organs have dual autonomic innervation that is critical for regulating gland function (4). GPNs have secretory and vasodilator effects in the parotid gland (6). The tympanic branch of the GPN known as Jacobson's nerve is located immediately after the jugular foramen and contributes to the tympanic plexus on the promontory, providing secretory innervation to the parotid gland (13). Parasympathetic, cholinergic innervation modulates the secretion of a large volume of watery serous saliva while sympathetic and adrenergic innervation stimulates the production of dense, low-volume, but enzyme-rich saliva (7).

Saliva is essential for taste perception and is required for initiating salivation, which is regulated by gustatory-salivary reflex networks in the brainstem (8) that include afferent limbs, salivary nuclei within the medulla, and an efferent limb comprising both sympathetic and parasympathetic secretomotor nerves. In addition, gustatory, visual, olfactory, psychic, masticatory, thermoreceptive, and possibly nociceptive stimulants induce saliva secretion (14). The GPN innervates taste receptors located on the epithelial folds of the foliate and circumvallate papillae in the posterior part of the tongue (15). The GPN as well as facial and vagal nerves have important roles in salivary secretion from salivary, respiratory, and intestinal glands. The GPN and vagus nerve are mixed nerves containing sensory, gustatory, motor, and autonomous (parasympathetic) fibers (16).

GPN lesions result in difficulty swallowing; impairment of taste over the posterior one-third of the tongue and palate; impaired sensation over the posterior one-third of the tongue, palate, and pharynx; loss of a gag reflex; and dysfunction of the parotid gland (17). Post-ganglionic parasympathectomy of rat parotid glands caused reductions in gland weight, acinar cell size, and glandular amylase expression (18).

In this study, parotid gland size was decreased in SAH animals. This was accompanied by loss of acinar, tubular, and supporting cells due to apoptosis, a decreased number of saliva filled vesicles, reduction in the total volume of saliva-filled vesicles, ductal epithelial cell injury related to ductal closing, and degenerative changes in intraparotid parasympathetic ganglia neurons (Fig. 3).

Unilateral parasympathetic denervation of ovine parotid glands in sheep 21–28 days after nerve resection reduced the mass of the ipsilateral gland while increasing that of the contralateral gland, to the extent that total gland mass was greater than in sheep with normally innervated glands. These results demonstrate that parasympathetic innervation to the parotid gland has important trophic effects (19). Bilateral resection of the glosso-pharyngeal nerves resulted in a 40%–50% decrease in enzyme activity of parotid glands over 7 days (20).

The results of this study reveal that unilateral parasympathetic denervation of ovine parotid gland induces substantial changes in parotid size, basal salivary flow, and protein expression. Both the size reduction and morphological changes observed following denervation were compatible with previously reports (21). In particular, nerve root- and ganglia-supplying arteries were more vasospastic in Groups IIIA and IIIB; these groups also showed greater neuronal apoptosis and GPN degeneration.

## CONCLUSION

Understanding the origin of fever in SAH patients may improve their functional outcomes. Decreased salivary secretion due to secretory gland degeneration resulting from GPN ischemia at the brainstem likely causes hyperthermia in SAH. However, it is also possible that ischemic degeneration of the GPN and facial and vagal nerves per se leads to decreased salivation, and that morphological changes in the parotid and other glands may be responsible for subsequent hyperthermic sequelae. This is the first study demonstrating decreased salivation by the parotid and other glands as causative to the development of hyperthermia following SAH

**Author contributions:** ME, IY, OT, NA, CO, MÇ, AE, MDA; Study design, Animal experiments, Literature search, AE; Writing article and revisions

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**Ethical issues:** All authors declare originality of research.

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## Assessment of radiation dose to pediatric patients during routine digital chest X-ray procedure in a government medical centre in Asaba, Nigeria

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### ABSTRACT

**Objective:** Radiation dose to pediatric patients have been widely reported, it is however necessary that imaging expert keep doses as low as possible to forestall long term cancer risk. This study is aimed at determining pediatric entrance surface dose (ESD), 75th percentile ESD, absorbed dose (D) and effective dose (E) for 0-15 years.

**Material and Methods:** The study used a digital radiography (DR) unit with a grid system for each chest X-ray. The thermoluminescent dosimeter (TLD) used was encapsulated in transparent nylon, it was then attached to the patient skin (chest wall) and the second was placed directly at the posterior end of it.

**Results:** The mean ESDs for the 4 age groups were as follows: 0- < 1 ( $1.54 \pm 0.74$ mGy), 1- < 5 ( $1.53 \pm 0.83$ mGy), 5- < 10 ( $0.55 \pm 0.39$ mGy) and 10- ≤15 ( $1.30 \pm 0.57$ mGy), with an overall mean of 1.23mGy. The 75th percentile ESD for each age group above 10 patients (excluding 5- < 10yrs) was 2.18, 2.19 and 1.75mGy respectively. The absorbed dose (D) ranged from 0.03-2.39mGy. The mean effective dose (E) for the 4 age groups was  $0.18 \pm 0.03$ mSv. There was a good correlation between ESD and D ( $P = 0.001$ ). A One-Way ANOVA shows that the field size and focus to film distance (FFD) affected the ESD and D ( $P < 0.001$ ) respectively. The risk of childhood cancer from a single radiograph was of the order of  $(1.54-23.4) \times 10^{-6}$ .

**Conclusion:** The 75th percentile ESD, E and childhood risk of cancer was higher than most studies it was compared with. The study reveals that machine parameters such as the field size and FFD played a major role in dose increase. Protocol optimization is currently needed for pediatric patients in the studied facility.

**Keywords:** Thermoluminescent dosimeter (TLD), Digital Radiography (DR), Entrance Surface Dose (ESD), Absorbed Dose (D), Exit Dose (ED), Effective Dose (E)

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### INTRODUCTION

The use of X-ray for pediatric radiography has increased over the years because it requires no invasive approach but radiation stochastic effects cannot be ruled out (1-3). Referrals for routine chest X-rays are common and it has served as the first line of diagnostic pathways for clinicians. Notably in pediatrics, about 40% of all images are of chest radiographs (4). Several medical conditions affecting the chest may occur from birth and the majority of these illnesses require chest X-rays for proper diagnosis and follow-up (prognosis) (5-7).

X-ray investigations involve the use of modalities that involves ionizing radiation. Medical exposure to radiation has been documented to carry some health risks especially in fast-dividing tissues and organs such as seen in children (8). Pediatrics has a higher average risk of incurring cancer, when compared with adults receiving the same dose (9). This is complicated by the tendency of longer life expectancy in children which allows more time to any harmful effect of radiation to manifest (10-12). Therefore, adequate care must be taken when imaging pediatric patients.

The report of the United Nations Scientific Committee on the Effect of Atomic Radiation (UNSCEAR), 2008 further indicates an increase in the use of X-rays globally especially since the advent of digital technology (8). Although digital techniques have the potential to minimize radiation dose to the patient and improve practice, it can increase the same due to its wide image range and post-processing window without easily adversely affecting the image quality. The risk of overexposure is not easily noticeable in digital radiography and this increases the risk to the patient. Although the detector current exposure Index (EXI) is a parameter currently used with the DR systems to determine if a radiograph is either optimal, overdose or underdose. (13).

Every radiological investigation involving the use of ionizing radiation must be justified and the procedure itself optimized based on the International Commission on Radiological Protection (ICRP) 60 (14). Radiation must not be used for medical investigations arbitrarily especially when other non-ionizing image modalities can offer better diagnostic values. Optimization requires the use of the lowest radiation as possible without profound adverse effects on the image quality. This concept is called ALARA (As Low As Reasonably achievable) principle. To ascertain that dose is constantly low, dose survey and audit is recommended by the ICRP. In practice, the entrance surface dose (ESD) or Entrance Surface Air Kerma can either be assessed using thermoluminescent (TL) chips, which is called the direct approach or can be computed using exposure parameters and mathematical software (indirect approach) as recommended by International Atomic Energy Agency (IAEA) and the International Commission on Radiation Units and Measurements (ICRU) (15, 16). This present study investigated the radiation dose to pediatric patients' between 0-15 years by determining their mean ESD, 75th percentile ESD, absorbed dose (D), effective dose (E) and cancer risk during chest procedures. Meanwhile, this research remains crucial considering the paucity of research in pediatric radiology in Nigeria.

## MATERIAL and METHODS

This study was undertaken using a direct digital (DR) X-ray machine (serial number 19021033). It has a total tube filtration of 3.3 mm Al equivalent at 75 kVp and peak kilovoltage (kVp) ranging from 40-150. Other specifications of the machine are presented [Table 1].

Table 1. Digital X-ray specifications

Digital Radiography machine specifications	
Manufacturer	RADIOLOGIA
Type	Ceiling Mounted Unit (DR System)
Serial Number	19030007
Machine Model	POLYRAD PREMIUM CS
Power Capacity	50kW
kVp Range	40-150kVp
mAs Range	0.1-630mAs
Maximum Current	3.5-1.6A
Minimum Filtration	2mmAl @75kVp
Focal Spot	1.2/0.6
Grid	Yes (14×17 inches)
Total Filtration	3.3mmAl
Line Voltage	115-240V
Phase	3, 50/60Hz
Target	Tungsten
Manufactured Date	February 2019

The study also used a calibrated meter rule for the measurement of height, a digital weighing balance, calibrated in kilogram (kg) for weight, TLD chips, a TLD Cube-400 reader and TLD furnace type LAB-01/400 annealing oven.

The ESD of 50 pediatric patients undergoing chest procedures was estimated using TLD-100 chips made of Lithium Fluoride, doped with Magnesium and Titanium (LiF: Mg, Ti) with sizes of 3.2 mm × 3.2 mm and thickness of 0.90±0.05.

It was preferred because of its tissue-equivalent nature. The TLD chips were calibrated at the secondary standard dosimetry laboratory (SSDL) using a Cesium (Cs)-137 source and the TLD element correction factor (ECF) and homogeneity were within the acceptable range for use (17, 18).

A total of 100 TLD (LiF: Mg, Ti) were placed in a TLD furnace type LAB-01/400 at a temperature of 400 °C for one (1) hour and allowed to cool to room temperature. In order to remove lower peaks, it was further subjected to another temperature of 100 °C for two (2) hours and was allowed to cool. After 48 hours (2 days) the chips were ready for use. The TLD chips were carefully placed in transparent nylon and were numbered serially.

It was attached to the patients' skin at the anterior and posterior end. After exposure the TLD chips were removed and read. A RadPro cube 400 manual TLD Reader (Friedberg Instruments GmbH, Germany) was used to determine the corresponding TLD count for the chips. The average background count was obtained from five (5) TLD chips that were not exposed to radiation (TLD<sub>0</sub>). Obtained TLD counts (TLD<sub>i</sub>-TLD<sub>0</sub>) were multiplied with a pre-determined X-ray calibration factor, which was previously determined (17).

Anthropometric parameter such as age, sex, height, and weight of the each patient were measured. Similarly machine parameter like kVp, mAs and Focus to Film Distance (FFD) was measured and recorded.

The patient effective dose (E) was calculated using the mathematical relation:

$$\text{Effective dose (E)} = \sum [\text{Tissue weighting factor (W}_T\text{)} \times \text{Equivalent dose (H}_T\text{)}] \quad [1]$$

The tissue weighting factor (W<sub>T</sub>) was determined using the International Commission on Radiological Protection (ICRP) report 103 and the equivalent dose (H<sub>T</sub>) was determined from the product of the absorbed dose and radiation quality factor for X-ray.

$$\text{Similarly, the Equivalent dose (H}_T\text{)} = \text{Quality factor (Q)} \times \text{Absorbed dose (D}_T\text{)} \quad [2]$$

In this case the radiation quality factor (Q) for X-ray ≡ 1.

## RESULTS

The distribution of males and females and the age range of patients' are presented [Figures 1 & 2].



The mean kVp, mAs, field size, height, weight, age, FFD and BMI among 13 female subjects from age 0- <1 years was  $54.77 \pm 4.02$  kVp,  $6.29 \pm 1.13$  mAs,  $488 \pm 180$  cm<sup>2</sup>,  $0.5 \pm 0.10$  m,  $4.9 \pm 1.66$  kg,  $0.18 \pm 0.29$  years,  $108.9 \pm 21.78$  cm and  $18.02 \pm 8.61$  kg/m<sup>2</sup> respectively, while that of 8 male subject was  $56.25 \pm 1.98$  kVp,  $5.98 \pm 0.60$  mAs,  $571 \pm 239$  cm<sup>2</sup>,  $0.57 \pm 0.14$  m,  $8.06 \pm 5.47$  kg,  $0.18 \pm 0.29$  years,  $103.9 \pm 10.96$  cm and  $23.5 \pm 8.83$  kg/m<sup>2</sup> respectively.

There was strong correlation between the ESD and D ( $P = 0.001$ ) [Table 2].

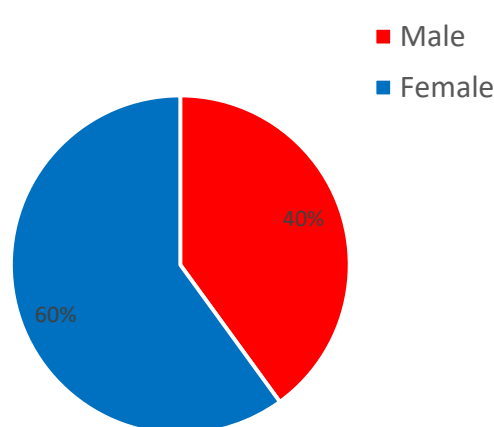
Also the mean kVp, mAs, field size, height, weight, age, FFD and BMI among 6 female subjects from age 1- <5 years was  $58.33 \pm 2.07$  kVp,  $8.53 \pm 0.74$  mAs,  $789 \pm 117$  cm<sup>2</sup>,  $0.83 \pm 0.17$  m,  $17.1 \pm 9.29$  kg,  $2.33 \pm 0.82$  years,  $128.8 \pm 31.59$  cm and  $24.79 \pm 11.6$  kg/m<sup>2</sup> respectively, while that of 6 male subject from was  $56.17 \pm 3.06$  kVp,  $6.78 \pm 1.68$  mAs,  $692.3 \pm 313.1$  cm<sup>2</sup>,  $0.80 \pm 0.18$  m,  $12.28 \pm 4.38$  kg,  $1.50 \pm 0.84$  years,  $109.7 \pm 23.7$  cm and  $20.69 \pm 9.67$  kg/m<sup>2</sup> respectively.

The ESD and D was correlated significantly ( $P < 0.001$ ) [Table 3].

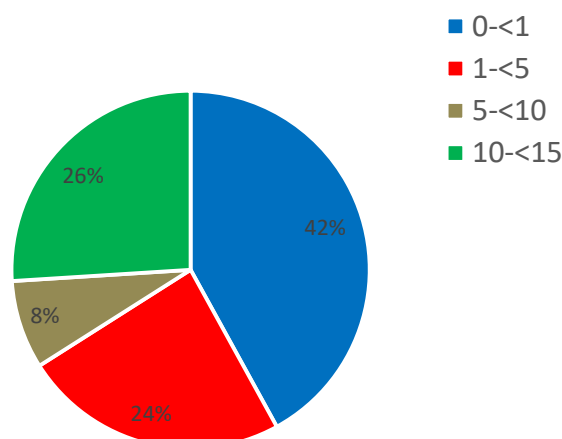
Furthermore, the mean kVp, mAs, field size, height, weight, age, FFD and BMI among 2 male subjects from age 5- <10 years was  $60 \pm 0.00$  kVp,  $8.20 \pm 0.28$  mAs,  $725 \pm 162.6$  cm<sup>2</sup>,  $1.21 \pm 0.15$  m,  $27 \pm 0.00$  kg,  $7.0 \pm 2.83$  years,  $164 \pm 8.49$  cm and  $19.02 \pm 4.65$  kg/m<sup>2</sup> respectively. There was no correlation in ESD and D ( $P = 0.254$ ) owing to the limited sample size [Table 4].

The mean kVp, mAs, field size, height, weight, age, FFD and BMI among 10 female subjects from age 10-  $\leq 15$  years was  $66.1 \pm 3.34$  kVp,  $12.31 \pm 0.82$  mAs,  $1126 \pm 227.5$  cm<sup>2</sup>,  $1.61 \pm 0.09$  m,  $57.9 \pm 12.85$  kg,  $14.8 \pm 0.63$  years,  $161.1 \pm 9.31$  cm and  $22.21 \pm 4.37$  kg/m<sup>2</sup> respectively, while that of 4 male subjects was  $66.25 \pm 4.79$  kVp,  $10.85 \pm 2.01$  mAs,  $850.25 \pm 228$  cm<sup>2</sup>,  $1.48 \pm 0.18$  m,  $58.3 \pm 20.49$  kg,  $12.5 \pm 2.89$  years,  $170 \pm 9.00$  cm and  $25.83 \pm 5.12$  kg/m<sup>2</sup> respectively. The ESD and D correlated significantly ( $P = 0.001$ ) [Table 5].

Lastly, comparison was made with similar studies. There was no correlation in ESD between this study and studies in Nigeria ( $P = 0.811$ ), Ethiopia ( $P = 0.926$ ), Sudan ( $P = 0.903$ ), and Brazil ( $P = 0.791$ ;  $P = 0.811$ ) [Table 6].



**Figure 1:** Gender classifications of the participants



**Figure 2:** Age range classification

**Table 2.** Anthropometric and exposure parameter for chest X-ray for age 0- <1 years

No	kVp	mAs	Field size (cm <sup>2</sup> )	Height (m)	Weight (kg)	Age (yrs)	Sex	FFD (cm <sup>2</sup> )	BMI (kg/m <sup>2</sup> )	ESD (mGy)	D (mGy)
1	56	6.3	304	0.5	3.4	0.008	F	100	13.6	0.89	0.04
2	57	6.3	690	0.7	5.2	0.75	F	100	10.6	1.68	0.57
3	49	5	368	0.6	3.9	0.3	F	100	10.8	1.53	0.51
4	49	5	810	0.4	3.8	0.003	F	100	23.8	2.88	1.35
5	57	6.3	705	0.6	4	0.005	F	100	11.1	2.21	0.78
6	57	6.3	413	0.6	4.8	0.07	F	100	13.3	2.74	1.95
7	50	5	192	0.4	4	0.02	F	100	25.0	0.76	0.75
8	54	6.3	630	0.5	4.5	0.005	F	100	18.0	1.24	0.29
9	55	5	414	0.4	4.4	0.003	F	100	27.5	0.41	0.15
10	50	8	442	0.5	9.8	0.25	F	100	39.2	1.88	0.92
11	58	8	520	0.6	6.2	0.083	F	100	17.2	0.52	0.16
12	60	6.3	530	0.58	4.3	0.041	F	158	12.78	0.57	0.38
13	60	8	330	0.69	5.4	0.83	F	158	11.34	1.18	0.38
14	55	5	930	0.7	18.3	0.75	M	100	37.3	2.56	1.38
15	58	6.3	506	0.7	14	0.25	M	100	28.6	1.38	0.60
16	54	6.3	389.5	0.5	5	0.008	M	100	20.0	2.55	0.26
17	58	6.3	361	0.5	3.9	0.005	M	100	15.6	2.11	0.38
18	54	6.3	930	0.4	5.5	0.03	M	100	34.4	1.61	0.92
19	57	5	329	0.4	3.2	0.003	M	100	20.0	1.52	0.46
20	59	6.3	525	0.6	4.9	0.055	M	100	13.6	1.06	0.41
21	55	6.3	594	0.73	9.7	0.92	M	131	18.2	1.09	0.80

kVp = Peak kilovoltage, mAs = milliamperes-seconds, FFD = Focus to film distance, BMI = Body mass index, ESD = Entrance surface dose, D = Absorbed dose

**Table 3.** Anthropometric and exposure parameter for chest X-ray for age 1- <5 years

No	kVp	mAs	Field size (cm <sup>2</sup> )	Height (m)	Weight (kg)	Age (yrs)	Sex	FFD (cm <sup>2</sup> )	BMI (kg/m <sup>2</sup> )	ESD (mGy)	D (mGy)
1	60	8.4	840	0.85	10.2	2	F	158	14.11	0.87	0.17
2	60	8	840	1.1	18.8	4	F	100	15.5	1.81	0.45
3	60	10	840	0.8	27	2	F	100	42.2	3.31	2.39
4	58	8	550	0.9	29	2	F	100	35.8	0.87	0.15
5	57	8.4	825	0.71	9.1	2	F	158	18.05	0.84	0.22
6	55	8.4	840	0.6	8.3	2	F	157	23.05	0.86	0.25
7	57	6.3	918	1	14.8	2	M	100	14.8	0.89	0.03
8	58	6	810	0.9	12	1	M	100	14.8	1.95	1.48
9	50	4	196	0.5	6	1	M	100	24	1.53	0.12
10	57	8	990	0.8	11.8	1	M	100	18.4	2.80	1.35
11	58	8	420	0.7	19	1	M	100	38.8	1.71	0.74
12	57	8.4	820	0.87	10.1	3	M	158	13.34	0.92	0.26

kVp = Peak kilovoltage, mAs = milliamperes-seconds, FFD = Focus to film distance, BMI =Body mass index, ESD =Entrance surface dose, D = Absorbed dose

**Table 4.** Anthropometric and exposure parameter for chest X-ray for age 5- <10 years

No	kVp	mAs	Field size (cm <sup>2</sup> )	Height (m)	Weight (kg)	Age	Sex	FFD (cm <sup>2</sup> )	BMI (kg/m <sup>2</sup> )	ESD (mGy)	D (mGy)
1	57	6.3	690	0.62	25	6	F	158	14.3	1.00	0.76
2	60	8	840	1.1	27	5	M	158	22.31	0.40	0.06
3	60	8.4	610	1.31	27	9	M	170	15.73	0.26	0.22

kVp = Peak kilovoltage, mAs = milliamperes-seconds, FFD = Focus to film distance, BMI =Body mass index, ESD =Entrance surface dose, D = Absorbed dose

**Table 5.** Anthropometric and exposure parameter for chest X-ray for age 10- ≤15 years

No	kVp	mAs	Field size (cm <sup>2</sup> )	Height (m)	Weight (kg)	Age (yrs)	Sex	FFD (cm <sup>2</sup> )	BMI (kg/m <sup>2</sup> )	ESD (mGy)	D (mGy)
1	60	12.5	572	1.6	52	15	F	180	20.3	1.65	1.23
2	68	12.8	1225	1.6	83.2	15	F	157	32.5	0.64	0.60
3	68	12.5	1225	1.6	52	15	F	157	20.3	1.36	0.80
4	68	12.5	1225	1.7	65.7	15	F	157	22.7	1.39	1.23
5	68	12.5	1225	1.6	58	15	F	157	22.7	1.00	0.85
6	68	12.5	1225	1.6	65	15	F	157	25.4	1.24	0.72
7	68	12.8	1225	1.6	57	15	F	158	22.3	1.82	0.62
8	68	12.5	1362	1.7	61	15	F	148	21.1	1.49	0.49
9	60	10	1050	1.4	33	13	F	170	16.83	2.10	1.19
10	65	12.5	930	1.7	52	15	F	170	17.99	1.68	1.14
11	60	8.4	621	1.21	29	10	M	170	19.8	0.33	0.16
12	70	10	1224	1.6	75	15	M	158	29.29	0.95	0.76
13	65	12.5	930	1.6	60	15	M	170	23.43	0.43	0.18
14	70	12.5	626	1.5	69.2	10	M	180	30.8	2.13	1.22

kVp = Peak kilovoltage, mAs = milliamperes-seconds, FFD = Focus to film distance, BMI =Body mass index, ESD =Entrance surface dose, D = Absorbed dose

**Table 6:** Comparison of ESD (mGy) of this work with other similar works

Age (years)	This study (TLD-100)	Nigeria <sup>22</sup> (DoseCal)	Ethiopia <sup>25</sup> (Tube output)	Sudan <sup>24</sup> (DoseCal)	Brazil <sup>21</sup> (TLD-100)	(CaSO <sub>4</sub> :Dy)
0-<1	1.54	0.110	1.82	0.057	-	-
1-<5	1.53	0.109	1.72	0.138	0.047	0.06
5-<10	0.55	0.109	3.40	0.220	0.09	0.06
10≤15	1.30	0.101	5.87	0.664	0.12	0.15

DoseCal = Software for computing ESD

## DISCUSSION

This study investigated radiation dose to pediatric patients undergoing chest X-ray (representing the most commonly referred cases) investigations in a non-dedicated X-rays unit using direct digital technology. In total, 50 patients from 0-15 years were studied. The EU (1996) recommends a sample of minimum of 10 patients for the survey to be statistically significant, however, this study can be said to be a good dose representative of the pediatric patients in Asaba metropolis, thus providing a reliable base-line data for subsequent researchers since this is a novel study of this category in the studied facility (17).

Findings from this study show that the 75th percentile ESD (2.00mSv) was 17 times higher in dose compared to the DRL in the American College of Radiology-American Association of Physicists in Medicine-Society for Pediatric Radiology (ACR-AAPM-SPR) report, where the pediatric dose was 0.15mSv (19, 20). Although there was a good correlation between ESD and D (P = 0.001), however, a One-Way ANOVA shows that the field size and focus to film distance (FFD) significantly affected ESD and D (P < 0.001) respectively.



In another study, the ESD for 0-1 (1.54mSv) and 1-5 years (1.53mSv) was higher than a study in Brazil by Mohamadain et al, where the ESD for AP chest X-ray for 0-1 and 1-5 was 0.07mSv with TLD (CaSO<sub>4</sub>:Dy) and the PA view was 0.05 and 0.06mSv with TLD-100 respectively. The variation in dose was between 129-132%, in comparison with our study. The same trend in dose variation (>100%) was observed for 1- 5, 5-10 and 10-15 years for AP chest X-ray in Mohamadain's study. The reason for this variation despite the use of similar TLD chips for surface dose measurements was due to the field size and FFD, which was noted to be statistically significant in this study. Other variations could be from the TLD properties and uncertainties (21).

In a similar study in Nigeria by Egbe et al, who used TLDs, the mean ESD from AP chest X-ray from 3 facilities studied between the age group of 0-1 years were 0.64, 0.07 and 1.1mGy (22). The variation between Egbe's work and this study was 64, 129 and 24%. Dose discrepancies are likely due to the total tube filtration which ranged from 2.5-2.7mm Al, against this study which was 3.3mm Al.

This study used a flat panel system and Egbe's study used a film-screen system. Other factors may be field size and FFD related (22). Also, TLDs were used in a study in Turkey by Olgar et al, where the obtained mean ESD was 0.07mGy. The variation was 129%, compared to our study (23).

The mean ESD for 0- >1 (0.057mSv), 1- > 5 (0.138mSv), 5- > 10 (0.220mSv) and 10- >15 (0.664mSv) from a study by Alatts and Abukhiar in Sudan with DoseCal Software was lower compared to our study. Variation in dose was 131, 118, 102 and 56% respectively (24). Machine parameters like the kVp, mAs, field size and FFD may contribute to the difference in the dose and Since the Dose Cal software is a mathematical human model, the ESD obtained may vary significantly with our study.

Conversely, the mean ESD for 0-1 (1.82mSv), 1-5 (1.72mSv), 5-10 (3.4mSv) and 10-15 (5.87mSv) from a study by Mesfin et al in Addis Ababa in Ethiopia with the tube output method was higher than our study. Variation in dose was 12, 8, 106 and 90% respectively (25). ESD from both studies was considered to be high in comparison with other studies. An independent student-t-test shows that both study showed no difference in age, weight, kVp, mAs and field size for 0-1 (P = 0.976), 1-5 (P = 0.947), 5-10 (P = 0.804) and 10-15 (P = 0.690) respectively.

The mean effective dose (E) for the 4 age groups (0.18±0.03mSv) was 28 times higher than the Health Protection Agency (HPA-CRCE-028) report, where the mean effective dose for 0-15 years was 0.0065mSv based on ICRP 60 report (26).

In a related study by Vilar-Palop et al, the effective dose (E) for <1 (0.05mSv), 1-5 (0.05mSv), 6-10 (0.05mSv) and 11-15 (0.06mSv) was lower than our study. It was identified from this study that the field size and FFD primary affected dose. This may be a reason for the disparity noticed; since the effective dose was calculated from the equivalent dose (27).

Finally, the risk of childhood cancer from a single radiograph was of the order of  $(1.54-23.4) \times 10^{-6}$ . This was higher than a study by Armpilia et al and Aliasgharzadeh et al, where their childhood cancer risk was  $(0.3-1.3) \times 10^{-6}$  (28) and

$(1.27-5.91) \times 10^{-6}$  (29). The above results imply that the risk of childhood cancer will increase.

## CONCLUSION

The local reference level for pediatric patients' for age 0-15 years based on the 75th percentile ESD was above studies it was compared with. The study identified the field size and FFD as major factors that contributed to the increase in patient dose. This increase affected the effective dose and cancer risk calculations. There is an urgent need for the facility to embark on protocol optimization in order to reduce cancer risk among pediatric patients'.

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## Antimicrobial resistance profiles of *Enterobacter cloacae* and *Klebsiella aerogenes* a tertiary hospital in Turkey: A five-years study

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### ABSTRACT

**Objective:** *Enterobacter cloacae* and *Klebsiella aerogenes* species have multiple drug resistance and antibiotic resistance is a growing problem regarding to treating infections.

**Objective:** The aim of this study was to determine and evaluate the antimicrobial resistance profiles of *E. cloacae* and *K. aerogenes* isolated from various clinical samples in our laboratory, retrospectively.

**Material and Methods:** Totally 223 patients who applied to Karabuk University Training and Research Hospital microbiology laboratory between October 2016-December 2020 were included in this study. Conventional methods and automated systems were used for the identification and antibiotic susceptibilities of strains. Antibiotic susceptibility results were evaluated as per the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.

**Results:** Total of 223 clinical samples (urine 68.6%, blood 12.6%, endotracheal aspirate 7.2%, wound 4.9%, sputum 3.6%, bronchoalveolar lavage fluid 2.7%, and ear fluid 0.4%) obtained from 223 patients; 119 (53%) females and 104 (47%) males, were analysed. The identified species were *E. cloacae* (132 strains, 59.2%) and *K. aerogenes* (91 strains, 40.8%). The *Enterobacter cloacae* and *Klebsiella aerogenes* positivity was detected as 30(13.4%) and 20(9.0%) in the samples. The highest resistance was found against cefixime at a rate of 60%; the lowest resistance was against amikacin, meropenem and imipenem ranged between 3% and 4% in both *E. cloacae* and *K. aerogenes* strains.

**Conclusions:** Amikacin, imipenem and meropenem were the most effective antibiotics against *E. cloacae* and *K. aerogenes*. We may prefer TMP-SMX and ciprofloxacin, as oral antibiotic agents in the treatment of *E. cloacae*/*K. aerogenes* infections. Amikacin, gentamicin and carbapenems may be the first choice for parenteral antibiotics therapy.

**Keywords:** Antibiotic resistance, *Enterobacter cloacae*, ESKAPE pathogen, *Klebsiella aerogenes*

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### INTRODUCTION

*Enterobacter cloacae* and *Klebsiella aerogenes* (formerly described *Enterobacter aerogenes*) are a facultative anaerobe, gram-negative rods, which include to the Enterobacteriales family. In recently, twenty-two species have been in the *Enterobacter* genus (*E. aerogenes*, *E. amnigenus*, *E. asburiae*, *E. arachidis*, *E. carcinogenus*, *E. cloacae*, *E. cowanii*, *E. dissolvans*, *E. gergoviae*, *E. helveticus*, *E. hormaechei*, *E. kobei*, *E. ludwigii*, *E. mori*, *E. nimipressuralis*, *E. oryzae*, *E. pulveris*, *E. pyrinus*, *E. radicincitans*, *E. soli*, *E. taylora*, and *E. turicensis*). Among these species, seven are called as “*E. cloacae* complex” (*E. cloacae*, *E. asburiae*, *E. hormaechei*, *E. kobei*, *E. ludwigii*, *E. mori*, and *E. nimipressuralis*) (1,2). Currently, whole genome sequence based comparative bacterial phylogenetic analyses of *E. aerogenes* demonstrated that *E. aerogenes* is more closely related to *Klebsiella pneumoniae* than to the *Enterobacter* species. Then, these bacteria formerly named as *E. aerogenes* was called as *Klebsiella aerogenes* (3).

*E. cloacae* and *K. aerogenes* are members of the respiratory tract and gastrointestinal microbiota of humans and often isolates as opportunistic pathogens in nosocomial infections, especially in neonates, immunocompromised patients and hospitalized in intensive care units (1,3).

*Enterobacter cloacae* cause neonatal meningitis, bacteraemia, lower respiratory tract infections, skin and soft tissue infections, and urinary tract infections. *Enterobacter* species are members of the ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter species*), which are described as the leading cause of resistant nosocomial infections (1, 2). *E. cloacae* and *K. aerogenes* associated with the contaminations caused by blood products, intravenous injection fluids, probes, catheters, respiratory therapy equipment, and colonized hands of healthcare workers. Invasive procedures, such as catheterization and intubation and a long-term duration of hospitalization which are frequently found in an intensive care unit (ICU), represent a main source of *Enterobacter* infection (3-5).

Antibiotic resistance is a growing problem regarding to treating *Enterobacter* infections. *Enterobacter* species (spp.) have multidrug resistance by means of porine loss, efflux system activation, AmpC cephalosporinase and metallo-beta-lactamase enzyme systems (6). The main mechanism of antimicrobial resistance in *Enterobacter* species is presence of beta-lactamases. They can hydrolyze the beta-lactam ring of penicillin and cephalosporins (6). Carbapenems are to be the most effective agent to treatments of multidrug-resistant *Enterobacter* infections. However, carbapenem-resistant *Enterobacter* species (CRE) and Extended-Spectrum beta-lactamase (ESBL) have been reported to cause serious nosocomial outbreaks in different countries with a high mortality rate (7-10)..

The World Health Organization announced a list of antibiotic-resistant bacteria in 2017, CRE was in the critical priority group for an urgent need to develop new antibiotics (4). Though bacterial comparative phylogenetics has demonstrated that *K. aerogenes* and *Enterobacter* species belong to different phylogenetic groups, the clinical impact of these genetic differences is unknown. The prevalence, clinical risk factors, antibiotic susceptibility patterns, and patient outcomes have not yet been determined since renaming *K. aerogenes*.

The aim of this study was to determine and evaluate the antimicrobial resistance profiles of *E. cloacae* and *K. aerogenes* isolated from various clinical samples in our laboratory between 2016-2020 retrospectively.

## MATERIAL and METHODS

In this cross-sectional study, antibiotic susceptibility results of 132 *E. cloacae* and 91 *K. aerogenes* strains obtained from outpatient or inpatient treated in Karabuk University Training and Research Hospital between January 2016- December 2020, five years period, were included. These results were obtained from the laboratory information system. The other bacteria's antibiotic susceptibility test results and repeated patient results were excluded from this study.

The identification and antibiotic susceptibility of strains were determined with the BD-Phoenix 100 (Becton-Dickinson, Sparks, MD, USA) fully automated system. Antibiotic susceptibility test results were interpreted as per the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (11).

*Escherichia coli* ATCC 25922 strains were used for quality control. The production of the extended-spectrum beta-lactamase (ESBL) enzyme was detected using the combined disk diffusion method (11).

## Statistical Analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS for IBM-PC, release 20.0; SPSS Inc., USA). The descriptive statistics were stated as the number, percentage, and median value. The Colmogorov- Smirnov test was used as normality test. The Mann - Whitney U test and Fisher's exact test were used to evaluate the data, and P-value  $\leq 0.05$  was considered statistically significant.

**Ethical Review of the Proposal and the Consent:** The ethics approval was obtained from the Non-Interventional Clinical Research Ethics Committee of Karabuk University (No: 2021/502).

## RESULTS

Among 223 patients, 119 (53%) were female and 104 (47%) were males. The median age of the patients was 57 (0-96) years. The numbers and age ranges of patients included in the study are shown in Table 1.

In our study, a total of 223 clinical specimens were examined over five years' time (2016-2020). The most common samples were urine (153 samples, 68.6%), blood (28 samples, 12.6%), ETA (16 samples, 7.2%). Wound (11 samples, 4.9%), sputum (8 samples, 3.6%), BAL (6 samples, 2.7%) and one ear fluid swab (0.4%) were also analyzed. The 132 of 223 (59.2%) strains were *E. cloacae* whereas 91 of them (40.8 %) were *K. aerogenes*. The distribution of the samples according to the *E. cloacae* and *K. aerogenes* positivity is shown in Table 2.

About 52% (n=116) of the strains were isolated from outpatients while 48% (n=107) were isolated from clinical or intensive care patients. Among outpatients, *E. cloacae* and *K. aerogenes* positivity were 53.4% (62/116) and 46.6% (54/116), respectively. Among clinics, intensive care patients *E. cloacae* and *K. aerogenes* positivity were 65.4% (70/107) and 34.6% (37/107), respectively. When the distribution of the *E. cloacae* and *K. aerogenes* positivity was examined according to clinics (outpatients or clinical or intensive care units), *E. cloacae* positivity was higher but there was not statistically significant ( $p>0.05$ ).

*Enterobacter cloacae* and *K. aerogenes* positivity rates are evaluated according to the clinics, where the samples were sent. The most frequently *E. cloacae* and *K. aerogenes* positivity was detected in 30 (13.4%) and 20 (9.0%) of the samples from the pediatric services, in 35 (15.7%) and 23 (10.3%) from an intensive care unit, in 25 (11.2%) and 25 (11.2%) of the samples from urology service, respectively.



When the distribution of the *E. cloacae* and *K. aerogenes* positivity was examined according to clinics, there was no statistically significant ( $P > 0.05$ ). The distribution of samples according to clinics is shown in Table 3.

Antibiotic susceptibility test was performed on all the samples with *E. cloacae* and *K. aerogenes* growth. Amikacin was found to be the most effective antibiotic against *E. cloacae* and *K. aerogenes*.

The highest resistance was found against cefixime as 60% and the lowest resistance was against amikacin, meropenem and imipenem ranged between 3% and 4% in both *E. cloacae* and *K. aerogenes* strains. Fosfomycin resistance rates were 27% among *E. cloacae* and 16% among *K. aerogenes* ( $P < 0.05$ ). The production of ESBL in *E. cloacae* strains was higher than *K. aerogenes* strains. ESBL rates were 26% among *E. cloacae* and 15% among *K. aerogenes*. It was statistically significant ( $P = 0.03$ ). Antibiotic susceptibility test results of *E. cloacae* and *K. aerogenes* strains are shown in Table 4.

**Table 1:** The gender and age of the patients

	Number of Patients	Median (Min.-Max)	Mean±SD
Female	119	51 (0-96)	47.8±33.0
Male	104	58 (0-95)	46.5±33.2
TOTAL	223	57 (0-96)	47.2±33.0

SD: Standart deviation Min: Minimum, Max: Maximum

**Table 2:** The distribution of the samples according to the *E. cloacae* and *K. aerogenes* positivity

Sample	<i>Enterobacter cloacae</i> n (%)	<i>Klebsiella aerogenes</i> n (%)	Total n (%)
Urine	86 (38.6%)	67 (30%)	153 (68.6%)
Blood	18 (8.0%)	10 (4.5%)	28 (12.6%)
ETA	9 (4.0%)	7 (3.1%)	16 (7.2%)
Wound	8 (3.6%)	3 (1.4%)	11 (4.9%)
Sputum	5 (2.3%)	3 (1.4%)	8 (3.6%)
BAL	5 (2.3%)	1 (0.4%)	6 (2.7%)
Ear fluid	1 (0.4%)	-	1 (0.4%)
TOTAL	132 (59.2%)	91 (40.8%)	223 (100%)

**Table 3:** The distribution of samples according to clinics

Clinics	<i>E. cloacae</i> n (%)	<i>K. aerogenes</i> n (%)	Total n (%)
Intensive care	35 (15.7%)	23 (10.3%)	58 (26.0%)
Pediatric	33 (14.7%)	22 (9.9%)	55 (24.6%)
Urology	25 (11.2%)	25 (11.2%)	50 (22.4%)
Internal medicine	11 (4.9%)	9 (4.0%)	20 (9.0%)
Chest diseases	6 (2.7%)	2 (0.9%)	8 (3.6%)
Palliative care	5 (2.2%)	3 (1.3%)	8 (3.6%)
Gyneacology	4 (4.0%)	3 (1.3%)	7 (3.1%)
Neurology	4 (4.0%)	-	4 (4.0%)
Infectious diseases	3 (1.3%)	2 (0.9%)	5 (2.2%)
Neurosurgery	2 (0.9%)	-	2 (0.9%)
Oncology	1 (0.4%)	1 (0.4%)	2 (0.9%)
General surgery	1 (0.4%)	1 (0.4%)	2 (0.9%)
Cardiovascular surgery	1 (0.4%)	-	1 (0.4%)
Otorhinolaryngology	1 (0.4%)	-	1 (0.4%)
TOTAL	132 (59.2%)	91 (40.8%)	223 (100%)

**Table 4:** Antibiotic susceptibilities of *E. cloacae* and *K. aerogenes* strains [(resistance rate %) number of resistant strains/ numbers of total strains]

Antibiotics	<i>Enterobacter cloacae</i> n (%)	<i>Klebsiella aerogenes</i> n (%)	Total n (%)	P value
CFM	60 (63/104)	61 (48/80)	60 (111/184)	0.81
CAZ	29 (39/132)	22 (20/91)	26 (59/223)	0.61
FOS	<b>27 (23/86)</b>	<b>16 (10/62)</b>	<b>29 (43/148)</b>	<b>0.03*</b>
ESBL	<b>26 (34/132)</b>	<b>15 (14/91)</b>	<b>22 (48/223)</b>	<b>0.03*</b>
TZP	26 (34/132)	23 (21/91)	25 (55/223)	0.56
CIP	20 (26/128)	13 (11/87)	17 (37/215)	0.71
TMP-SMX	14 (18/132)	22 (20/91)	17 (38/223)	0.79
GN	7 (9/132)	10 (9/91)	9 (19/223)	0.56
IPM	4 (4/89)	3 (2/62)	4 (6/151)	0.82
MEM	4 (4/89)	3 (2/62)	4 (6/151)	0.82
AK	4 (5/122)	0 (0/88)	2 (5/210)	NA

FOS: Fosfomycin, AMP: ampicillin, AMC: amoxicillin-clavulanic acid, CFM: cefixime, TMP-SMX: trimethoprim/sulfamethoxazole, GN: gentamicin, AK: amikacin, CIP: ciprofloxacin, CAZ: ceftazidime, IPM: imipenem, MEM: meropenem, TZP: tazobactam, ESBL: Extended spectrum beta-lactamase, NA: not applicable. \*p<0.05

## DISCUSSION

Increasing antibiotic resistance emerges as an important health problem worldwide. Antimicrobial resistance is widespread in Enterobacteriales family, especially *E. coli*, *Klebsiella* and *Enterobacter* species. In 2009, the Infectious Disease Society of America (IDSA) included these three genera into ESKAPE pathogens (12).

*Enterobacter* species are intrinsic resistant to aminopenicillins, first and second-generation cephalosporins because they can produce chromosomally derived AmpC beta-lactamase (7). On the other hand, ESBL-producing *Enterobacter* species emerged due to the overuse of third-generation cephalosporins (13). Since ESBL-producing isolates can hydrolyze penicillin, cephalosporins, and monobactams, treatment options are limited. In this study, we found the rate of ESBL in *Enterobacter* species to be 22%. This ratio was significantly higher in *E. cloacae* strains than *K. aerogenes* and was 26% and 17%, respectively (P = 0.03). The ESBL rates are highly versatile among countries. For instance, it has been reported as 7.5% (14) in the Netherlands and 72.7% (15) from Bosnia. This may be due to the difference in antibiotic using strategies among countries and the isolates' collection date. The treatment options of ESBL-producing *Enterobacter* infections are limited. Carbapenems are often the first choice. However, as a result of the overuse of carbapenem, carbapenem-resistant *Enterobacter* isolates have emerged. Carbapenem resistance has been reported between 0%-35.1% in *Enterobacter* isolates globally (13,15-17). One hundred-thirty *Enterobacter* spp. isolated in India, MBL was detected in 36.9% of the strains and it has been reported that they carry VIM-2, VIM-6, and NDM-1. Also, Omp 35 and Omp 36 porin loss were found associated with carbapenem resistance (18).

This study detected 4% resistance to imipenem and meropenem in *E. cloacae* and *K. aerogenes* strains. Yazıcı et al. (19) reported 2.4% in 2004, Aksaray et al. (20) found a resistance rate of 4% in 2006, whereas Ozcan et al. (21) reported 11.4% carbapenem resistance among *Enterobacter* spp. in 2020. Accordingly, the carbapenem resistance rate is low in our study. However, it is noteworthy that all seven carbapenem-resistant strains were isolated in 2020.

In a China study, carbapenem resistance in *E. cloacae* strains was 1% in 2007, whereas this rate was reported as 6.8% in 2017 (22). On the other hand, Nedjaci et al. from Algeria have reported no carbapenem resistance in *E. cloacae* strains in 2013 (13). Besides, Cui et al. (16) 11.5% from China, Uzunovic et al. (15) 7.1% from Bosnia, and Ghanavati et al. reported as 35.1% resistance rate in Iran (17).

Aminoglycoside antibiotics are good therapeutic options for both carbapenem-resistant and ESBL-producing isolates. It has been reported that aminoglycoside resistance develops through aminoglycoside-modifying enzymes in *Enterobacter* species (7). In this study, we have found 4% resistance to gentamicin and 2% to amikacin. Gentamicin resistance was reported ranging from 21.1%-44% in Turkey (20,21,23). However, its highly variable ranging from 9.4%-85.9% worldwide (15,16,22,24,25). Therefore, we should determine empirical antibiotic treatment protocols according to regional antibiotic resistance rates and follow.

Cefixime is an oral third-generation cephalosporin frequently prescribed in children and pregnant women. In this study, we found 60% resistance to cefixime. In 2014, Khosravi et al. have reported 71% resistance to cefixime in urinary *Enterobacter* strains in Iran (26). On the other hand, we found resistance at a rate of 17% to TMP-SMX and ciprofloxacin, which are oral antibiotics commonly used in the treatment of urinary infections. In 2020, Ozcan et al. from Turkey has reported a resistance rate of 27.3% to ciprofloxacin and 19.3% to TMP-SMX (21). In previous studies, ciprofloxacin resistance is reported between 2.4%-8% (19,20) in Turkey. It has reported ranging from 13.3%-78.6% worldwide (15-17). TMP-SMX resistance is between 18.9% and 79.7% in studies reported from China (16,22,24).

Although fosfomycin was found in 1960, it was discontinued over time. However, it has become popular today as it is effective against most multidrug-resistant bacteria. It can be used both oral and intravenously. In this study, we found resistance to fosfomycin at a rate of 27% in *E. cloacae* isolates and 16% in *K. aerogenes* strains. Demir et al. (27) from Turkey have reported the fosfomycin resistance rate as



44% in urinary *Enterobacter* isolates and Fajfi et al. reported at a rate of 74.4% from the Czech Republic (28). The IDSA does not recommend antibiotics with a resistance rate of more than 20% in empirical antibiotic therapy (29). Accordingly, TMP-SMX and ciprofloxacin may be preferred instead of cefixime and fosfomycin in the empirical treatment of *Enterobacter* infections in our region.

This study has some limitations. It is a retrospective, single-center study based on laboratory data. Also, we did not include the patients' clinical features, diagnosis, and treatment protocols.

## CONCLUSION

*Enterobacter cloacae* and *K. aerogenes* have many intrinsic and acquired resistance determinants. Besides, it was observed that antibiotic resistance gradually increases over time. In our region, TMP-SMX and ciprofloxacin may be preferred for urinary infections caused by these species. Aminoglycoside antibiotics and carbapenems can be the first choice to treat systemic infections. Apart from determining antibiotic resistance profiles, it should be monitored regularly.

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**Ethical issues:** All authors declare originality of research.

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## Investigation of the Value of Coagulation Parameters in Thromboembolic Events Among Patients Not Receiving Anticoagulant Therapy

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### ABSTRACT

**Objective:** The main causes of thromboembolic events are atherosclerosis, vascular endothelial injury, and hypercoagulability. Coagulation is activated through two basic mechanisms, including intrinsic and extrinsic pathways, leading to thrombin production as a result of a series of enzymatic reactions. The intrinsic pathway is evaluated with activated partial thromboplastin time (APTT) and extrinsic pathway with prothrombin time (PT). This study aimed to investigate the relationship between thromboembolic diseases and coagulation parameters.

**Material and Methods:** Patients diagnosed with acute ischemic stroke (AIS) (n=216), acute coronary syndrome (ACS) (n=25), pulmonary thromboembolism (PTE) (n=15), and patients without an emergency pathology (n=71) (Control Group) in the emergency department were retrospectively reviewed in the period from 01 November 2016 to 31 March 2019.

**Results:** The APTT (25.61±5.93 sec), PT (12.05±2.26 sec), and INR (1.04±0.19) values of the AIS group were statistically significantly lower compared to APTT (27.98±3.21 sec), PT (12.58±2.18 sec), and INR (1.10±0.15) values of the control group (p = <0.001, <0.001 and <0.001 respectively). Similarly, the APTT (27.15±8.97 sec), PT (12.26±2.75 sec), and INR (1.03±0.25) values of the ACS group were statistically significantly lower compared to those of the control group (p=0.012, 0.030, and 0.001, respectively). There was no statistically significant difference between the PTE group and control group in terms of APTT, PT, and INR values (p= 0.133, 0.758, and 0.711, respectively).

**Conclusion:** Shortened APTT levels in cases without a history of anticoagulant use at the time of admission can be considered to be a predictive and effective tool for clinicians in arterial embolic events (AIS and ACS).

**Key words:** Activated Partial Thromboplastin Time, Acute Coronary Syndrome, Acute Ischemic Stroke, International Normalized Ratio, Prothrombin Time, Pulmonary Thromboembolism

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### INTRODUCTION

Despite great progress in the diagnosis and management of thromboembolic diseases, the recognition of them still challenging for emergency physicians. The main causes of thromboembolic events are atherosclerosis, vascular endothelial injury, and hypercoagulability. Coagulation is activated through two basic mechanisms, including intrinsic and extrinsic pathways, leading to thrombin production as a result of a series of enzymatic reactions. The intrinsic and common pathways are evaluated with activated partial thromboplastin time (APTT) and extrinsic and common pathways with prothrombin time (PT)(1). The APTT is a medical test that characterizes blood coagulation and measures the time necessary to generate fibrin from initiation of the intrinsic pathway. Normal APTT times require the presence of the following coagulation factors: I, II, V, VIII, IX, X, XI, and XII. Dysregulation of the intrinsic pathway would be expected to contribute to thromboembolic disease (2,3).

Shortening of the APTT is considered to have clinical relevance with an increased risk of thromboembolism (4).

This study aimed to investigate the relationship between thromboembolic diseases and coagulation parameters.

## MATERIAL and METHODS

Patients diagnosed with acute ischemic stroke (AIS), acute coronary syndrome (ACS), and pulmonary thromboembolism (PTE) in the emergency department in the period from 01 November 2016 to 31 March 2019 were retrospectively reviewed. This study included a total of 327 patients, including those diagnosed with AIS (n=216), ACS (n=25), and PTE (n=15) in the emergency room, who did not receive anticoagulant therapy for any reason in the past, and 71 control patients with no history of stroke, coroner artery disease, or pulmonary thromboembolism.

Patients with, coagulopathic disorders, renal failure and use of anticoagulants (warfarin and heparin) were excluded. The APTT, PT, and international normalized ratio (INR) values of the patients, which were measured at the time of admission, were recorded and compared between the groups for statistical analysis.

**Laboratory Assays:** PT-INR, and APTT (Sysmex CS-2500, Kobe 651-0073 Japan) were measured in the emergency biochemistry laboratory.

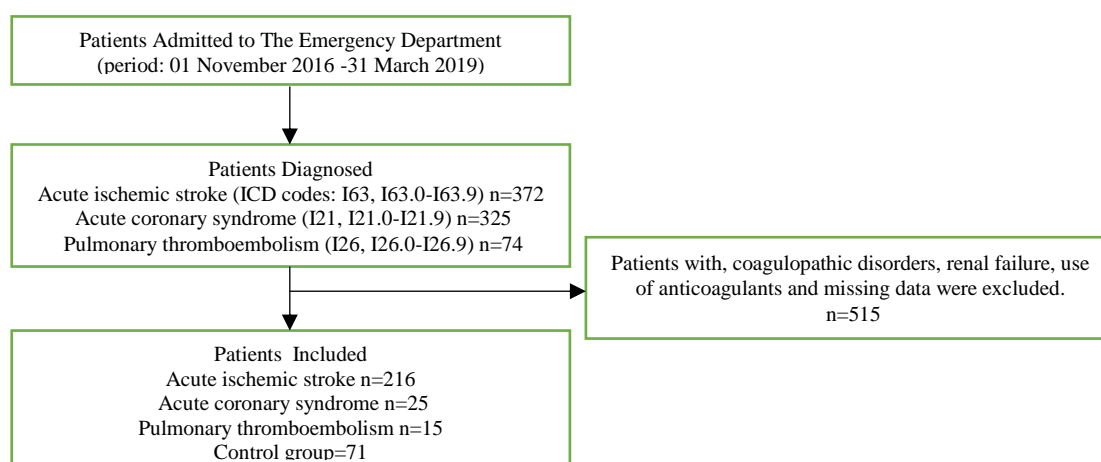
**Power analysis:** The sample size was calculated with an alpha value of 0.05, 80% power, an enrollment ratio of 1, and APTT values of 28.5 in group 1 (control group) and 26.9 in group 2 (patients with venous thromboembolism), yielding that 48 patients in either group making a total of 96 participants would be included in the study (5).

**Statistical analyses:** The categorical variables were presented as frequency and percentages. Continuous variables were presented as mean  $\pm$  standard deviation. The Kolmogorov Smirnov test was used for testing whether the variables were distributed normally. Student's t-test was used for binary comparison of continuous variables conforming to the normal distribution, Mann-Whitney U test for non-compliant ones, and Chi-square test for comparison of categorical data. A p-value of  $< 0.05$  was considered statistically significant. The statistical analysis software was SPSS Statistics for Windows, version 22.0 (SPSS Statistics for Windows, Version 22.0. IBM Corp., Armonk, N.Y., USA)..

## RESULTS

Two hundred and sixteen AIS patients, 25 ACS patients, 15 PTE patients and 71 controls were included in the study. The clinical characteristics of patients and controls are presented in Table 1. Significant differences were observed between the controls and patients in age, sex, hypertension, and diabetes mellitus. The APTT ( $25.61 \pm 5.93$  sec), PT ( $12.05 \pm 2.26$  sec), and INR ( $1.04 \pm 0.19$ ) values of the AIS group were found to be statistically significantly lower compared to the APTT ( $27.98 \pm 3.21$  sec), PT ( $12.58 \pm 2.18$  sec), and INR ( $1.10 \pm 0.15$ ) values of the control group (p values were  $<0.001$ ,  $<0.001$ , and  $<0.001$ , respectively). Similarly, the APTT ( $27.15 \pm 8.97$  sec), PT ( $12.26 \pm 2.75$  sec), and INR ( $1.03 \pm 0.25$ ) values of the ACS group were found to be statistically significantly lower compared to those of control group (p values were 0.012, 0.030, and 0.001, respectively). No statistically significant difference was observed between the APTT ( $27.89 \pm 9.76$  sec), PT ( $12.66 \pm 1.42$  sec), and INR ( $1.12 \pm 0.16$ ) values of the PTE group and those of the control group (p values were 0.133, 0.758, and 0.711, respectively) (Table 2).

**Figure 1:** Study Flow Chart



**Table 1:** Demographics of The Patients

	Patients (AIS, ACS, PTE) n=266	Control Group (n=71)	P value
Age, year	69.4±12.7	65.4±17.1	0.035*
Male Sex, %	63.9 (170)	46.4 (33)	0.013**
Hypertension, %	59 (157)	43.7 (31)	0.03**
Diabetes mellitus, %	40.9 (109)	21.1 (15)	0.003**

ACS: acute coronary syndrome, AIS: acute ischemic stroke, APTT: activated partial thromboplastin time, INR: international normalized ratio, PT: prothrombin time, PTE: pulmonary thromboembolism \*Student t test, \*\*Chi-square analysis

**Table 2:** Comparison of the Groups

Coagulation parameters	AIS (n=216)	ACS (n=25)	PTE (n=15)	Control group (n=71)	(p*)	AIS vs Control (p **)	ACS vs Control (p **)	PTE vs Control (p **)
APTT (sec.)	25.61±5.93	27.15±8.97	27.89±9.76	27.98±3.21	<0.001	<0.001	0.012	0.133
PT (sec.)	12.05±2.26	12.26±2.75	12.66±1.42	12.58±2.18	<0.001	<0.001	0.030	0.758
INR	1.04±0.19	1.03±0.25	1.12±0.16	1.10±0.15	<0.001	<0.001	0.001	0.711

ACS: acute coronary syndrome, AIS: acute ischemic stroke, APTT: activated partial thromboplastin time, INR: international normalized ratio, PT: prothrombin time, PTE: pulmonary thromboembolism, \*Kruskal Wallis, \*\*Mann-Whitney U Test

## DISCUSSION

There are studies in the literature examining the relationship between APTT AIS, ACS, and PTE (1,5,6). To the best of our knowledge, our study is the first study to examine these three diseases at once.

The most important cause of stroke occurs when a thrombus occludes or obstructs a brain artery. Lin et. al investigated the association of the shortened APTT with AIS in their study. They concluded that shortened APTT was a marker for thrombosis and could be used to evaluate AIS severity and neurological prognosis. In their study, the reference range for APTT was 23.3-39.3 seconds and the mean normal value was 28.4 seconds. Therefore they defined the shortened APTT as the APTT of fewer than 28.4 seconds (1). Similarly, in our study, it was found that the APTT time was significantly shorter in the AIS group compared to the control group.

In the study investigating the relationship between APTT and ACS, Abdullah et al. reported that the presence or absence of shortened APTT can be used as a positive or negative predictive marker in patients with suspected coronary artery disease (7). In the study investigating the relationship between APTT and ACS by Anvari et al., it was reported that significantly shortened APTT times were detected in STEMI or non-STEMI patients compared to the control group (6). Similar to both studies, in our study, the APTT time was significantly shorter in the ACS group compared to the control group.

Venous thrombosis and arterial thrombotic disease have traditionally been thought of as separate processes; however, they share many similarities in pathophysiology and risk factors (8). In their study, Tripodi et al. compared the relationship between VTE and APTT. Shortened APTT was evaluated as a risk factor for VTE regardless of thrombophilia and factor 8 level (5). Differently, in our study, when the VTE group was compared with the control group, no statistically significant difference was found between them in terms of APTT durations. We consider that the reason we obtained different results is due to the difference in assessment methods.

Tripodi et al. used the APTT ratio (test value/reference value) as the evaluation parameter and determined 0.87 as the cut-off value. In our study, we compared the groups in terms of mean APTT results without determining any cut-off value.

**Limitations:** This study has the following limitations. First patients were tested after their thrombotic episodes. Second, the role played by high levels of coagulation factors in shortening the APTT could not be evaluated, even if the conditions were known to affect the test (patients with, coagulopathic disorders, renal failure, and use of anticoagulants) were excluded.

## CONCLUSION

Shortened APTT levels in cases without a history of anticoagulant use at the time of admission can be considered to be a predictive and effective tool for clinicians in arterial embolic events (AIS and ACS). We recommend that multicentre prospective cohort studies with a larger patient population investigating coagulation parameters in cases with PTE, which is induced by venous occlusion should be conducted in the future.

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**Author contributions:** OT; Study design, experimental studies, Literature search, Data analyzes OT; Writing article and revisions

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**Ethical issues:** All authors declare originality of research.

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## Investigation of the change in the biological structure of the tumor in metastatic breast cancer

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### ABSTRACT

**Objective:** When metastasis develops in some breast cancer patients, hormone receptors (HR) and Human Epidermal Growth Factor-2 (Her-2) status can change and the tumor alters its character. We tried to determine the rate of these changes in tumor biology in 110 patients that we followed in our clinic and performed the change of the biopsy from the metastatic site (re-bx). We aimed to determine the biological changes of tumors and, contribute to the literature by examining the relationship of these changes with the adjuvant endocrine treatments (ET) or chemotherapy type (CT).

**Material and Methods:** We included 110 metastatic breast cancer patients in our study. These patients had previously completed their local treatments followed by CT, and those with positive HR completed ET. After the first metastasis developed in the patients, we performed metastasectomy or biopsy from the metastatic site.

**Results:** The median ki-67 value was 25% at the time of primary diagnosis and 30% in re-bx. 20.9% of patients estrogen receptor (ER), 31.8% of patients progesterone receptor (PR) and 26.3% of patients Her-2 changed when metastasis developed.

**Conclusions:** We found that the metastatic tumor has more aggressive properties than the primary tumor. Adjuvant chemotherapy and endocrine treatments or the location of metastasis did not make a significant difference in tumor biology.

**Keywords:** Breast cancer, metastatic breast cancer, change of hormone receptors

### INTRODUCTION

Among women, breast cancer is the most common type of cancer all over the world and second among the causes of cancer-related death (1). As in all other types of cancer, multidisciplinary treatment is a sine qua non for success in the treatment of breast cancer (2). In recent years, with the increasing number of molecular predictive biomarkers, personalized treatments have come to the fore in breast cancer. In both early-stage and advanced-stage disease, the parameters including hormone receptors (HR) such as Estrogen (ER) and Progesterone Receptor (PR), Human Epidermal Growth Factor-2 (Her-2), grade, and ki-67 proliferation index are the most important markers that determine the treatment decision (3). HR and Her-2 status both assess treatment options and provide information about the prognosis of the disease. Therefore, according to HR and Her-2 status, breast cancer is divided into three subgroups: Hormone receptor-positive, Her-2 positive, and triple-negative (4). While ER positivity is around 75%, PR positivity 60%, and Her-2 receptor positivity 20% in breast cancer, the rate of negativity of all three is about 15% (5). However, when metastasis develops in some patients, the tumor alters its character, and HR and Her-2 status can change. The exchange of these receptors necessitates radically changing the treatment strategy. Parameters that change with the development of metastasis are not limited to HR and Her-2. Still, we can add markers showing tumor behavior such as grade and ki-67 proliferation index to this list.

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We tried to determine the rate of these changes in tumor biology in 110 patients that we followed in our clinic and performed biopsy from the first metastatic site (re-bx). Also, we aimed to contribute to the literature by examining the relationship of these changes with the endocrine treatments (ET) or chemotherapeutic type (CT) that patients received in the adjuvant period.

## MATERIAL and METHODS

We included 110 metastatic breast cancer patients in our study. These patients had previously completed their local treatments and adjuvant chemotherapy for early breast cancer, and those with positive hormone receptors completed endocrine treatments or continued maintenance endocrine therapy. After the first metastasis developed in the patients, we performed metastasectomy or biopsy from the metastatic site. We excluded the patients undergoing cytological examination, those with isolated malignant pleural effusion, malignant ascites, and cerebrospinal fluid, and those younger than 18 years old. HR, Her-2, grade, ki-67 were studied immunohistochemically (IHC) in the samples taken. We compared these results with the results in the primary tumor and examined the rates of change. We discussed the relationships of these changes with the age and sex of the patients, CT in the adjuvant period, ET, local recurrence, and metastatic organ. ER and PR positivity was defined as 1% and above. Her-2 positivity was accepted as IHC: +3 or Fluorescence In Situ Hybridization (FISH) positivity.

**Statistical analysis:** Statistical analysis was performed using the SPSS statistical software package (Version 25.0, SPSS Inc., Chicago, IL, USA). We checked each continuous variable with Kolmogorov Smirnov and Shapiro-Wilk tests and histograms. The expression of all numerical data was median values (Minimum-Maximum) or ratio. We used the Chi-Square or Fisher Exact test to analyze the categorical variables between the groups. We compared the groups using the Student T-test or the One Way ANOVA for normally distributed data and using the Mann Whitney U test or the Kruskal Wallis test for non-distributed data. After the preliminary measurement, we performed the Mc-Nemar test or Wilcoxon test.  $P < 0.05$  value was considered statistically significant.

## RESULTS

As summarized in table-1, the youngest of the patients was 25 years old and the oldest one 81. The median age was 51 years old. The average time from primary diagnosis to metastatic period was 49 months. 108 patients (98.2%) were female, and 2 (1.8%) were male. The most frequently been metastasized site was the liver (27.3%), followed by bone (20%). Only 8 patients had a local recurrence (7.3%) ER was positive in 78.2% of patients in primary diagnosis, and 73.6% in metastasis biopsy. PR positivity was 64.5% in primary diagnosis and 56.4% in metastasis biopsy. Her-2 was positive in 34.5% of patients in primary diagnosis and 35.5% in metastasis biopsy. 0.9% of the patients were grade 1, 43.6% grade 2, and 55.5% grade 3 ant initial . In the metastasis biopsy, these rates were 0%, 31.8%, and 68.2%, respectively. 28.2% of the patients received Tamoxifen, 41.8% aromatase inhibitors (AI), and 9.1% gonadotropin-releasing hormone (GnRH) analog as adjuvant ET. 20.9% of the patients had not

received adjuvant ET. 61.8% of the patients received adjuvant Taxanes, and 84.5% received adjuvant Anthracyclines. The proportion of patients who received adjuvant Trastuzumab was 38.2%. The median ki-67 value was 25% at the time of primary diagnosis, and 30% in re-bx. Among all patients, the ki-67 value of 22 patients (20%) decreased in re-bx, while the ki-67 of 12 (10.9%) did not change and that of the other 76 (69.9%) increased ( $p=0.0001$ ). There was no statistically significant difference between the results of ER, PR, and Her-2 at the primary tumor and re-bx.

As summarized in table-2, ER became negative when metastasis developed in 14 (16.2%) of 86 patients who were initially ER positive. In 9 (37.5%) of 24 patients who were initially ER negative, then ER became positive. In general, in 23 (20.9%) of 110 patients, ER status in metastatic focus biopsy changed according to the primary diagnosis. 22 of the 71 patients (30.9%) who were initially positive for the PR, then the PR became negative. In 13 (33.3%) of 39 patients who were initially negative for the PR, the PR was positive in the metastatic site. In total, PR status changed in 35 of 110 patients (31.8%). Her-2 receptors became negative in 14 (36.8%) of 38 patients who were initially positive for Her-2 receptors. In 15 (20.8%) of 72 patients who were initially negative for Her-2 receptors, then Her-2 became positive. In total, Her-2 changed in 29 of 110 patients (26.3%).

As summarized in table-3, there was only one patient who was in grade 1 at the beginning, and his grade in re-bx increased to 3. In 28 (58.3%) of 48 patients who were initially grade-2, it increased to grade 3 in re-bx, and in 20 (41.6%) of them, the grade did not change. In 54 (88.5%) of 61 patients who initially graded 3, the grade was the same again in re-bx, and in 7 (11.4%), grade-2 was detected. Overall, tumor grades increased significantly after metastasis ( $p = 0.007$ ). There was no statistically significant relationship between the difference in ki-67 and CT, ET or location of metastasis ( $p = 0.877$ ). CT did not have a statistically significant relationship with the metastatic region. As summarized in table-4, there was no statistically significant relationship between ET and change of HR and Her-2. Also, there was no statistically significant relationship between CT and change of HR and Her-2. Her-2 results of 68 patients who did not receive trastuzumab were initially negative, while 22% were positive in re-bx ( $p=0.0001$ ). Her-2 of 38 patients who received trastuzumab were initially positive, 36.8% of these patients were negative in re-bx ( $p=0.0001$ ).

**Table 1.** Patient characteristics

		n	%
<b>Gender</b>	Female	108	98,2
	Male	2	1,8
<b>Metastasis Location</b>	Bone	22	20,0
	Lymph nodes	16	14,5
	Liver	30	27,3
	Lung	6	5,5
	Brain	8	7,3
	Soft tissue	5	4,5
	Opposite breast	8	7,3
<b>Local Recurrence</b>	Other	15	13,6
	Yes	8	7,3
	No	102	92,7

**Table 2:** Change of hormone receptors and Her-2

		Re-bx		p
		Positive	Negative	
Primary ER	Positive	72	14	0,405
	Negative	9	15	
Primary PR	Positive	49	22	0,175
	Negative	13	26	
Primary HER-2	Positive	24	14	1,000
	Negative	15	57	

**Table 3:** Change of the grade

		Re-bx Grade			p
		1	2	3	
Initial Grade	1	0	1		0,007
	2	28	20		
	3	7	54		

**Table 4:** Change of HR and HER-2 according adjuvant endocrine therapy

		Not received		Tamoxifen		LHRH analog		AI	
		Re-bx positive	Re-bx negative	Re-bx positive	Re-bx negative	Re-bx positive	Re-bx negative	Re-bx positive	Re-bx negative
Initial ER	Positive	1	3	23	5	9	1	39	5
	Negative	5	14	3	0	0	0	1	1
Initial PR	Positive	1	1	19	7	6	3	23	11
	Negative	3	18	4	1	1	0	5	7
Initial HER-2	Positive	9	4	6	6	1	1	8	3
	Negative	1	9	3	16	2	6	9	26

## DISCUSSION

Breast cancer is the most common cancer among women, with approximately 2 million new cases annually and more than 500,000 deaths worldwide, and the second cause of cancer-related deaths in women (6-8). The main determining factor in breast cancer treatment is tumor biology. Parameters such as the status of HR, Her-2, grade, and ki-67 proliferation index are among the variables that enable us to have an idea about tumor biology. In particular, the HR and Her-2 status are predictive biomarkers that show what treatments may be more beneficial to the patient while guiding in terms of prognosis. Grade and ki-67 proliferation index, provide information about tumor aggressiveness and therefore prognosis. Generally, approximately 20-30% of patients with early breast cancer become metastatic in the future (6). HR and Her-2 may change later in some patients with metastasis. Although scientists have not fully determined the reason for this change, they have put forward some theories. The most important ones of these are tumor heterogeneity, clonal selection induced by the treatments, and the change in the genotype and phenotype of the tumor (9-11). Therefore, in an early-stage breast cancer patient, re-bx from the metastatic site when it is the first metastasis is now a standard approach worldwide. There are three rational reasons for this practice. The first is to make a malignant or benign distinction in the suspected lesion. The second reason is to distinguish a second primary tumor or breast cancer metastasis if it is malignant. The third and perhaps most important reason is to plan the treatment of breast cancer metastasis according to the new HR and Her-2 status. Published studies are showing that biopsy from metastatic focus increases survival (12-14). The discordance between the HR and Her-2 states of the primary and metastatic focus has been the subject of numerous studies before (9,15,16).

The most comprehensive of these studies is the meta-analysis performed by Aurilio G. et al. In this meta-analysis of 48 studies published between 1983-2012, the rate of ER change was 20%, PR change rate was 33%, and Her-2 change rate was 8% (15). In another more recent study, these change rates in ER, PR, and Her-2 were 18%, 34%, and 14%, respectively (17). In our study, these rates resulted in 20%, 31%, and 26%, respectively. While the hormone receptor change had similar results with the literature in our study, the rate of change in Her-2 was generally higher than in the literature. In this study, the rate of patients who were ER-positive at the beginning and then negative was 13%, and the rate of patients who were negative initially and then positive was 37%. Similarly, in our study, these values were determined as 16.2% and 37.5%, respectively. In the study mentioned above, the rate of patients who became negative when PR was initially positive was 38%, and those who became positive when they were negative at the beginning was 29%. In our study, these rates were similar, with 30.9% and 33.3%, respectively. As a result of the present study, the proportion of patients who became negative when Her-2 was positive at the beginning was 33%, and those who became positive when initially negative were only 1%, these rates were 36.8% and 20.8%, respectively in our results. As can be seen in our study, the proportion of patients who initially became negative for Her-2 and later became positive is significantly higher. The difference may arise from the change of standards of Her-2 evaluation or differences of chemotherapy agents and anti-Her-2 treatments. When the ki-67 values between the primary and metastatic disease were compared, the median Ki-67 value was 25 at the beginning, whereas in the metastatic disease this value was 30. This difference was also statistically significant. The grade of 65% of patients resulted

similarly, 6% decreased from 3 to 2, 18% increased from 2 to 3 and 1% increased from 1 to 3 in the metastatic site according to initial diagnosis. The grade was increased statistically significant in metastatic disease. However, we could not find any study in the literature where we can compare these results. The data we observed for Her-2, ki-67, and grade can be interpreted as the tumor gains more aggressive biology when metastasis develops. In our study, one of the questions was whether CT or ET affected the change in HR. According to our results, the differences between neither ET or CT associated with HR, Her-2, grade, and ki-67 changes. Again, in our study, the difference between metastasized organs was not associated with HR, Her-2, grade, and ki-67 changes. Constanze V. et al. also sought the answers to these questions in their studies (17). In this study, the chemotherapy regimens were investigated separately as Taxanes and Anthracyclines, which are the most frequently used agents in the adjuvant period. As in our study, the chemotherapy regimens did not make a significant difference in HR and Her-2 changes. However, in this trial unlike our results patients who received adjuvant Tamoxifen had more statistically significant HR receptor changes. The variation in HR status was again higher in those using Letrozole, and this change was from positive to negative. These changes were found statistically significant. The difference between studies can be due to follows: First, in the present study, AI was examined as a group and not divided into Letrozole, Anastrozole, or Exemestane. Second, the duration of ET in both studies is unknown. However, CT had been completed for both Taxanes and Anthracyclines recipients, and these durations were probably similar in groups of the patient in both studies. Perhaps this is why both studies have produced similar results for CT, and conflicting results for ET. However, we need extensive studies, and so we can compare this information.

## CONCLUSION

In our study, we found the rate of HR change between primary tumor and metastatic site similar to other published studies. However, the proportion of patients who later became positive when Her-2 was negative was significantly higher in our study. We found that the metastatic disease has more aggressive properties in tumor biology than the primary tumor. This result was a significant difference that distinguished our study from other studies in the literature. Again, we showed that CT or ET the location of metastasis did not make a significant difference in tumor biology. We also think that our study is essential for the data from our country and region.

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## Ultrasonographic assessment of lower uterine segment thickness and perinatal Outcomes in patients with a previous cesarean history

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### ABSTRACT

**Objective:** The purpose of the study was to establish the validity of the abdominal evaluation of lower uterine segment (LUS) thickness in patients having a previous cesarean section (CS) in predicting uterine rupture, and to evaluate if there is any effect of the number of a previous surgeries on adverse maternal and perinatal outcomes.

**Materials and methods:** A prospective case-control study was carried out between December 2017 and June 2018 in Dr. Zekai Tahir Burak Women's Health Education and Research Hospital. 555 patients were classified into three groups; Group 1: patients having one previous CS, Group 2: patients having two previous CS, Group 3: patients having more than the two previous CS). Ultrasonographic measurements of the LUS, intraoperative findings and, adverse pregnancy outcomes were assessed.

**Results:** LUS thickness of group 2 was significantly less than the LUS thickness of the other groups ( $p=0.022$ ). The feeling of pain was significantly more in the patients of group 2, when compared with the other groups ( $p=0.019$ ). Pregnancy interval was the only parameter that had significant predictivity for adverse pregnancy outcomes for group 1 ( $\#CS=2$ ) and group 3 ( $\#CS\geq 4$ ) ( $p=0.042$ , and  $p=0.021$ , respectively). In group 2 ( $\#CS=3$ ), age, the thickness of LUS, and thickness of subcutaneous adipose tissue were found to have significantly high predictivity for adverse pregnancy outcomes ( $p=0.012$ ,  $p\leq 0.001$ , and  $p=0.007$ , respectively).

**Conclusion:** Measurement of LUS, in the patients who had previous CS, can be used for risk assessment and management. It is a non-invasive, reliable and, easily applicable method. Standardization of the measurement technique is a necessity.

**Keywords:** Cesarean, Ultrasonography, Lower, Uterus, Segment, Thickness

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### INTRODUCTION

Uterine rupture is an intrapartum emergency, characterized by the disruption of muscular integrity of the uterine wall, which causes high maternal and neonatal mortality and morbidity (1). Its incidence in an unscarred uterus is 0.033 %, whereas it occurs in 1 of 100 scarred uterus. The main factor is the previous uterine surgery, mainly cesarean section (CS), whose incidence increases worldwide (2, 3). Uterine rupture risk is approximately 0.3 % in patients who had a previous CS, without regarding the delivery route (4).

The complications may be severe like maternal hemorrhage needing a blood transfusion, or hysterectomy, bladder injury, maternal death, as well as fetal prematurity, lower Apgar scores, and death (5, 6).

The poorer outcomes may result from the delayed diagnosis and management because of the unexpectedness and rareness. Although it is not enough for a definite diagnosis, the patients who had previous CS may be evaluated for their higher rupture risk, and adverse pregnancy outcome with the following criteria; advanced maternal age, a gestational week beyond 40 weeks, estimated fetal weight more than 4000g, the short period between two surgeries, and previous surgical technique (7).

In 1988, Fukuda et al. reported that ultra-sonographic examination could detect the thinner part of the lower uterine segment (LUS) and predict uterine scar dehiscence at repeat CS (8). Systematic reviews and meta-analyses of several studies confirmed the relation between LUS thickness and uterine scar defects (scar dehiscence or scar rupture) (9-15).

The purpose of this study was to establish the validity of the abdominal evaluation of LUS thickness in patients having previous CS in predicting uterine rupture and to evaluate if there is any effect of the number of previous surgeries on adverse maternal and perinatal outcomes.

## MATERIAL and METHODS

A prospective case-control study was carried out between December 2017 and June 2018 in Dr. Zekai Tahir Burak Women's Health Education and Research Hospital. The study protocol was performed according to the principles of the Declaration of Helsinki and approved by the local Ethical Committee of our hospital. Informed consent was taken from all patients.

555 patients, who had one previous CS, within 37<sup>th</sup> and 40<sup>th</sup> weeks of gestation, were recruited in the study. All patients had an elective repeated CS (not in labor). Exclusion criteria were; active labor (4–6 cm dilatation), multiple pregnancies, abnormal amniotic fluid volumes, low lying placenta, leiomyoma in LUS of the uterus, previous classical CS, or other uterine surgeries (myomectomy, hysterotomy, polypectomy, lysis of uterine synechia, or hysteroscopic metroplasty).

Patients were classified into three groups; Group 1: patients having one previous CS, Group 2: patients having two previous CS, Group 3: patients having more than two previous CS). All the patients underwent ultra-sonographic evaluation for their LUS before the scheduled surgery. The measurement was made by the surgeon who operated.

For decent imaging of the LUS, the transabdominal sonographic examination was implemented with a full urinary bladder (in this way the patient had the urge barely). When the uterine contraction was detected during the ultrasonographic examination, the examination was stopped, then resumed after the contraction had moderated. Along the cervical canal at the midsagittal plane, the thinnest zone of a lower segment was spotted visually. The distance between the bladder wall and the amniotic cavity was defined as full LUS thickness.

It was measured by placing one caliper at the link between the urine and bladder wall and the other at the link between amniotic fluid and decidual endometrium.

At least 3 measurements were made, and the lowest value was taken as the full LUS thickness (15).

We compared the ultrasonographic measurements with the intraoperative findings of the LUS to identify women who have higher risk of uterine scar dehiscence and rupture.

Uterine scar dehiscence is also called a uterine 'window' was defined as loss of integrity of the myometrial layer without whole rupture of the LUS. Rupture was defined as total separation of the uterine scar causing transmission between the peritoneal cavities and uterine. Adverse pregnancy outcomes (scar dehiscence, uterine rupture, adhesions, low Apgar score, need for neonatal intensive care unit, or maternal blood transfusion) were assessed for all patients.

**Statistical analysis:** Data analysis was performed by using SPSS for Windows, version 17 (SPSS Inc., Chicago, IL, United States). Data were shown as mean (95% Confidence Interval) or number of cases and (percentage), where applicable. The mean differences between groups were compared by Student's T-test.

Nominal data were analyzed by Pearson's chi-square test. The variables for the three groups were analyzed with one-way ANOVA. Multiple logistic regression analyses were applied for calculating odds ratios and 95% confidence intervals for the prediction of adverse pregnancy outcomes in groups. A p-value less than 0.05 was considered statistically significant.

## RESULTS

555 patients were included in the study, 230 of which had 2 previous CS, (group 1), 276 of which had 3 previous CS (group 2), and 46 of which had 4 or more CS (group 3). The clinical and laboratory parameters of the patients were demonstrated in table 1.

The increase in CS rate with increasing age was significant ( $p=0.001$ ). LUS thickness of group 2 was significantly less than the LUS thickness of the other groups ( $p=0.022$ ). The feeling of pain was significantly more in the patients of group 2, when compared with the other groups ( $p=0.019$ ) (Table 1).

Multivariate and univariate logistic regression analyses were performed to detect risk factors effective on the adverse gestational outcome (presence of 2 of the following criteria were needed: scar, dehiscence, uterine rupture, adhesion, neonatal unit admission, need for neonatal respiratory support, need for maternal blood transfusion).

Pregnancy interval was the only parameter that had significant predictivity for adverse pregnancy outcome for group 1 (#CS=2) and group 3 (#CS $\geq$ 4) ( $p=0.042$ , and  $p=0.021$ , respectively) (Table 2).

In group 2 (#CS=3), age, the thickness of LUS, and thickness of subcutaneous adipose

tissue were found to have significantly high predictivity for adverse pregnancy outcome ( $p=0.012$ ,  $p<0.001$ , and  $p=0.007$ , respectively) (Table 2).

**Table 1:** Demographic, Clinical, and Laboratory Parameters of the Groups

	Group 1 (#CS=2) N=230	Group 2 (#CS=3) N=276	Group 3 (#CS≥ 4) N=49	p value*	p value
Age *	26.28 ± 5.36	29.66 ± 5.51	33.16 ± 4.50	p(12)<0.001 p(13)<0.001 p(23)<0.001	<b>&lt;0.001</b>
Body Mass Index*	29.66 ± 5.48	28.58 ± 5.18	29.44 ± 6.71	p(12)=0.027 p(13)=ns p(23)=ns	0.078
Weight Gain *	12.50 ± 5.01	11.37 ± 4.96	12.37 ± 7.05	p(12)=0.015 p(13)=ns p(23)=ns	0.255
Interval*	4.33 ± 2.64	4.32 ± 2.67	4.34 ± 6.71	p(12)=ns p(13)=ns p(23)=ns	0.376
Lower Uterine Segment *	3.96 ± 1.34	3.66 ± 1.19	3.88 ± 1.13	p(12)=0.006 p(13)=ns p(23)=ns	<b>0.022</b>
Cervical Dilation (cm)**	[0-2]	[0-2]	[0-2]	p(12)=ns p(13)=ns p(23)=ns	0.782
Thickness of Subcutaneous adipose tissue (cm) *	3.375 ± 1.17	3.56 ± 1.21	3.50 ± 1.26	p(12)=ns p(13)=ns p(23)=ns	0.501
Uterine contraction***					
(+)	45 (19.6%)	55 (19.9%)	11 (22.4%)	p(12)=ns p(13)=ns p(23)=ns	0.900
(-)	185 (80.4%)	221 (80.1%)	38 (77.6%)	p(12)=ns p(13)=ns p(23)=ns	
Feeling of Pain***					
(+)	88 (38.3%)	140 (50.7%)	21 (42.9%)	p(12)= 0.005 p(13)= ns p(23)=ns	<b>0.019</b>
(-)	142 (61.7%)	136 (49.3%)	28 (57.1%)		
Weight of Newborn (grams)*	3235.59 ± 539.29	3188.42 ± 554.21	3257.35 ± 397.11	p(12)=ns p(13)=ns p(23)=ns	0.517
Hemoglobin level before caesarian*	11.87 ± 1.27	11.76 ± 1.47	11.89 ± 1.33	p(12)=ns p(13)=ns p(23)=ns	0.618
Hemoglobin level after caesarian*	10.97 ± 1.22	10.99 ± 5.63	10.71 ± 1.19	p(12)=ns p(13)=ns p(23)=ns	0.901

## DISCUSSION

In the present study, mean LUS measurements in all three groups were thicker than the 3.5 mm which cut-off value introduced for foreseeing complications varied between 2.0 and 3.5 mm in the literature (9-13). Sonographic measurement of LUS thickness near term is correlated inversely with the risk of uterine scar defect, including uterine scar dehiscence and/or uterine rupture (10-15). A systematic review of 12 studies, including 1834 women, with a 6.6% rate of uterine scar defects, confirmed the strong association between the degrees of measure of LUS thickness in the third trimester of pregnancy and the risk of uterine scar rupture/dehiscence. The cut-off value proposed for predicting these complications varied between 2.0- 3.5mm (14). A precise cut-off value has not been able to be determined due to the heterogeneity of the studies. Another recent meta-analysis of 21 studies also confirmed these findings (15).

In our study, when we compared the LUS measurements of the groups, we observed that it was significantly thinner in group 2 (p=0.022). The studies reported that prior CS is associated with a sonographically thinner LUS when compared with those with prior vaginal delivery (11).

However Ginsberg et al. reported that neither the number of deliveries nor the presence of previous CS did change the wall thickness; if it was not a post-term pregnancy (16). Landon et al. reported that uterine rupture risk was 0.7% in women who had one previous CS; whereas it was 0.09% in patients who had more than one CS; and proposed that the number of the previous CS was not relevant to the complication of uterine rupture (17).

On the other hand, they had observed a higher maternal mortality rate and need for hysterectomy in the group in which the patients had multiple previous CS. LUS thickness had a high predictive value for proposing uterine rupture, in Group 2, which had thinner LUS (p<0.001) (17).

Complete uterine rupture is a rare peripartum complication, often associated with a ruinous outcome for both the fetus and pregnant woman. CS-based scarred uterus considerably increases the risk of uterine rupture. Qureshi graded the intraoperative scars into four.

**Table 2:** Univariate and Multivariate Regression Analyses for Adverse Pregnancy, Outcome In Groups (GROUP 1: # CS=2, GROUP 2: # CS= 3 and GROUP 3: # CS ≥4)

		GROUP 1 (# CS=2)				GROUP 2 (# CS=3)				GROUP 3 (# CS≥4)			
		Univariate OR (95% CI)	P value*	Multivariate OR (95% CI)	P value*	Univariate OR (95% CI)	P value*	Multivariate OR (95% CI)	P value*	Univariate OR (95% CI)	P value*	Multivariate OR (95% CI)	P value*
Age		1.028 (0.979- 1.080)	0.263			0.946 (0.902- 0.993)	<b>0.025</b>	0.938 (0.892- 0.986)	<b>0.012</b>	0.938 (0.824- 1.066)	0.326		
Body Mass Index		1.016 (0.969- 1.066)	0.506			1.018 (0.972- 1.066)	0.454			1.055 (0.964- 1.154)	0.248		
Weight Gain		1.009 (0.957- 1.063)	0.740			0.975 (0.929- 1.023)	0.305			1.021 (0.942- 1.108)	0.608		
Interval		1.114 (1.006- 1.234)	<b>0.037</b>	1.112 (1.004- 1.232)	<b>0.042</b>	1.037 (0.948- 1.134)	0.425			1.607 (1.102- 2.344)	<b>0.014</b>	1.569 (1.070- 2.302)	<b>0.021</b>
Lower Uterine Segment		1.065 (0.875- 1.296)	0.530			1.487 (1.199- 1.842)	<b>&lt;0.001</b>	1.538 (1.232- 1.920)	<b>&lt;0.001</b>	1.407 (0.833- 2.376)	0.201		
Feeling of Pain		0.725 (0.423- 1.241)	0.241			1.220 (0.756- 1.968)	0.415			0.682 (0.219- 2.126)	0.509		
Presence of Contractions		0.779 (0.405- 1.500)	0.455			0.856 (0.472- 1.551)	0.608			2.406 (0.601- 9.632)	0.215		
Cervical Dilation (cm)		1.154 (0.842- 1.581)	0.375			1.189 (0.864- 1.634)	0.288			1.540 (0.733- 3.235)	0.254		
Thickness of Subcutaneous Adipose Tissue (cm)		0.874 (0.696- 1.098)	0.248			0.793 (0.647- 0.972)	<b>0.026</b>	0.745 (0.601- 0.922)	<b>0.007</b>	0.676 (0.418- 1.094)	0.111		
Hemoglobin Level Before Caesarian		1.074 (0.872- 1.323)	0.502			0.965 (0.821- 1.135)	0.669			0.940 (0.614- 1.440)	0.776		
Hemoglobin Level After Caesarian		1.017 (0.821- 1.260)	0.878			0.907 (0.749- 1.097)	0.314			1.086 (0.675- 1.748)	0.732		

Grades III and IV (incomplete or complete dehiscence) are occasionally observed in elective CS (18). Fortunately, the maternal as well as fetal outcome is unaffected in such situations. Other reports have shown that uterine dehiscence is a high risk condition for uterine rupture (19). Therefore, measurement of the LUS thickness before the onset of labor may have clinical significance if it can identify the uterine dehiscence. When we evaluated the adverse outcomes in our study, we observed that as the age of the mothers increased, the number of CS increased. Especially in group 2, this result had high predictivity for adverse pregnancy outcomes. Similarly, other studies confirmed that as the age of the mother got older than 30 years, there were a 2-3 times increased risk for complications (20). A short interpregnancy interval has been hypothesized to be linked with deficient healing of the scar in the uterine and thus an increased risk for rupture in the uterine and adverse pregnancy outcomes (21, 22). These findings were confirmed by our results. In group 2 which has thinner LUS measurements, there was a statistically significant difference in pain of the suprapubic region compared to other groups. Cohen and colleagues (23), observed that abdominal pain can not be the strong predictor of scar rupture alone, but it has a nearly 60 % positive predictive value for rupture with an additional sign or symptom. In our study, the patients of group 2, which had thinner LUS, felt significantly higher pain. The varied results of group 2 by sonographic measurement of LUS are

assumed to be due to pain with contractile state of the uterus and the engaged status of the fetal head may stretch the LUS further and makes the LUS thinner possibly leading to uterine dehiscence or rupture. Thus, the quantitative risk of rupture associated with scar thickness has not been determined, a thin LUS might have a low positive predictive value for rupture (9) and women with normal lower uterine segment thickness have gone on to rupture their uterus when in labor (24,25).

Several authors described the techniques of measuring the myometrial thickness and full LUS in the third trimester. LUS thickness can be measured on ultrasound by using a transabdominal or a transvaginal probe (13, 26, 27). When the examinations were done with full-bladders, a strong connection was observed between the two approaches (13); whereas no correlation was observed when the bladders were empty (28). While the transvaginal technique can be considered as the best approach to visualize scar defects located in the lowest part of the LUS, for the women who had previous CS performed late in the first or second stage of labor, we can observe that the transabdominal technique can detect scar defects located high on the LUS, for the women who had previous CS performed in early weeks of gestation and/or before labor (29). We preferred to make the examinations by transabdominal route with the full bladder, to evaluate LUS better; because we could not reach all of the information of the previous CS, i.e. surgical technique, emergent conditions, and labor period. However, the studies



done with the transabdominal approach showed greater margin values than the ones done with the transvaginal approach for predicting uterine scar dehiscence/rupture (10,14). The best way to measure LUS thickness is believed to be the combination of both approaches as the experts suggest.

In our study, we used full LUS measurement instead of myometrial thickness. Because myometrial thickness can be difficult to determine the limits of the myometrial layer in the LUS, which can be causing a less accurate measurement (14,15). Both of the measurement approaches were found to be linked to the risk of uterine scar dehiscence/rupture (10,15,26,27).

The methodical review of Jastrow et al. (14) suggests that full LUS thickness is more predictive than myometrial thickness for the envision of uterine scar dehiscence/rupture. More to that, the recent meta-analysis of Kok et al.(15) found no meaningful differences between these measurements for the envision of uterine scar dehiscence/rupture.

Observations of present study corroborate the fact that measuring LUS either with transabdominal or transvaginal technique is vital to save many pregnant women and babies from significant morbidity and mortality. Early diagnosis of uterine scar dehiscence/rupture, followed by neonatal resuscitation and expeditious laparotomy is crucial for reducing the leading morbidity and mortality. An observational study showed a potential upper limit for nonhypoxic neonatal delivery of 18 minutes from suspected uterine rupture to delivery (30).

It is important to acknowledge that scar dehiscence may be asymptomatic in up to 48 % of patients, and the classic triad of a complete uterine rupture (pain, vaginal bleeding, and fetal heart rate abnormalities) may present in less than 10% of cases (31). Though there was no accurate clinical prediction or prevention for uterine rupture; awareness and response at the right time could reduce maternal and neonatal morbidity rates.

One of the limitations of the study is that the number of the patients included to the study. We believe that further studies would help decision making on this topic

## CONCLUSION

Measurement of LUS, in the patients who had previous CS, can be used for risk assessment and management, without regarding the delivery route. Standardization of the measurement technique is a necessity.

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## Total or less than total thyroidectomy for multinodular goiter long term follow-up

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### ABSTRACT

**Objective:** Multinodular goiter is a common surgical disease. There is no common consensus regarding the extent of thyroidectomy for multinodular goiter. This study aims to present personal experience on treating patients with multinodular goiter and to compare complication rates and results of total and partial thyroidectomy for multinodular goiter.

**Material and Method:** Three hundred fifty patients underwent thyroidectomy for multinodular goiter between May 2003 and October 2010. All patients were diagnosed as multinodular goiter and were referred to surgery by one endocrinologist. All operations were also performed by one surgeon using microsurgical techniques. Partial thyroidectomy (bilateral subtotal or unilateral total thyroidectomy and contralateral subtotal thyroidectomy) was performed in 65 patients (Group-1) and extracapsular total thyroidectomy was performed in 285 patients (Group-2). All patients are being followed followed from the day they were diagnosed until now by the same endocrinologist. Fisher exact test was used for statistical analysis.

**Results:** In Group-1, one patient had transient vocal-cord palsy and but none had hypoparathyroidism. On the other hand, in Group-2, two patients had transient vocal-cord palsy, five had hypocalcemia (one was permanent), and one had a hematoma. Mortality and wound infection were absent in both groups. The histopathological studies showed that 40 incidental thyroid carcinomas occurred among Group-2 patients. During long-term follow-up, 13 patients had goiter recurrence (n = 65, 20%) in Group-1, whereas none had goiter recurrence in Group-2.

**Conclusion:** There were no statistically significant differences in the complication rate between subtotal and total thyroidectomy groups ( $p>0.05$ ). However, the recurrence rate was higher (statistically significant) after subtotal thyroidectomy than after total thyroidectomy ( $p<0.05$ ). Total thyroidectomy eliminated future recurrence of the disease and is also curative in incidental thyroid carcinomas. In addition, it can be safely performed using microsurgical techniques.

**Keywords:** Total thyroidectomy, partial thyroidectomy, microsurgical technique

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### INTRODUCTION

Multinodular goiter is one of the most common surgical diseases. It was reported that goiters affect 5% of the world population (1), and 10%-15% of goiter patients eventually require thyroidectomy (2). Thyroidectomy is frequent surgical procedure worldwide, and severe complications, such as recurrent laryngeal nerve (RLN) palsy and hypoparathyroidism, may occur postoperatively. These complications may account for various serious medicolegal cases. In many patients, it is very hard to distinguish whether recurrent laryngeal nerve palsy or hypoparathyroidism occurs due to a complication or negligence. In private practice, many surgeons prefer to perform subtotal or partial thyroidectomy to avoid complications and medicolegal problems. Subtotal thyroidectomy has long been the preferred treatment for multinodular goiter (MNG). This is because of the common belief that subtotal thyroidectomy is easier to perform and causes fewer complications than total thyroidectomy. However, subtotal thyroidectomy has a high recurrence rate of up to 50% (3, 4).

In recent years, total thyroidectomy has increasingly replaced subtotal thyroidectomy (5). We present our experience with 350 cases and compare and discuss the results

## MATERIAL and METHODS

Between May 2003 and October 2010, 350 patients with multinodular goiter were referred to surgery by one endocrinologist. All operations were performed by one surgeon using microsurgical techniques. were operated on by one surgeon in private practice.

They are being followed by the same endocrinologist since then. There were 271 women and 79 men. The age distribution (mean  $\pm$  standard deviation (SD)) was  $44 \pm 13$  years (range 15–78 years). Preoperative work-up, such as thyroid function tests, serum calcium levels, and routine biochemistry, were performed in all patients. Vocal-cord functions were examined routinely before and after operations by an otolaryngologist.

The routine surgical procedure between May 2003 and November 2005 was partial thyroidectomy along with frozen section for patients with MNG. Patients with positive or suspicious frozen-section results were excluded from the Group-1 patients (Partial thyroidectomy group). 70 patients underwent partial thyroidectomy.

During this period, five patients had false-negative frozen-section results. These patients were excluded from Group-1; thus, the study included 65 such patients, including bilateral subtotal thyroidectomy in 16 cases and Dunhill procedure in 49 cases. Before November 2005, only 30 patients with MNG underwent total thyroidectomy.

After the November 2005, the surgical procedure was changed, and extracapsular total thyroidectomy was performed on a routine basis for patients with MNG. 285 patients underwent total thyroidectomy. Total thyroidectomy or total lobectomy was always performed by extracapsular dissection under the inferior and lateral traction of the upper pole by an Allis clamp.

All terminal vessels were ligated or sealed in close proximity to the thyroid capsule. This maneuver helps the surgeon to identify and protect the superior laryngeal nerve and upper parathyroid gland.

In the procedure for total thyroidectomy, upper pole dissection was always terminated before reaching the lower edge of the cricothyroid muscle, because it is the entry area of the recurrent laryngeal nerve to the larynx. After the procedure on the upper pole, the attention was turned to the lower pole.

Once again, all terminal vessels close to the thyroid capsule were ligated and sealed. During this part of the procedure, the second parathyroid gland was usually visualized and was preserved in situ with its own blood supply. The thyroid was suspended from both upper and lower poles, using two Allis clamps, and the median thyroid vein was then ligated. Extracapsular dissection was advanced down to the tracheoesophageal groove.

During this period, the microsurgical technique was always used with a 2.5 $\times$  magnification surgical loupe and microsurgical instruments (jewelry forceps, detectable peanut sponge, and right-angle clamp) and RLN was usually identified posterior or anterior to the inferior thyroid artery. (Figure 1, 2)

Whenever RLN was exposed, it was dissected and protected until its entry point to the larynx. If the parathyroid glands were inadvertently damaged, they were implanted in the sternocleidomastoid muscle.

The Fisher exact test was used for statistical analysis, and  $P < 0.05$  was considered to indicate statistical significance.

## RESULTS

In Group-1, 65 patients underwent partial thyroidectomy. Patient age was (mean  $\pm$  SD)  $40 \pm 11$  years (range 20 – 70 years); there were 15 males and 50 females. All these patients were followed up for  $\leq 14$  years (median 11 years, range 9 – 14 years).

In this group, only one patient had transient vocal-cord palsy, and none had hypocalcemia or wound infection. In the long-term follow-up, 13 patients who underwent subtotal thyroidectomy had recurrent thyroid nodules  $>1$  cm. Twelve of these patients were recommended for or underwent a secondary thyroidectomy procedure and one patient with recurrent toxic multinodular goiter received radioactive iodine therapy.

In Group-2, 285 patients underwent total thyroidectomy. Patient age was  $45 \pm 13$  (range 15 – 78 years); there were 64 males and 221 females. All these patients were followed up for  $\leq 16$  years (mean 7 years). After total thyroidectomy, two patients had transient vocal-cord palsy. Five patients had postoperative hypocalcemia, one showed permanent hypocalcemia and one had a hematoma that required additional surgery.

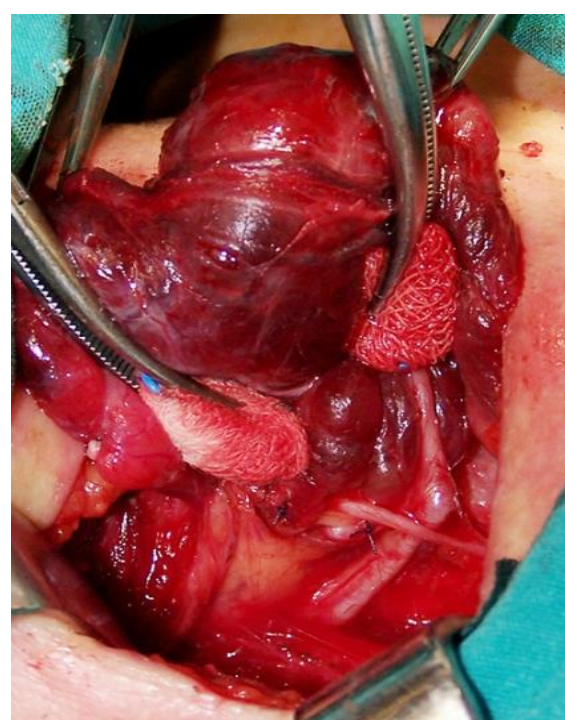
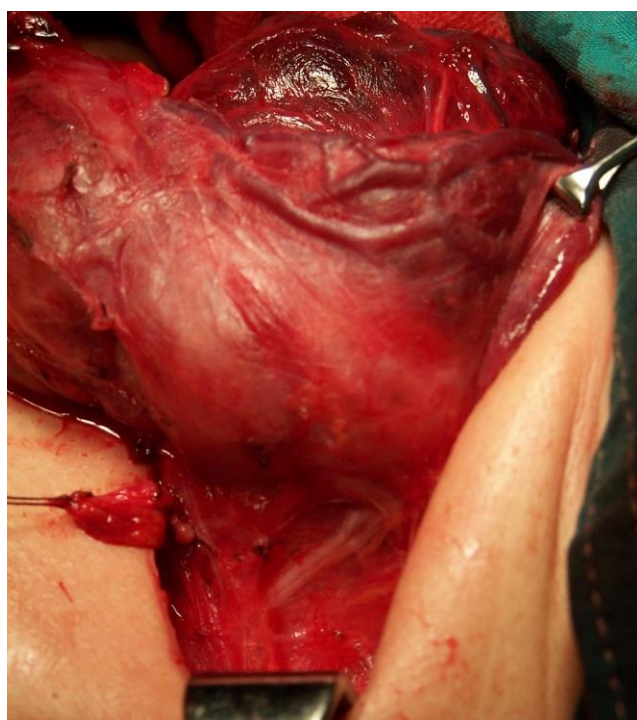
Among Group-2 patients, there was no mortality, wound infection, disease recurrence, or bilateral vocal-cord palsy, but incidental thyroid carcinomas were found in 40 cases (14.0%) (33 papillary, 3 follicular, 2 medullary, 1 well-differentiated and 1 hurthle cell carcinoma). 10 of 40 thyroid carcinomas were multicentric, 7 of them had bilobar tumor and 16 of 40 thyroid carcinomas were smaller than 10 mm.

The incidence of vocal-cord palsy was 1.5% in Group-1 patients and 0.7% in Group-2 patients; the difference between the groups was not statistically significant ( $p > 0.05$ ). Although the incidence of hypocalcemia was higher in Group-2 patients than in Group-1 patients (1.75% vs. 0.0%, respectively), there was no statistically significant difference between the two groups.

The incidence of goiter recurrence was significantly higher in Group-1 patients than in Group-2 patients ( $p < 0.05$ ). (see Table 1)

**Table 1:** Complications of partial thyroidectomy (Group-1) & total thyroidectomy (Group-2) patients (n = 350)

Complications	Group-1 (n = 65) Partial Thyroidectomy		Group-2 (n = 285) Total Thyroidectomy	
	Count	Rate (%)	Count	Rate (%)
<b>Hypocalcemia</b>	-	<b>0.0%</b>	<b>5</b>	<b>1.75%</b>
Temporary	-	0.0%	4	1.4%
Permanent	-	0.0%	1	0.35%
<b>Recurrent laryngeal nerve palsy</b>	<b>1</b>	<b>1.5%</b>	<b>2</b>	<b>0.7%</b>
Temporary	1	1.5%	2	0.7%
Unilateral	1	1.5%	2	0.7%
Bilateral	-	0.0%	-	0.0%
Permanent	-	0.0%	-	0.0%
Unilateral	-	0.0%	-	0.0%
Bilateral	-	0.0%	-	0.0%
<b>Other</b>	-	<b>0.0%</b>	<b>1</b>	<b>0.35%</b>
Hemorrhage	-	0.0%	1	0.35%
Wound infection	-	0.0%	-	0.0%
<b>Total complications</b>	<b>1</b>	<b>1.5%</b>	<b>8</b>	<b>2.8%</b>
Temporary	1	1.5%	7	2.45%
Permanent	-	0.0%	1	0.35%

**Figure 1:** RLN passes posterior to inferior thyroid artery **Figure 2:** RLN passes anterior to inferior thyroid artery

## DISCUSSION

There are four main surgical options frequently used for multinodular goiter: bilateral subtotal thyroidectomy, near-total thyroidectomy, total thyroidectomy, and Dunhill procedure (unilateral total thyroidectomy plus contralateral subtotal thyroidectomy). In all options other than total thyroidectomy, surgeons intentionally leave some thyroid tissue to prevent complications, such as recurrent nerve injury and hypocalcemia.

Dralle et al reported that 43 of 75 thyroidectomy malpractice claims involved RLN palsy (21 unilateral and 22 bilateral RLN palsies), with a 45% tracheostomy rate for bilateral RLN palsy. Twenty-one claims involved permanent hypoparathyroidism (6). Is it in fact true that leaving residual tissue after partial thyroidectomy can prevent these complications?



Generally, it is believed that the extent of the thyroidectomy may affect the complication rate. Thomusch et al reported that total thyroidectomy was associated with an increased rate of RLN palsy and hypoparathyroidism; only 88 patients underwent total thyroidectomy, whereas 5,107 patients underwent subtotal thyroidectomy (7). On the basis of Thomusch et al's report, we can state that even some high-volume surgeons frequently perform partial thyroidectomy. Sosa et al reported that high-volume surgeons frequently performing total thyroidectomy procedures have lower complications rates (8). It is possible to speculate that high-volume surgeons who do not frequently practice total thyroidectomy may have higher complication rates in performing total thyroidectomy. On the other hand, an increasing number of studies recommend total thyroidectomy with a low complication rate for multinodular goiter (9, 10, 11, 12). Cao et al reported that the nodule recurrence rate after total thyroidectomy for multinodular goiter was lower than that after partial thyroidectomy, and that total thyroidectomy did not increase permanent complications (13). In his review of the literature, Agarwal concluded that total thyroidectomy is a safe procedure for benign multinodular goiter (14). In the present study, one patient had transient vocal-cord palsy and none had permanent complications among the partial thyroidectomy patients; on the other hand, two patients had transient vocal-cord palsy, four had transient hypocalcemia, and one had permanent hypocalcemia in the total thyroidectomy patients. In brief, only one patient had a permanent complication in the total thyroidectomy group. The present study showed that the complication rates were not statistically significant ( $p > 0.05$ ) in the total and partial thyroidectomy groups. This study suggests that microsurgical technique, knowledge of thyroid anatomy, and the performance of total thyroidectomy on a routine basis is more important than the extent of thyroidectomy.

Routine RLN dissection was performed during the procedures. I used a surgical loupe with 2.5× magnification and microsurgical instruments, using microsurgical techniques. Microsurgical dissection of RLN is one of the most important steps of the total thyroidectomy procedure. It has long been established that routine identification of the nerve decreases the risk of iatrogenic injury (15). Moreover, intraoperative neuromonitoring might be a supportive tool for the early detection and identification RLN (16). However, it has been reported that routine usage of intraoperative neuromonitoring does not decrease the incidence of RLN palsy (17). Many authors reported that using loupe magnification and microsurgical techniques during thyroidectomy improved the results and decreased the complication rate (18, 19, 20, 21). The RLN dissection technique I used in the total thyroidectomy procedure is similar to that used in the experimental orthotopic liver transplantation and heterotopic heart transplantation in hamsters and rats (except anastomosis), as described by Kamada (liver transplantation), Ono, and Lindsey (heart transplantation) (22, 23). These experimental transplantation procedures require a highly delicate technique performed at a brisk pace. Without the necessary pace and delicacy, heart or liver transplantation procedures performed on the subject rodent will be unsuccessful. Practicing these experimental transplantation procedures, the surgeon gains the necessary technique and knowledge to perform dissections in narrow

areas with small structures. Successful RLN dissection requires the same delicacy required by those experimental transplantation procedures. In the present study, using this technique, only three patients showed transient RLN palsy. Two of them had the complication after total thyroidectomy and one had it after the Dunhill procedure, on the subtotal side, where RLN dissection was not performed. The same microdissection technique was used for in situ preservation of parathyroid glands. That is if the parathyroid glands were inadvertently damaged, they were implanted into the sternocleidomastoid muscle.

After partial thyroidectomy, nodule recurrences are always possible because of residual thyroid tissue. However, it was reported that thyroid nodule recurrences occurred not only after partial thyroidectomy but also after total thyroidectomy (24). Many surgeons believed that it was related to the completeness of the total thyroidectomy. D'Andrea reported that 66.7% total thyroidectomies were really total, whereas 33.3% were subtotal or near-total thyroidectomies (25). What, in fact, do surgeons actually leave behind after partial thyroidectomy? Karakoyun et al their study showed that residual tissue in 62% subtotal thyroidectomy patients and 56% near-total thyroidectomy patients had abnormal pathological findings, including conditions ranging from micronodules to thyroid carcinoma. There are high rates of microscopic pathological findings on residual tissues, after both subtotal and near-total thyroidectomy (26). Delbridge et al noticed that the peak incidence of secondary thyroidectomy for recurrent multinodular goiter occurred 13 years after that for subtotal thyroidectomy for multinodular goiter in Australia (27). In the current study, 13 of the 65 patients who underwent subtotal thyroidectomy had recurrent multinodular goiter, whereas no patients had recurrent goiter after having total thyroidectomy with a follow-up period of >10 years. This case series showed that subtotal thyroidectomy resulted in a higher recurrence rate than total thyroidectomy. ( $n = 65$ , 20% v  $n = 285$ , 0%) ( $p < 0.05$ ) Many authors reported a similar or higher recurrence rate after subtotal thyroidectomy (3,4,28,29).

Another important issue is the increased incidental thyroid carcinoma rate in patients operated on for benign thyroid disease. Although it is reported that the incidence of thyroid carcinoma in benign thyroid disease was approximately 5%, recent reports showed that the incidental thyroid carcinoma rate has increased dramatically (30). In this case series, incidental thyroid carcinoma after total thyroidectomy was seen in 40 patients. ( $n = 285$ , 14.0%) (Among these 40 patients with thyroid carcinoma, only one patient with medullary carcinoma had undergone secondary surgery after total thyroidectomy (bilateral neck dissection); the remaining patients had not undergone further thyroid surgery (31). During subtotal thyroidectomy, frozen section may be helpful in the diagnosis of incidental thyroid carcinoma. In this study, within the partial thyroidectomy group, five false-negative frozen section results were observed, and all of them underwent secondary thyroidectomy ( $n = 70$ , 7.1%) for completion of thyroidectomy. The declining role of frozen section during thyroidectomy was reported previously (31) and it carries high false-negative results risk (32). We discontinued performing routine frozen section examination to determine the extent of surgery for bilateral multinodular



goiter nearly 10 years ago, as frozen section carries false-negative result risk and does not prevent secondary thyroidectomy. In this paper 10 of 40 patients with thyroid carcinomas had multicentric tumors and 7 of 10 multicentric tumors were bilobar. Total thyroidectomy removes all pathologic thyroid tissue including incidental multicentric tumors.

Finally, an important issue remains: who should perform total thyroidectomy? Although many authors advocate high-volume endocrine surgeons should perform total thyroidectomy, surprisingly many high-volume endocrine surgeons frequently perform less than total thyroidectomy for multinodular goiter. We completely agree with Delbridge about this condition. With appropriate supervision, even surgical residents can perform total thyroidectomy just as safely as experienced surgeons (27). We believe surgical skill, knowledge of thyroid anatomy, and the quality of training are the most important prerequisites to safely perform total thyroidectomy. In this case series, total thyroidectomy had 2% transient complication rate and a 0.3% permanent complication rate, comparable with that for high-volume endocrine surgeons.

## CONCLUSION

Microsurgical techniques during RLN dissection, and in situ parathyroid preservation, are safe ways to avoid RLN palsy and hypocalcemia. Total thyroidectomy is the procedure of choice for bilateral multinodular goiter, removing all pathological thyroid tissue, including incidental thyroid carcinoma, and preventing future goiter recurrence

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## A rare lesion of cervical lymph nodes: Angiomyomatous hamartoma

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### ABSTRACT

**Objective:** Angiomyomatous hamartomas are extremely rare, tumor-like lesions of the lymph nodes. They are usually seen in the inguinal region lymph nodes. They are rarely seen in the lymph nodes of the cervical region. Histopathologically, fibrous tissues, smooth muscle cells, and vascular structures are seen in the lymph node structure. It is important to distinguish it from benign and malignant lesions of the lymph node.

**Case:** A 1 cm diameter lymph node excision material removed from the cervical region of a 26-year-old male patient was sent to the pathology laboratory with a pre-diagnosis of lymphadenitis. 4- micron sections were taken from the paraffin blocks prepared from the tissues belonging to the lesion. The samples were examined by staining Hematoxylin-Eosin. In histopathological examination, it was found that almost all of the lymph node structure consisted of vascular structures and smooth muscle cells located on a fibrous ground. The case was reported as angiomyomatous hamartoma.

**Conclusion:** Angiomyomatous hamartomas are extremely rare lesions of the cervical lymph nodes and their consideration in differential diagnosis will reduce the risk of possible diagnostic error.

**Keywords:** Angiomyomatous hamartoma, cervical region, lymph node

### INTRODUCTION

Angiomyomatous hamartoma is a benign vascular tumor of the lymph node with unknown etiology (1). The hamartomatous condition is defined as the irregular development of smooth muscle and vascular structures (1).

Angiomyomatous hamartoma in the lymph node was first reported in 1992 by Chan et al. (2). Some studies suggest that lymphatic flow disturbance is a factor associated with the pathogenesis of angiomyomatous hamartoma (3).

This rare tumor usually involves the femoral and inguinal lymph nodes (4). It can rarely be seen in femoral, cervical, popliteal, and postauricular lymph nodes (4). Local excision is sufficient for treatment (5).

### CASE REPORT

A 26-year-old male patient was admitted to the hospital with the complaint of swelling in the left cervical region for about 1 year. Physical examination revealed that the mass was a semi-mobile mass, approximately 1 cm in diameter. No pathology was found in the patient's blood tests and chest X-ray.

Lymph node excision material was sent to the pathology laboratory with a pre-diagnosis of lymphadenitis. 4- micron sections were taken from the paraffin blocks prepared from the tissues belonging to the lesion.

The samples were examined by staining Hematoxylin-Eosin. In histopathological examination, it was found that almost all of the lymph node structure consisted of vascular structures and smooth muscle cells located on a fibrous ground (Figure 1).

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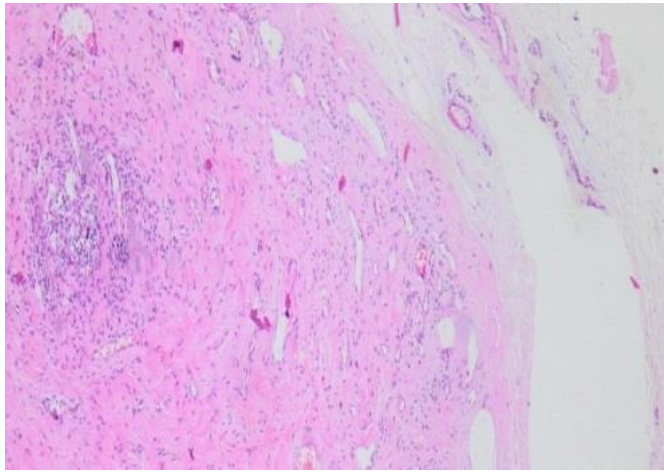
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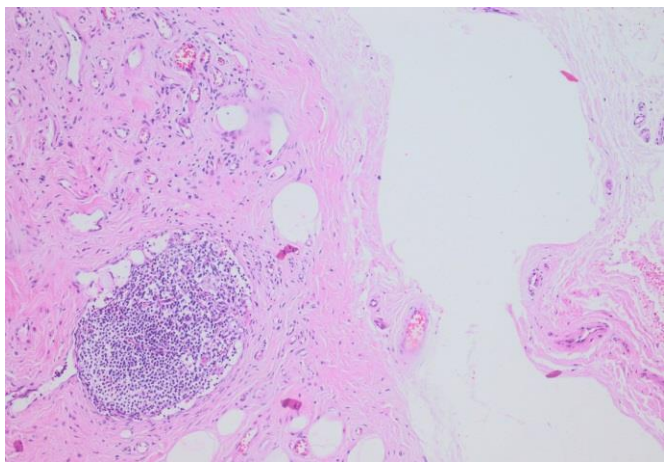
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**Figure 1:** Vascular structures and smooth muscle cells located on a fibrous ground. (HEx100)

Pleomorphism, necrosis, atypical mitotic activity was not observed. Smooth muscle cells and vascular walls were stained with Smooth muscle actin in the immunohistochemical study. There were few residual lymphoid follicles in the tissue (Figure 2).



**Figure 2:** Few lymphoid follicles in the tissue. (HEx100)

Biclonal staining with CD3 and CD20 was observed in the lymphoid tissue that remained in a small number of the ground. The case was reported as angiomyomatous hamartoma.

## DISCUSSION

Angiomyomatous hamartoma of the lymph nodes is a rare benign lesion of the lymph node consisting of smooth muscle proliferation and thick-walled blood vessels in the collagen stroma (2). Lymph nodes contain few residual lymphoid follicles (6). It is most common in middle-aged patients in the inguinal lymph nodes (3). They are rare lesions and the number of cases reported in the literature is around 50 (7,8,9). Few case reports on the head and neck region have been published in the literature (10,11). Local excisions are sufficient for treatment (5). It may lead to the development of a secondary lesion at the excision site after resection due to impaired lymphatic flow (8). The case is presented because of its extremely rare occurrence in cervical lymph nodes. Angiomyomatous hamartoma should be considered when

proliferating smooth muscle cells and vascular structures and few lymphoid tissues are seen in lymph nodes.

## CONCLUSION

In conclusion, angiomyomatous hamartomas are extremely rare lesions of the cervical lymph nodes. It is important to distinguish it from benign and malignant lesions of the lymph node. Considering it in differential diagnosis will eliminate possible problems in the diagnosis and treatment of patients.

**Author contributions:** MGB, MT; Patient examination, Literature search and study design, data collection and analyzes MG; Writing article and revisions

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**Ethical issues:** All authors declare originality of research.

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## A Rare Entity in Cushing's Disease: Severe Hypokalemia and Metabolic Alkalosis

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### ABSTRACT

**Objective:** This case report aimed to highlight the prominence of considering Cushing's disease (CD) in the differential diagnosis of severe hypokalemia and metabolic alkalosis.

**Case Report:** A 63-year-old woman who was admitted to the emergency room with fatigue and severe weakness of extremities. Biochemistry results indicated severe hypokalemia (potassium = 1.2 mmol/L) and metabolic alkalosis (pH = 7.83) and based on further endocrinological investigations, the final diagnosis of CD was confirmed, and magnetic resonance imaging revealed a microadenoma in the right side of the pituitary gland. Transsphenoidal surgery was performed. After surgery, biochemical assessments showed normal potassium levels and corrected metabolic alkalosis without any further treatment. The hypothalamic–pituitary–adrenal axis recovered in nearly eight months and the patient was in remission.

**Conclusions:** Although hypokalemia could be present in CD, none of the previous studies have reported hypokalemia as severe as in this case. This case report highlighted the prominence of considering CD in the differential diagnosis of severe hypokalemia and metabolic alkalosis that could be a crucial part of biochemical features in CD.

**Keywords:** Cushing's Disease, Hypokalemia, Metabolic Alkalosis

### INTRODUCTION

Cushing's syndrome (CS) is an endocrine disease characterized by chronic exposure to glucocorticoids and the most common reason for CS is therapeutic administration of exogenous glucocorticoids (1). ACTH-dependent CS (ADCS) is mostly caused by a pituitary corticotrope adenoma [Cushing's disease (CD)] in approximately 80% of cases, and less frequently by an extra pituitary tumor (ectopic ACTH syndrome). In addition to that, CS may be ACTH independent (AICS) when it is caused by excess production of cortisol by unilateral adrenocortical tumors or by bilateral surreal hyperplasia or dysplasia (2). CS can precipitate several biochemical alterations such as hypokalemia, metabolic alkalosis, and hypernatremia, this may be caused by mineralocorticoid effects of cortisol. However, these alterations caused nonspecific clinical findings and their role in CD is still matter of debate. Hypokalemia and metabolic alkalosis are found rarely in CD although it is present 57% in ectopic ACTH syndrome (3). Previous limited studies have suggested that many patients with AICS are associated with hypokalemia and metabolic alkalosis, there are a few numbers of cases reported with hypokalemia and metabolic alkalosis in CD (4-7). The patient presented here is an unusual case of CD in which severe hypokalemia and metabolic alkalosis were the presenting findings.

### CASE REPORT

A 63-year-old woman was admitted to the emergency room with a two-week history of fatigue, severe weakness of the lower and upper extremities, mild hypertension, and confusion.

### Case Report

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Her medical history consisted of hypertension well controlled with perindopril and indapamide. She was diagnosed with type 2 diabetes mellitus (HbA1c 11.2%) 4 months ago and treated with metformin 1000 mg twice daily, insulin glargine 24 units plus insulin aspart 8 units with meals three times daily. She was a non-smoker and non-drinker, and her family history was insignificant.

Her physical examination revealed facial puffiness with plethora, petechiae over her abdomen, purple striae on the inner surfaces of the upper arms and obesity with a protuberant abdomen. Blood pressure was 150/90 mmHg with a pulse rate of 82/min respiratory rate was 10/min, and the temperature was 37.1°C in the supine position. The patient was 162.3 cm tall and weighed 76 kg. Neurological findings showed that she was confused without a sign of lateralization or focalization. Initial biochemistry results indicated severe hypokalemia and metabolic alkalosis (Table 1).

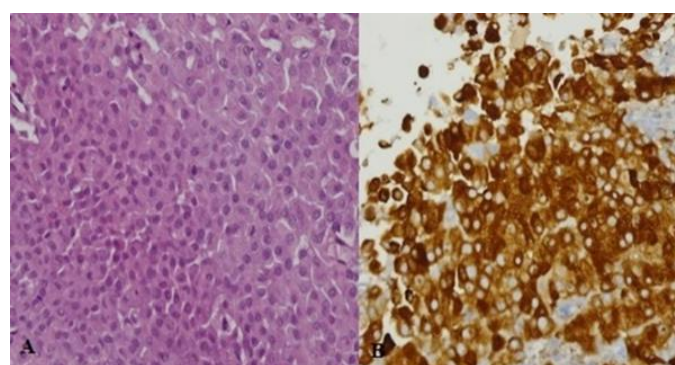
**Table 1:** Initial Biochemical Parameters

	Plasma value	Reference Range
Na (mmol/L)	136	136-145
Cl (mmol/L)	70	98-107
K (mmol/L)	1.2	3.5-5.1
Glucose (mg/dL)	234	70-100
Creatinine (mg/dL)	0.8	0.5-0.9
Urea (mg/dL)	50	10-50
Leukocyte (x10 <sup>3</sup> /μL)	7.9	3.8-10
Hemoglobin (gr/dL)	11.6	11.5-15.5
Platelet (x10 <sup>3</sup> /μL)	205	150-400
pH	7.83	7.35-7.45
PCO <sub>2</sub> (mmHg)	57.2	32-45
PO <sub>2</sub> (mmHg)	77	75-100
SaO <sub>2</sub> (%)	97.9	95-100
HCO <sub>3</sub> (mmol/L)	68.1	24-28
C-reactive protein (mg/dL)	0.2	0-0.5

CS was suspected and endocrinological investigations (urinary free cortisol, 1 mg, and 2 days 2 mg dexamethasone suppression test) showed ACTH-dependent hypercortisolism and 8 mg dexamethasone suppression test indicated a pituitary origin of ACTH secretion (Table 2).

Pelvis, chest, and abdomen CTs were insignificant. Bilateral inferior petrosal sinus sampling (BIPSS) showed a maximal central / peripheral plasma ACTH ratio of 2.1 before and 3.7 after the intravenous administration of Corticotrophin-releasing hormone (CRH) 100 μg, also showed a significant right to the left gradient of 14.9 times (higher on right side). Magnetic resonance imaging (MRI) of the pituitary gland determined a 7 mm x 5 mm the late enhancement area in the right side.

Imaging assessment confirmed that the patient has a pituitary microadenoma without any symptoms by local mass effect. She underwent transsphenoidal surgery (TSS) with 300 mg hydrocortisone infusion and the histological findings were consistent with a pituitary adenoma. Immunohistochemistry showed strong positivity with ACTH and revealed weak immunostaining for prolactin, follicle-stimulating hormone also p53 stain shows rare positivity and Ki-67 was 2% (Figure 1).



**Figure 1** A) Pituitary adenoma stained with H&E (X200) B) ACTH immunostaining of the pituitary adenoma (X200), heavy brown staining of cells indicating ACTH production.

After surgery, hormonal evaluation verified the lack of hypercortisolism, and biochemical assessments showed normal potassium levels and corrected metabolic alkalosis without any further treatment for these conditions (Table 2). Postoperative early cortisol level was 1.7 μg/dL and patient received 5mg/day prednisolone treatment after surgery. In follow-up examinations, the hypothalamic-pituitary-adrenal axis recovered in nearly eight months and prednisolone treatment was stopped. Plasma cortisol level was suppressed to 0.9 μg/dL with 1mg dexamethasone suppression test and the patient was in remission.

**Table 2:** Initial and Follow-up Endocrinological and Biochemical Parameters

	Initial	Pre-op*	Post-op	Reference Range
Plasma Active Renin (uIU/ml)	28.1	-	-	4.2-69.7
Plasma Aldosterone (ng/dL)	3.8	-	-	3.7-31
Serum Cortisol (μg/dL)	67.7	36.7	1.7	4.6-22.8
Urinary free cortisol (μg/24h)	3846.4	-	-	36-137
Plasma ACTH (pg/mL)	133	89.2	38.6	7.2-63.3
Serum cortisol after 1 mg dexamethasone (μg/dL)	6.98	-	-	<1.8
Serum cortisol after 2 mg dexamethasone (μg/dL)	10.3	-	-	<1.8
Serum cortisol after 8 mg dexamethasone (μg/dL)	37 (55%suppression)	-	-	**
Ph	7.83	7.76	7.42	7.35-7.45
HCO <sub>3</sub> (mmol/L)	68.1	62.2	27.4	24-28
K (mmol/L)	1.2	2.6	3.6	3.5-5.1

ACTH: Adrenocorticotrophic hormone, \* Patient received intravenous administration of 10 mEq/h potassium chloride infusions and oral potassium chloride (40 mEq/8 h.), \*\*Suppression of plasma cortisol by at least 50% indicates Cushing's Disease

## DISCUSSION

CD is a rare endocrine disorder caused by ACTH-secreting pituitary tumors with an incidence of 80-85% in all patients with ADCS. The major manifestations of CS are centripetal obesity, facial plethora, skin atrophy and wide purplish striae, proximal muscle weakness, glucose intolerance, hypertension, and psychological changes (2). After performing initial diagnostic tests for CD, BIPSS is the most accurate test for diagnosing and lateralization, with a sensitivity of up to 97% and specificity of up to 100% when CRH stimulation is used (8). We performed BIPSS which showed a significant right to left gradient and MRI revealed a microadenoma in the right side of the pituitary. Successful TSS was performed to the patient and treatment was achieved.

The main point of this case was severe hypokalemia and metabolic alkalosis, despite these are more expected findings in ectopic CS than CD. Torpy et al. identified 58 patients with ectopic CS in a retrospective case review and they showed hypokalemia was much more related with ectopic CS than in patients with other causes of CS, affecting 57% of patients. Additionally, they found a significant relationship with 24-hour urine cortisol excretion and the presence of hypokalemia (3). Giraldi et al. evaluated gender-related differences in the presentation of CD, as regards biochemical results of hypercortisolism, and found hypokalemia was independently associated with 24-hour urine cortisol levels (9). Titan et al. have reported deep hypokalemia and metabolic alkalosis in a patient with ectopic adrenocorticotrophic hormone syndrome and have suggested possible mechanisms (10). Consistent with this finding, 24-hour urine cortisol level was elevated to 30 times normal values in our patient with severe hypokalemia. To our knowledge, none of the previous studies have reported hypokalemia as severe as in our patient. This finding suggests that severe hypokalemia should be considered not only in patients with CS but also in patients with CD.

Plausible mechanisms have been postulated to explain the reasons of hypokalemia and metabolic alkalosis in CS. The influence of CS on these biochemical alterations and pathological mechanisms involved are still not clarified. High cortisol concentration which has an in vitro binding affinity to the mineralocorticoid receptor (MR) similar to aldosterone could play a role as a mineralocorticoid. This mineralocorticoid effect of cortisol is insignificant in normal situation because of 11 beta-hydroxysteroid dehydrogenase 2 (11 $\beta$ -HSD2) enzyme which converts cortisol to inactive cortisone. High levels of cortisol concentration may saturate 11 $\beta$ -HSD2 and mineralocorticoid effects appear (11). Moreover, a deficient 11 $\beta$ -HSD2 enzyme activity causes by mutations in the 11 $\beta$ -HSD2 gene results in cortisol saturation of MR. This excess stimulation of MR causes renal sodium retention, hypokalemia, and decreased of plasma renin and aldosterone secretion (12). In the light of these findings, a possible mutation or polymorphism in 11 $\beta$ -HSD2 gene may be the reason for differences in blood pressure levels and variations of serum potassium and sodium levels in patients with CS.

Another mechanism to explain hypokalemia and metabolic alkalosis in CD could be that direct ACTH contribution to the

mineralocorticoid excess-like state of ADCS. ACTH can inhibit 11 $\beta$ -HSD2 both directly and/or triggering an inhibitory adrenal product (3). In consistence with these, it was suggested that increased levels of corticosterone and deoxycorticosterone, which controls mineralocorticoid activity, could be correlated with hypokalemia in CD (13, 14). Further investigations are needed to explain the role of 11 $\beta$ -HSD2 gene and its relation to biochemical and clinical findings in patients with CS.

## CONCLUSION

In conclusion, this case highlighted the prominence of considering CD in the differential diagnosis of severe hypokalemia and metabolic alkalosis that could be a crucial part of biochemical features in CD. Large series are needed to assess the true incidence and severity of hypokalemia and metabolic alkalosis and to explain the mechanisms involved in CD.

**Author contributions:** ST, MI, RS, MA, RK; Patient examination, Biochemical analyzes, Literature search and study design, data collection and analyzes ST; Writing article and revisions

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**Ethical issues:** All authors declare originality of research.

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