MSD Feb. 2021, Vol 8, No 2

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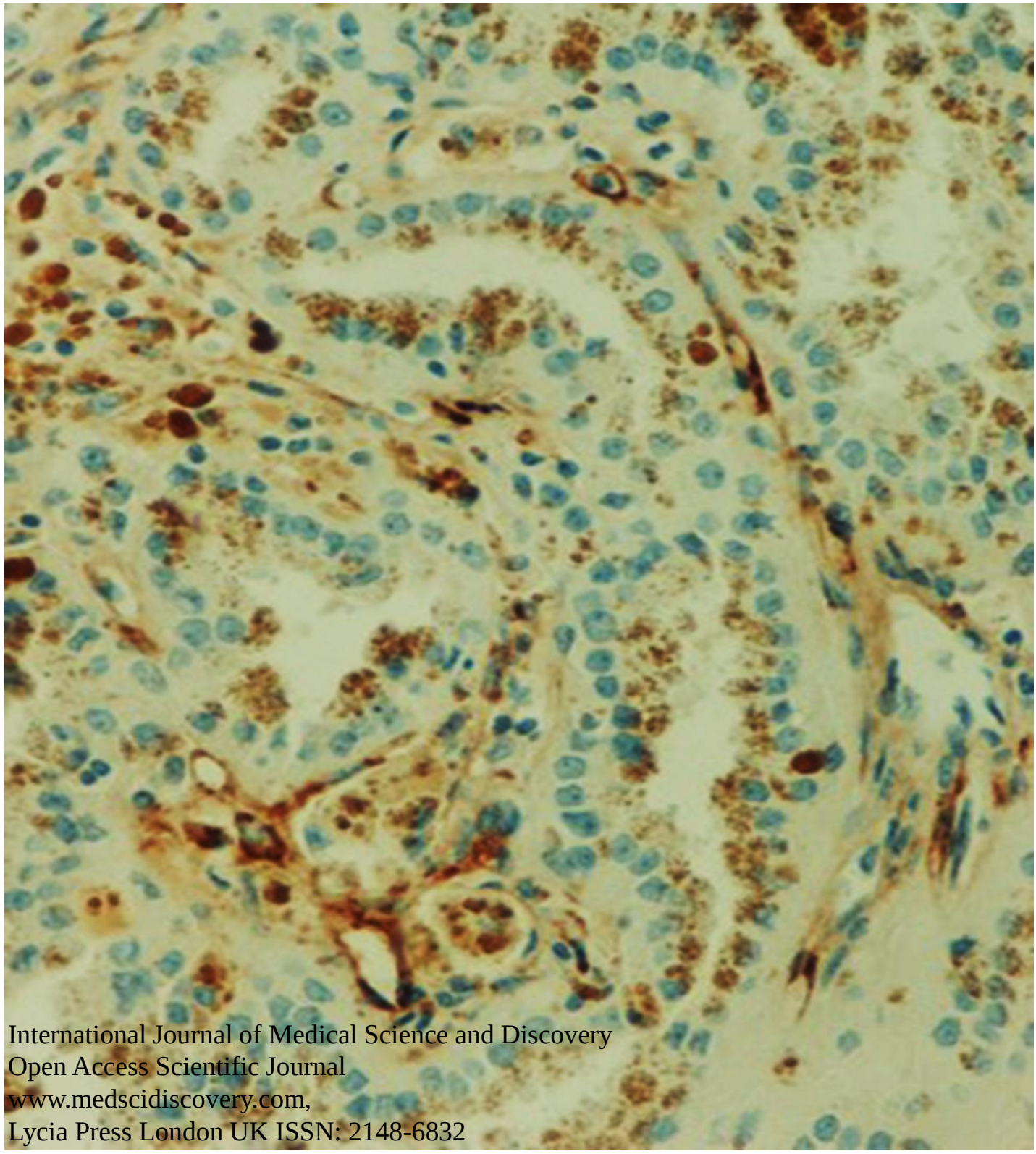

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ISSN: 2148-6832 (Print) E-ISSN: 2148-6832 (Online)

Category: Multi Disciplinary Health Science Journal

Abbreviated key title: Med. Sci. Discov.

Frequency: Monthly

Review System: Double Blind Peer Review

Circulation: Globally, Online, Printed

Article Processing Charge (APC): Free

Licensing: CC-BY-NC 4.0 International License Environmental

Editor-in-Chief: Assoc. Prof. Dr. Dr. Ahmad Rajabzadeh, Anatomical Department of lorestan, University of Medical Sciences, Tabriz, Iran

Established: 30.04.2014

Web address: www.medscidiscovery.com

E-mail : editor [at] medscidiscovery.com

Phone : +44 020 3289 9294

Design and preparation of PDFs, Language editing, Web site design, Graphical design Services of international Journal of Medical Science and Discovery has been contracted with Lycia Press LONDON, UK (as Publisher), by the MSD Board of Directors

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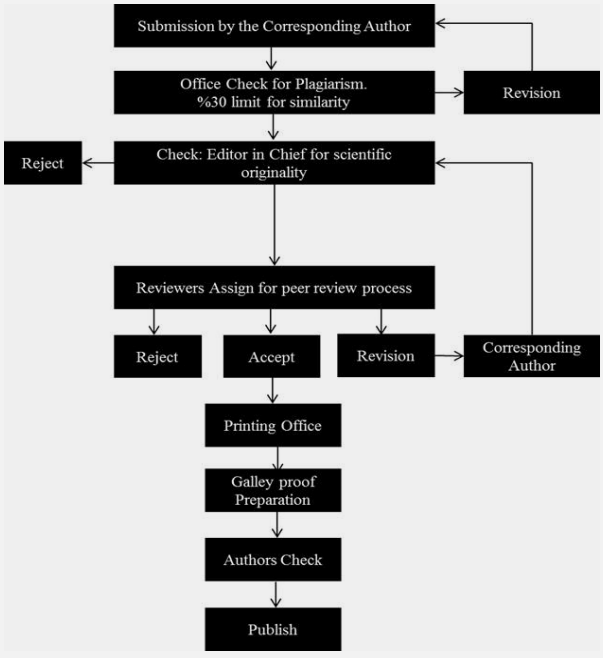

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Medical Science and Discovery, February 2021, Vol.8, No.2

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Research Articles

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Medical Science and Discovery
ISSN: 2148-6832

Waiting time analysis in a paediatric outpatient clinic in South East Nigeria

Onyinye Uchenna Anyanwu[1], Thecla Ezeonu[1], Maria Lauretta Orji[1], Obumneme Ezeanosike[1], Charles Ikegwuonu[1]*, Nnaemeka Kenneth Omeje[1], Uzoma Vivian Asiegbu[1], Ogah Emeka Onwe[1], Nnamdi Benson Onyire[1]

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ABSTRACT

Objective: Waiting time is a resource investment by the patient for the desired goal of being attended to by the physician. It is the time taken or spent in waiting to be attended to by a physician in a health facility. It is important because waiting time is an essential determinant of patient satisfaction in health care practice, and its study would expose the bottleneck areas in patient's time-flow so that the facility can improve services with that regard.

Materials and Methods: A cross-sectional study of time spent by paediatric patients in the outpatient department of Alex Ekwueme Federal University Teaching Hospital Abakaliki by secretly following the patients from arrival at CHOP till after consultation. Means were calculated of time spent in various areas.

Results: Of the 384 patients observed, the mean (SD) total time spent in the hospital was 142.58 (23.17) minutes while waiting time and consultation time were 113.15(18.01) and 24.43 (10.38) minutes respectively. The mean time spent at the nurse's bay was 23.79 (6.47) minutes, while that spent at the queue was 22.94 (8.98) minutes. The time spent at the records unit was the highest, with a mean time of 47.2 (17.42) minutes.

Conclusion: The long waiting time obtained from the current study is mostly attributable to delays from the records/registration unit, therefore conceited efforts aimed at improvement of service delivery in this unit will reduce patient waiting time and invariably patient satisfaction.

Keywords: waiting time, outpatient clinic, paediatrics, consultation time, AE-FUTHA

INTRODUCTION

Ensuring timely access to health care is a major policy concern in Nigeria. A patient time-flow analysis is a process that tracks patient flow, time spent, in addition to the use of personnel time in clinic settings (1). Its usefulness resided in the clarity with which it identified portions of the visit where excessive waits appeared to occur (1). Time-flow studies are used to identify problems in patient flow, as well as personnel needs to improve patients' timely access and continuity of health care. It identifies bottlenecks, system flaws, and hospital protocols that may cause a delay in health care delivery (2).

In 2001, the final report of the Quality of care in America project gave recommendations for improving the health care delivery system (3). Six aims were defined to help improve the quality of health care, and these were outlined in a framework of guiding principles that would help health institutions stay abreast of an increasingly competitive health care market (3). Timeliness was one of these six aims and involved providing timely care to patients that would help reduce harmful delays. Timeliness which is better recognized as waiting time in many literatures, although an understudied aspect of health care has been described as an essential determinant of patient satisfaction in health care practice (4), particularly because long wait times have been found to result in negative perceptions of the quality of services provided in outpatient departments (5,6) and the resultant decrease in patient satisfaction, in turn, may influence the return rate to outpatient departments, an essential element in the treatment of complex and chronic conditions (7).

Research Article

Received 07-01-2021

Accepted 02-02-2021

Available Online: 06-02-2021

Published 24-02-2021

http://dx.doi.org/10.36472/msd.v8i2.459

The recommendation by the Institute of Medicine (IOM) is that at least 90% of patients should be seen within 30 min of their scheduled appointment time (8). Long waiting time cuts across countries whether developed or developing as several studies have shown that patients spend 2-4 h in the outpatient departments before seeing the doctor (9-13). In the USA, there is an average of 188 min in Michigan (9). Singh et al (10) in Trinidad and Tobago reported an average waiting time of 160 minutes while several studies in Nigeria, reported an average waiting time range from 73-173minutes (11-14).

Time spent waiting is a resource investment by the patient for the desired goal of being attended to by the physician and therefore may be moderated by the outcome. Huang (15) observed that patients appear satisfied if they waited not more than 37 minutes when arriving on time for the appointment and no more than 63 minutes when late for appointments.

Patient's satisfaction is increasingly becoming a yardstick for the measurement of quality of health care delivery. Prolonged waiting time leads to a reduction in patient's satisfaction, increased risk of leaving without being seen by the doctor, and ultimately resulting in poor health outcomes (16). The frustration experienced by patients as a result of prolonged waiting time may explain the reduction in utilization of available health care services as patients seek alternatives to orthodox health care. Goodacre and Webster (17) observed that time of presentation rather than the individual characteristics were the most powerful predictor of waiting time. This finding was corroborated by Bamgboye et al (14) who noted that patients who presented at night and weekends had longer waiting time in the children's emergency room. On the contrary, Ofili and Ofovwe (10) and Ocheet al (13) attributed the long waiting time observed in their studies to large numbers of patients waiting to see relatively few doctors and hospital bureaucratic bottlenecks.

The Paediatrics department of Alex-Ekwueme Federal University Teaching Hospital Abakaliki (AE-FUTHA) is committed to providing children with the highest level of care, a mission that is impossible if patients are not seen in a timely manner. No assessment of waiting time among patients in the paediatrics outpatient department has been done to the best of our knowledge. This study aims to describe the waiting time for patients while identifying the bottlenecks and spots that delay timeliness in health care in the outpatient department.

MATERIAL AND METHODS

Study design: This was a cross-sectional descriptive study

Study area: Abakaliki is the capital city of Ebonyi State in the southeastern part of Nigeria. It has an area of 452sq kilometers. The inhabitants are mainly Igbo-speaking though other ethnic groups may be found. The metropolis has a total population of 149,683 inhabitants according to the 2006 census records (18). 57,029 are children and adolescents. Alex Ekwueme Federal University Teaching Hospital Abakaliki(AE-FUTHA) is a tertiary health institution located in Abakaliki the capital of Ebonyi State in Southeast Nigeria. It is the only tertiary health institution in the state that offers paediatric care to Ebonyi indigenes and other persons from nearby states.

The Paediatrics department has the ambulatory unit known as the children outpatient clinic where patients are seen on a daily basis except on weekends. Several paediatric subspecialty clinics hold concurrently in CHOP as follows: Infectious Diseases unit and Adolescent/Social Paediatrics unit on Mondays, Neurology and Endocrinology units on Tuesdays, Paediatric Respirology, and Gastroenterology units on Wednesdays, Haemato-oncology and Nephrology units on Thursdays while Cardiology unit holds on Fridays. The daily Children Outpatient clinic(CHOP) of AE-FUTHA has an average daily client turnover of 106 clients usually seen by 8 physicians of different cadre averagely. Every year, averages of 19,109 clients are seen. It also has the inpatient unit which consists of the emergency and the non-emergency units. The Children outpatient clinic (CHOP) of AE-FUTHA is open to see patients from 8am to 4pm from Mondays through Friday. However, it most often stays on till 6pm or until the last patient is seen.

The following describes patients flow in CHOP in AE-FUTHA,

Paediatric patients who come into the hospital are first received at the reception area of CHOP. From there they are directed to the general Records Department where they either obtain a new case note or retrieve an old one and go back to CHOP. The general records unit serves all patients who present for care irrespective of subspecialty and has an average of 4 staff per shift. The record staff takes case notes of patients periodically to CHOP where these case notes are registered by attendants. After this, nurses take the vital signs of the patients and document the same on the patients' case notes. The case notes are taken back by the attendants again who document the vitals in their books of record as written by the nurse. Nurses then move case notes into doctors consulting rooms and each patient takes a number in the queue to see the doctor. At his turn, the patient goes in to see the doctor and after the consultation, nurses/orderlies take the case notes again to the attendants to record findings and plans. Patient has now referred appropriately to one of the following according to the doctor's request, Pharmacy, Laboratory, Home, Paediatric ward/Children emergency department, Neonatal ward, or to another subspecialty unit.

Study population: Patients who present at the children outpatient department of AEFUTHA for ambulatory care.

Sample size calculation: The desired sample size was determined by the formula for sample size appropriate for an infinite population (19).

$$n = \frac{Z^2 pq}{d^2}$$

n = sample size when the target population is above 10,000

Z = Z statistic for a level of confidence, usually set at 1.96 (95% confidence level)

P = expected prevalence or proportion. In this case taken as 0.5 since there is no previous study

q= (1-P) = 1- 0.5= 0.5

d = precision

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(In proportion of one; if 5%, d = 0.05).

$$n = \frac{Z^2 pq}{d^2}$$

$$= \frac{(1.96)^2(0.5)(1-0.5)}{(0.05)^2}$$

$$= \frac{0.9604}{0.0025} = 384.16$$

Approximately 384 subjects was the minimum sample size used for the study

Sampling technique/ subjects selection

A minimum sample size of 384 was calculated and this number was recruited over 1 year. We, therefore, recruited 32 clients monthly (384/12months = 32). This number was shared among the five days of the clinic.

Hence we recruited 7 clients on Mondays and Tuesdays which had the largest number of clients the preceding year and 6 clients on Wednesday, Thursday and Fridays.

The second week of each month was selected for this study arbitrarily.

Six or seven patients were randomly selected daily.

Inclusion criteria: All clients who presented to the paediatrics children outpatient clinic (CHOP) of AE-FUTHA.

Exclusion criteria: Clients who presented to CHOP but found to need emergency care were referred to the Children emergency room of FETHA.

Ethical consideration: Ethical approval was not required for this study because it was an observatory, and had no direct involvement with human or animal participants.

Duration of study: The study was carried out between August 2018 and July 2019.

Research assistants: Three research assistants were recruited. These assistants were Doctors who had just finished housemanship and were waiting to do NYSC

Data collection: Selected clients were identified on arrival at CHOP reception area. The following time were recorded in a proforma .

T1. Time of arrival at CHOP

T2. Time of arrival at records department for folder retrieval

T3. Time case note arrived at chop (usually piled up till a significant is available to be carried to chop by an orderly)

T4. Time client finished with the attendants and nurses at CHOP, then took a number to see the doctor

T5. Time patient was ushered in to see a doctor

T6. Time client finished the consultation with the doctor

T7. Time client finished with nurse and record attendants and gets referred to the appropriate place

From these the total time (TT), time spent waiting to see the doctor (WT), and time spent consulting the doctor (CT) was calculated. Other times calculated were, time spent at general hospital records for folder retrieval (RT), time spent with nurses for vital signs and attendants for recording (NT), and time spent on the queue to be consulted (WsT).

These were calculated as follows:

TT=T1-T7

WT=T1-T5

CT=T5-T6

RT=T2-T3

NT=T3-T4

WsT=T4-T5

The numberof doctors on duty each of the days of study was also noted

The total number of patient seen each week day was also recorded, from this the average number of patient seen each day (mean daily patient load) of the week was calculated.

Data analysis: The data obtained was transferred into an electronic data base using the Statistical Package for Social Sciences (SPSS) version 22.0. descriptive analysis was done with calculations of means. A comparison of means was done using the ANOVA. All calculations was based on a significant level of $p<0.05$.

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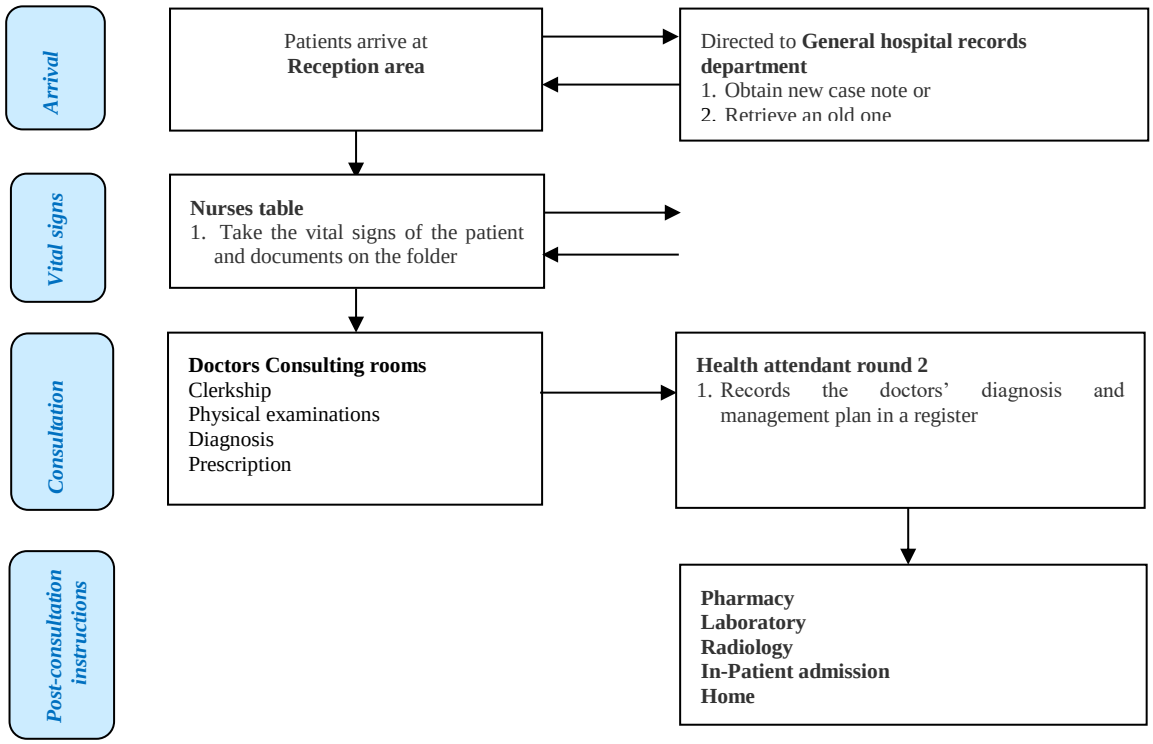
Table I: Patient Time Flow Diagram



RESULTS

Total clients reviewed were 384. Table I shows the distribution of clients followed on each day of the week. It also shows that the total number of patients who were seen in CHOP during the one year study period was 19,558. These clients were seen over 51 weeks. The number of days the clinic opened in each day of the week as well as, the total and means of each week of the daysarenoted in Table I. Mondays had the greatest mean attendance of 103 clients while Fridays had the least client attendants, 53.

The mean age of clients seen as well as the mean time spent in the different areas, waiting time as well as the total time spent in the hospital is represented in Table II.

Table III and IV show the descriptive analysis as well as the comparison of mean time spent on the different days of consultation Mean total time, mean consulting time, and mean queue time was greatest on Tuesdays while mean nursing time was the least on Tuesdays.

Tuesdays and Wednesdays seemed to have the greatest time spent and these were statistically significant. Also Figures 1-5 are shows the graphical representations of the different times studied.

Table II: Descriptive analysis of time spent for all patients

	Total clients	Mean	Min	Max	SD
Age of patient(years)	384	6.13	1	16	±4.59
Total Time spent (TT)	384	142.58	100.00	197.00	± 23.17
Waiting time (WT)	384	113.15	80.0	160.00	± 18.01
Consultation time (CT)	384	24.43	12.00	50.00	± 10.38
Time spent at record (RT)	384	47.20	20.00	90.00	± 17.42
Time spent at nursing bay (NT)	384	23.79	15.00	40.00	± 6.47
Time spent on the queue (WsT)	384	22.94	10.00	40.00	± 8.98

*all time is in minutes

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Table III: Comparison of means on the different days of consultation

	Day patient was seen	Mean daily patient load	Mean time (seconds)	Standard deviation	F_2	p
Total time spent	Monday	103	135.28	15.21	12.201	0.000
	Tuesday	77	154.14	23.14		
	Wednesday	71	145.74	27.08		
	Thursday	94	132.83	26.23		
	Friday	53	144.21	15.71		
Waiting time	Monday	103	106.00	12.68	14.237	0.000
	Tuesday	77	119.00	12.21		
	Wednesday	71	121.07	22.02		
	Thursday	94	105.33	22.19		
	Friday	53	114.57	13.48		
Consulting time	Monday	103	24.29	10.89	12.027	0.000
	Tuesday	77	30.14	12.44		
	Wednesday	71	19.67	6.59		
	Thursday	94	22.50	8.60		
	Friday	53	24.64	8.88		
Records time	Monday	103	44.86	14.19	17.486	0.000
	Tuesday	77	55.00	20.12		
	Wednesday	71	55.28	21.43		
	Thursday	94	38.00	7.00		
	Friday	53	44.28	12.84		
Nursing/recording time	Monday	103	23.86	4.08	19.553	0.000
	Tuesday	77	19.14	2.04		
	Wednesday	71	24.10	4.54		
	Thursday	94	26.33	10.98		
	Friday	53	26.31	4.66		
Queue time	Monday	103	22.86	9.26	2.427	0.048
	Tuesday	77	24.86	10.36		
	Wednesday	71	21.69	4.97		
	Thursday	94	21.00	10.83		
	Friday	53	23.99	7.49		

The means of total time, waiting time, consultation time, record time and nursing time were significantly different for the various days with different daily patient load (F2 = ,p<0.001)

Table IV: Represetation of the comparison of means of various times to the different days of presentation (ANOVA)

		Sum of Squares	df	Mean Square	F	Sig.
total time spent	Between Groups	23448.044	4	5862.011	12.201	.000
	Within Groups	182087.290	379	480.441		
	Total	205535.333	383			
waiting time	Between Groups	16228.934	4	4057.234	14.237	.000
	Within Groups	108010.306	379	284.988		
	Total	124239.240	383			
consulting time	Between Groups	4648.200	4	1162.050	12.027	.000
	Within Groups	36620.040	379	96.623		
	Total	41268.240	383			
records time	Between Groups	18101.784	4	4525.446	17.486	.000
	Within Groups	98085.175	379	258.800		
	Total	116186.958	383			
nursing/record time	Between Groups	2742.579	4	685.645	19.553	.000
	Within Groups	13290.169	379	35.066		
	Total	16032.747	383			
queue time	Between Groups	770.787	4	192.697	2.427	.048
	Within Groups	30094.835	379	79.406		
	Total	30865.622	383			

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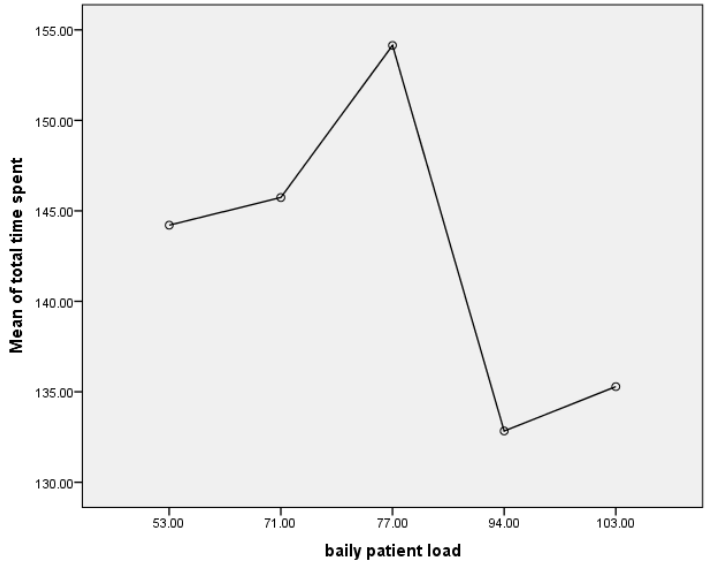

Figure I: graphical representation of comparison of mean of total time spent on the various days with varying patient load

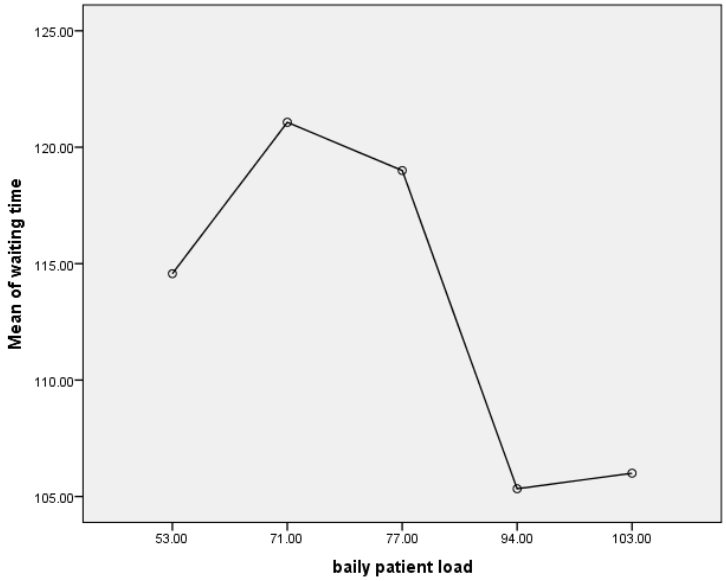

Figure II: graphical representation of comparison of mean of waiting time on the various days with varying patient load

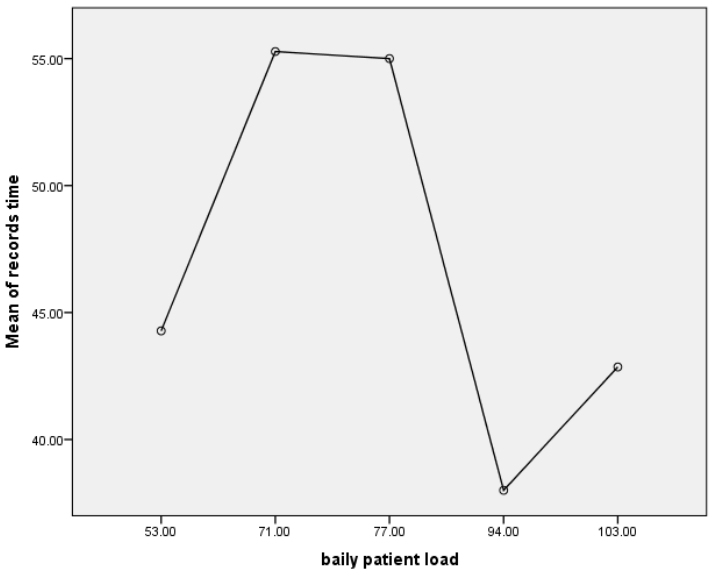

Figure III: Graphical representation of comparison of mean of time spent in records department on the various days with varying patient load

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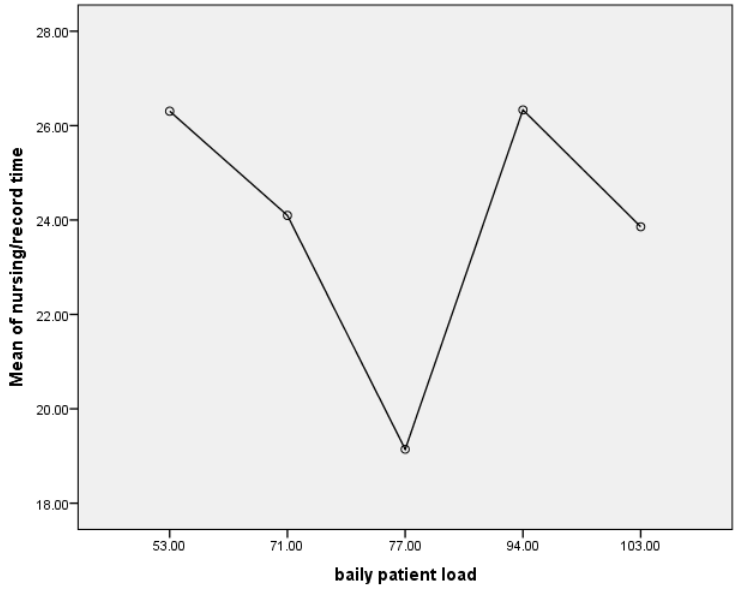

Figure IV: Graphical representation of comparison of mean of nursing time spent on the various days with varying patient load

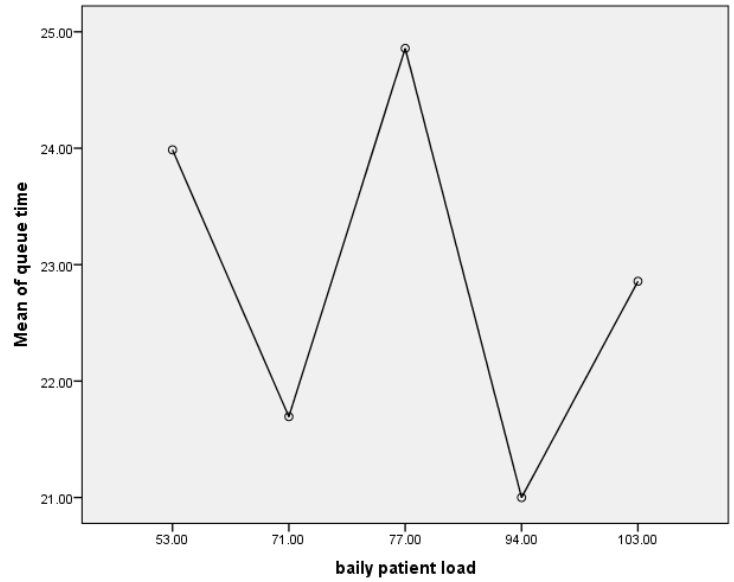

Figure V: Graphical representation of comparison of mean of time spent on the queue on the various days with varying patient load

DISCUSSION

From this study, one can appreciate various hurdles a client or patient seeking to access health care in hospital would have to jump, from the entrance into the out-patient department to departure. It entails movement and waiting from arrival at CHOP to arrival at records department for folder retrieval and registration; and eventual arrival of folder or case note at the CHOP, where client waits to be called by the Nurses for a record of vital signs. This is followed by a wait on a queue until the patient is ushered into the consulting room. After the consultation, the client returns to the nurses' station and gets referred to the appropriate station eg pharmacy or laboratory as the case may be. The mean total waiting time as shown in this study was 142.58 minutes, from arrival to exit from the outpatient department with most of this time spent at the records/registration department.

Oche& Adams in their study noted an even longer waiting time of 168 minutes, similarly having most of the time spent at the registration end (13).

Waiting time specifically has been defined as the length of time between arrival in the clinic/unit to when being attended to (20). In this study, the actual time spent before the client gets to see the doctor was recorded as 113.15 minutes. This is somewhat less than the time recorded by some other authors, such as Ofowve&Ofili, who recorded waiting time as long as 173 minutes (11) and Hasanpoor-Azghdy et al (21) in Iran who reported an average total time of 161 minutes, from entry to exit (21).

Some other studies on the contrary have reported that patients waited for a period of time ranging between 30 and 45 minutes to get the needed treatment (22-24).

As a standard, the Institute of Medicine (IOM) recommends that, at least 90% of patients should be seen within 30 minutes of their scheduled appointment time, but the findings in this paper are way behind the recommendation (8).

A further breakdown of the time intervals has shown that registration specifically took 47.20 minutes. This is quite long and different from findings by some authors in India where 10-20 minutes was spent at registration (25,26).

On the other hand, Oche& Adamu (11), in their study in Northern Nigeria, recorded registration time as high as 60–120 minutes in a majority of the clients studied, which they attributed to a few record clerks. Notably, Babalola et al have reported that three major factors associated with a long wait time are registration time, insufficient number of physicians, and insufficient number of counter staff (27). Some literature have associated this long wait at registration to the time of the day, specifically between 8am – 10am (28). Others added not just the time of visiting the counter, but also patients flow in hospital and numbers of registration counter etc (25).

From our study, the consultation time was an average of 24.43 minutes. This is more than the findings of 13.35 and 13 minutes by Sharma & Chowhan (29) and Wafula et al (30) respectively. It may be stated here that consultation time may depend on factors such as peculiarity of illness, the age of the patient, the type of services needed, the expertise of the medical officer and possibly the nature of the health institution. Indeed, Oche& Adamu (11) noted that the reason for higher consultation time in some tertiary hospitals may not be unconnected to the fact that in such an area the doctors use the opportunity of their interaction with patients to teach medical students undergoing various clinical postings, thus increasing the time spent.

Current study has shown notable variations in the time flow across days of the week, whereby the longest mean waiting time was on Wednesdays despite relatively lower average patient load and the least waiting time spent was on Thursday, although the registration time component was longest on Tuesday. Patel and his companion noted the influence of day of visit to the hospital on registration time (25). This provokes a thought that this longer waiting time disparity may be a problem with human resource capacity or the work attitude of the workers involved on some particular days. The consultation time was longest on Tuesday with mean of 30.14 minutes. The specialties that consult on Tuesday are the Paediatric Neurology and the Paediatric Endocrinology. The relatively longer consultation time might depend on the type of cases seen on that specific day or the degree of services needed, or time spent on training medical students during the consultation. It may also depend on the skills/expertise of the doctor being consulted.

Generally, anything that will make a patient spend so much time in the hospital may have a deleterious effect on the health seeking behaviour. Various studies have been able to show that prolonged waiting time for people has negative effects on health-seeking actions and that better patient satisfaction leads to better utilization of health services and contented patients are also more willing to adhere to the healthcare provider (31,32). McCarthy et al reported that patient dissatisfaction due to a long wait has been a reason why some of them will not return to the unit/clinic for care in future, instead, they resort to expensive, small private hospitals (33). Not only does waiting time influence patient satisfaction, it also serves as an indicator, used to evaluate the health care institution and its quality of services.

It is important to note that the outpatient department is the window to a hospital's services, and a patient's impression of the hospital, begins there (25). Although our study did not explore the possible causes of a long wait time, it is necessary to mention possible causes of undue waiting time in our health care setting. Inadequacies in equipment and manpower may often lead to prolonged and stressful waiting time for the patients (34). In our study area, efforts are continuously being made to computerize hospital records or patient's folders, but this is yet to be achieved as at the time of this study. This has put much stress on the staff and the clients, as time is spent sorting folders, following or trying to trace back folders. It is even worse with the interdepartmental movement of folders occasionally from the surgical units to the paediatric units depending on the case. There is need for a successful transition to computerized documentation of patient records with the provision of necessary up to date equipment and assurance of relatively constant electric supply to power the equipment. Inadequacies in manpower come in the form of quantity and quality. With enough personnel at the registration counter or the various service ends, to serve the teeming population of clients, waiting time should be significantly reduced. In addition, well trained, experienced personnel can maximize time with better work efficiency.

Oguet al included inadequate staffing with attendant excessive work load experienced by health providers (35), as Okonofua et al added poor attitudes of care providers as causes of long waiting time (36).

Having to see 50 – 100 clients on a clinic day as seen in this study may take a toll on the human resources and the available equipment. It demands adequate capacity planning and proper coordination of services to reduce frustration for the provider as well as the consumer. Some authors have attributed the availability of high workload, work procedures and environment, employee attitude and beliefs, employee's supervision management problems, and medical and administrative facilities availability to be common causes of waiting time problems in healthcare settings (37,38).

This study has been carried out as a simple review of patient time flow in the children's outpatient department of our Tertiary Health care institution, with the aim of discovering areas that need improvement for better quality of service to our clients.

Recommendation: Having noted that patient registration and card sorting takes the bulk of the patient's time flow and time spent on registration prolongs the average waiting time, it is hereby recommended that decentralization and computerization of the patients' record will effectively reduce waiting time. Capacity building for the records staff on quick, efficient data entry and folder retrieval may help on the interim and always. Where waiting is in evitable, clients can be made more comfortable while they wait through the use of

Television, newspaper, periodicals, drinking water, heating and cooling facilities etc.It may not be out of place to serve some simple refreshments to the children while they wait with their parents. These gestures allay clients' frustration and possibly may improve clients' willingness to return.

It is a fact that people generally do not like waiting especially for a service they have paid for. People tend to value their time more for some other economic gain. Conceited efforts must therefore be made to ensure minimum waiting time and patient satisfaction. In order to improve the health seeking behaviour of our people in the developing world, and boost their confidence in the tertiary health facility, waiting time must be effectively tackled, and time spent must be shown to be worthwhile.

CONCLUSION

In conclusion, from our study, most of the delay in the clients waiting time is from the records/registration unit. It calls for concerted effort to improve and mobilize resources for more efficient service delivery at this unit, which will ultimately result in much shorter waiting time and better patient satisfaction.

Acknowledgment: We thank the almighty God for the conceptualization and completion of this work. We also express our gratitude to the management of the Alex Ekwueme Federal University Teaching Hospital Abakaliki (AE-FUTHA) and all the research assistants for their support and immense contributions towards the actualization of this work.

Disclosure: There was no conflict of interest and the research was solely funded by the research authors

Limitation: None

Author contributions: OUA, TE, MLO, OE, CI, NKO, UVA, OEO, NBO; Design of the study and data collection. **CI;** review of the literature, analyzes and writing of the manuscript.

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. This research did not receive and specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical issues: All authors declare originality of research.

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MSD

Medical Science and Discovery
ISSN: 2148-6832

Breast diseases and breast cancer screening in pregnant and puerperal in Şanlıurfa province

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ABSTRACT

Objective: Pregnancy-associated breast cancer is breast cancer that occurs during pregnancy or within 1 year after birth. It occurs in one out of 3000-10000 pregnancies and is the most common cancer occurring during pregnancy and the postpartum period. It was aimed to reveal the incidence of pregnancy-associated breast cancer in pregnant and lactating patients in a city with high fertility rates.

Material and Methods: Patients who presented with breast pain and palpable mass in the breast in the first year of pregnancy and lactation between December 2018 and November 2020 were prospectively recorded. The 314 patients were included in the study.

Results: The mean age of the patients was 28.7 ± 6.1 years. 258 (82.1%) of the patients were Turkish and 56 (17.8%) were Syrian nationals. The most common complaints were pain in the breast, palpable mass, redness, and breast swelling. A palpable mass in the breast was detected in 39 (12.4%) patients. As a result of the examinations and tests performed in both patients, a diagnosis of malignancy was made.

Conclusion: Breast cancer risk increases in pregnant and breastfeeding patients. To reduce the incidence of breast cancer, it is important to perform a breast examination by a physician before or at the beginning of pregnancy and breast self-examination. From the moment of diagnosis, general surgery, obstetrics and oncology clinics should be followed with a multidisciplinary approach.

Keywords: Breast cancer, pregnancy, Lactation

INTRODUCTION

Many malignant and benign diseases can occur during pregnancy. Pregnancy-associated breast cancer (PABC) is breast cancer that occurs during pregnancy or within 1 year after birth (1). PABC occurs in one out of 3000-10000 pregnancies, and is the most common cancer occurring during pregnancy and the postpartum period (2).

The average incidence age is 32-34. Although it is more common in developed countries, its incidence has increased in the last 30 years. 10% of breast cancers under the age of 40 are pregnancy-related breast cancer. The incidence of breast cancer increases with age. Breast cancer is 2-3 times higher in patients who have their first pregnancy over the age of 30 compared to those under 20. Nowadays, an increase in the incidence is likely with the increase in the age of conception. No difference was found in terms of frequency compared to trimesters during pregnancy. However, GIMT is diagnosed at a higher rate during breastfeeding (3). The effect of hormones on the development of breast cancer is known. These hormones have proliferating effects on the glandular and ductal tissues of the breast. In addition, estrogen and prolactin are known to increase breast cancer growth. In addition, it is known that the increase in growth hormone and corticosteroid concentrations accelerates the spread of the tumor by decreasing immunity in animal experiments (4).

Breast examination during pregnancy is difficult due to physiological changes. After a detailed physical examination, ultrasonography (USG) is the first method to be used in diagnosis. Mammography can also be done by protecting the baby with a lead apron. Oblique shooting should be done first, and if it is not enough, it should be taken from other directions. Milk should be drained from the breast before shooting.

Research Article

Received 14-12-2021

Accepted 10-01-2021

Available Online: 08-02-2021

Published 24-02-2021

http://dx.doi.org/10.36472/msd.v8i2.451

The differential diagnosis includes lactational adenoma, fibroadenoma, fibrocystic change, galactocele and abscess. Trucut biopsy is 90% diagnostic. Pregnant patients with breast cancer have similar clinicopathological characteristics to those who are not, and yield similar survival results with non-pregnant patients at the same stage (**3**). Although the idea of termination of pregnancy in patients diagnosed with breast cancer has been defended for many years, it is now outdated. GIMT treatment aims to provide local control of the disease and to prevent systemic metastases, as in non-pregnant patients. In the treatment planning, the fetus should be considered as an individual and protective measures should definitely be included. The stage of the disease and the week of gestation are important criteria to be considered in treatment management. Treatment should be planned individually for each patient. In this study, we aimed to reveal the incidence of PABC in pregnant and lactating patients who applied to the general surgery clinic in a city such as Sanliurfa where the fertility rate is high.

MATERIAL AND METHODS

Study Design and Patients: Patients who were admitted to the general surgery outpatient clinic of Şanlıurfa Training and Research Hospital between December 2018 and November 2020 with complaints of breast pain and palpable mass in the breast during the first year of pregnancy and lactation were recorded prospectively. The study was conducted in accordance with the Declaration of Helsinki. Ethics committee approval was obtained for the study (HRÜ / 19.03.42 decision). Between these dates, 314 patients were examined in our outpatient clinic.

RESULTS

A total of 314 patients who met the study criteria were included in the study. The mean age of the patients was 28.7 ± 6.1 years. 258 (82.1%) of the patients were Turkish and 56 (17.8%) were Syrian nationals (Table-1).

Table 1: The demographic characteristics of the patients.

Variable		N	(%)
Age (year) (median)	28.7 years		
National	Turkish	258	82.1
	Syrian	56	17.8
Smoking		29	9.2
Pregnant		66	21
Breastfeeding period		248	78.9
Children of patients (median)		3.5	

When the patients were examined in terms of complaints of presentation, 96 (30.5%) patients had pain in the right breast, 104 (33.1%) patients had left breast pain, 32 (10.1%) had bilateral breast pain, 33 (10.5%) patients had breast pain and palpable mass, 9 (2.8%), pain and redness in the breast in 22 (7%) patients, swelling in the breast in 22 (7%) patients, nipple retraction, cracking and bloody nipple discharge in 4 (1.2%) patients, size difference in the breast in 2 (0.6%) patients, It was observed that 12 (3.8%) patients had swelling and pain in the armpit (Table-2).

Table 2: Application complaints of patients

Variable	N	(%)
Right breast pain	96	30.5
Left breast pain	104	33.1
Bilateral breast pain	32	10.1
Pain and palpable mass	33	10.5
Pain and redness	9	2.8
Swelling	22	7
Axillary pain and swelling	12	3.8
Other	6	1.9
Total	314	100

The 29 (9.2%) patients were smoking, alcohol use was not observed. In the anamnesis, 11 (3.5%) patients had a family history of breast ca It was observed that 66 (21%) of the patients were pregnant and 248 (78.9%) were in the breastfeeding period. It was observed that 89 (28.3%) patients were in the first 3 months of breastfeeding, and 42 (13.3%) of these patients were in the first one-month postpartum period. The average number of children of the patients was 3.5 ± 2.2 (min: 0- max: 11). When examination findings were examined, normal breast examination in 172 (54.7%) patients, fullness in the breast in 35 (11.1%) patients, a palpable mass in the breast in 39 (12.4%) patients, increased warmth and redness in the breast in 22 (7%) patients. and tenderness, nipple crack in 11 (3.5%) patients, axillary lymphadenopathy in 7 (2.2%) patients, and accessory breast in 4 (1.2%) patients (Table-3).

Table 3: Physical examination findings

Variable	N	(%)
Normal physical examination	172	54.7
Fullness	35	11.1
Palpable mass	39	12.4
Redness and sensitivity	22	7
Nipple crack	11	3.5
Other	35	11.1
Total	314	100

In the radiological examinations of the patients, 163 (51.9%) patients had normal breast USG findings, 83 (26.4%) patients had dilated ducts, 5 (1.5%) patients had galactocele? the lesion, 26 (8.2%) patients with benign breast lesions such as cyst, fibroadenoma, lipoma, 16 (5%) patients with edematous breast, mastitis? Lesion, fluid collection in 5 (1.5%) patients abscess? lesions, 5 (1.5%) patients had accessory breasts, 9 (2.8%) patients had axillary reactive lymph nodes, and 2 (0.6%) patients had lesions with a suspicion of solid malignancy (Table-4). Mammography was performed in 2 patients and no finding in favor of malignancy was observed. Invasive ductal carcinoma was observed as a result of tru-cut biopsy of the patients. Breast-conserving surgery + Sentinal lymph node sampling was performed on the patient who was pregnant in the second trimester. The patient received chemotherapy from the medical oncology clinic afterwards, and his radiotherapy was postponed until after birth. Breast-conserving surgery + Sentinal lymph node sampling was performed in the other patient who was breastfeeding. After surgery, chemotherapy and radiotherapy was planned for the patient by the medical oncology clinic.

http://dx.doi.org/10.36472/msd.v8i2.451

Table 4: Ultrasonography Findings

Variable	N	(%)
Normal breast USG	163	51.9
Dilated ducts	83	26.4
Cyst, fibroadenoma etc.	26	8.2
Galactocele	5	1.5
Mastitis	16	5
Abscess and loculation	5	1.5
Solid mass lesion (Malignancy?)	2	0.6
Total	314	100

DISCUSSION

During pregnancy and lactation, dramatic physiological changes occur in the breast with the effect of the circulating hormones estrogen, progesterone and prolactin. Estrogen and prolactin are known to increase breast cancer growth (**5**). Physiological changes such as increased density, increased volume, thickening of the tissue, hypervascularity and congestion are observed in the breast. 90% of the masses during pregnancy and lactation are diagnosed by self-examination. Every mass exceeding 2–4 weeks should be investigated. 80% of the masses during pregnancy and lactation are benign. 92.8% of the masses detected in our study were found benign.

As the gestational week progresses, breast examination becomes difficult, if the mass is detected in the first months of pregnancy, close follow-up is recommended because as the pregnancy progresses, it may be confused with the physiological stiffness that develops due to pregnancy (**6**). Therefore, breast examination should be the first step in determining GIMT. The best time for breast examination is the first trimester, and it is recommended that the basic breast examination be performed at the prenatal visit, if possible (**7**).

When a mass is detected in the breast during pregnancy or lactation, ultrasonography is recommended as the first imaging method. Ultrasonography can show simple cysts, galactoceles and lymph nodes in the breast.

If ultrasonography shows a solid mass, biopsy should be done. If the biopsy result is malignant, mammography can be performed on the patient by taking necessary measures (**6, 8**). Examinations were performed gradually according to the risk of malignancy.

The first goal of treatment in pregnant patients with breast cancer is to control the disease and prevent metastasis. Treatment should be planned according to the week of gestation, the stage of the disease, and the condition of the patient and fetus (**9**).

The risk of metastasis of breast cancer increases with hormonal effects during pregnancy (**4**). Surgery is the first treatment possible in PABC treatment, and it is safe during pregnancy (**10**). The treatment is similar to the protocol in non-pregnant patients, delay in treatment is risky in terms of metastasis. Chemotherapy to the mother during pregnancy should be done by considering the balance between the risk of the fetus and the prognosis of the disease. Chemotherapy and radiotherapy have the risk of teratogenicity and adverse effects on fetal development. Therefore, if possible, radiotherapy should be postponed until after birth. Modification of standard chemotherapy for the healthy baby can worsen the prognosis. It has been reported that after the first trimester of chemotherapy, pregnancy resulted in 95% normal live birth and morbidity was low (**11**). Surgical treatment was applied to patients with breast cancer. Both patients received chemotherapy. Radiotherapy of the pregnant patient was postponed until after delivery. Pregnancy negatively affects the prognosis of breast cancer. Compared with non-pregnant women in the same age group, the 4-year prognosis of PABC is worse (**12**).

In 73.8% of the patients' application complaints, the complaint of breast pain is remarkable. Breast examination was found to be normal in 54.7% of the patients. In the breast USG examination, 163 (51.9%) patients were also normal. Am I breast cancer in women with breast pain? It draws our attention that consulting a doctor is too much for fear. Self-examination of the person is important for breast cancer.

CONCLUSION

As a result, the risk of breast cancer increases, especially in pregnant and breastfeeding patients. In order to reduce the incidence of breast cancer, it is important to perform a breast examination by a physician before or at the beginning of pregnancy and breast self-examination. From the moment of diagnosis, general surgery, obstetrics and oncology clinics should be followed with a multidisciplinary approach.

Author contributions: MP, DAÇ, TG; Design of the study Patient examinatins and data collection. **MP;** review of the literature, analyzes and writing of the manuscript.

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. This research did not receive and specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical issues: All authors declare originality of research. Ethics committee approval was obtained for the study (HRÜ / 19.03.42)

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MSD

Medical Science and Discovery
ISSN: 2148-6832

Simple, safe, and fast Ponseti cast removal procedure in pes equina varus patients

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ABSTRACT

Objective: In our study, it is aimed to remove the cast more easily and safely without using the cutting tools by leaving the cast ends marked by folding in the idiopathic clubfoot patients treated with Ponseti method.

Material and Methods: Forty feet of 29 patients treated for Pes Equinovarus were included in the study. Patients were followed up in two groups. The group treated with Ponseti method by cast marking were named as “modified group” and cast wrapped group without marking were named as “classical group”. Neurological, teratologic and syndromic clubfoot patients were not included in the study. During the six series of casting, cast removal times for each extremity are recorded in minutes and it is noted that whether any additional cutting tool is used during cast removal or not. A summary of the data was presented as mean, standard deviation and percentage. Comparisons of the categorical characteristics were analysed by using the Chi-square test and the Mann-Whitney test. IBM-SPSS 20 program was used for analysis. In all tests, the level of significance was adjusted to 0.05.

Results: Thirteen (44.8%) of the 29 patients were male and 16 (55.2%) were female. While the mean time to start treatment for the 15 patients in the modified group was 3.46 (2-7) days, mean time for the 14 patients in the classical group was 3.78 (2-10) days. While the mean cast removal time of the 20 extremities of 15 patients in the modifying group was 10.9 minutes (8-14.3 min);it was 22.2 minutes (17.1-29.5 min) for the 20 extremities of 14 patients in the classical group. While no additional cutting tool was used during cast removal in the modified group, additional cutting tools were used during removal of cast in 75% (15/20) of the patients in the classical group and statistically significant difference was found between two groups in terms of the use of cutting tools ($p<0.001$).

Conclusion: We found that the cast ends’ being marked by folding during plastering in idiopathic clubfoot patients treated with Ponseti technique is costless, easy to apply, significantly shortens cast removal time, does not require the use of cutting tools, and thus is a notably safe method for these patients.

Keywords: congenital clubfoot, Ponseti method, soaking the cast, cast removing

INTRODUCTION

The idiopathic clubfoot is the most common congenital musculoskeletal disease and is seen at an average of 1/750 in eachnew-born. Most orthopaedists agree that initial treatment should be initiated immediately after birth and with non-surgical methods. Several studies published in the literature over the past few years have shown that Ponseti method provides 95% successful correction of clubfoot treatment (**1-2**). This method requires an average of six series of casting so that the deformity can reach the desired correctionon each plane.Although all the steps of the casting technique have been described in detail in the literature, the issue of cast removal has been mentioned very little (**3-5**). Ponseti cast removal is usually done by soaking and then unwrapping the plaster, but it is often very difficult and time consuming to find the end edge of the plaster (**6**).

Research Article

Received 15-01-2020

Accepted 30-01-2021

Available Online: 08-02-2021

Published 24-02-2021

http://dx.doi.org/10.36472/msd.v8i2.464

There is a limited number of studies on this subject in the literature. So, we believe that the simple, safe and fast cast removal technique we have demonstrated in this study will help solving the difficulties experienced during cast removal in Pes equinovarus patients treated with Ponseti, method.

MATERIAL AND METHODS

Forty feet of 29 patients treated for Pes Equinovarus in our hospital between 2015 and 2016 were included in the study. Patients were followed up in two groups. The group treated with Ponseti method by cast marking were named as “modified group” and cast wrapped group without marking were named as “classical group”. All patients were followed prospectively. After reaching 20 idiopathic clubfoot patients treated with casting by Ponsetimethod in both groups, new patient intake was stopped. Fifteen patients with 20 idiopathic clubfoot in the modified group and 14 patients with 20 idiopathic clubfoot in the classic group were obtained. Neurological, teratologic and syndromic clubfoot patients were not included in the study. During the study all the patients were followed and treated by 2 different orthopaedic specialists. While marking by folding was applied during casting in modifying group (Figure 1A-B-C), casting was applied without marking in the classical group. All the patients were treated with apercutanachillotomy to correct the equinusdeformity under local anaesthesia after six series of casting. Then the seventh cast that will stay for three weeks was applied. At the end of the third week, all patients were treated with Denis-Browne abduction orthosis to maintain the position of the corrected foot.

All families have been trained in soaking and unwrapping Ponseti plaster. The families were requested to record the cast removal times for each extremity in minutes during six series of casting (the time passed from soaking the extremity to the complete removal of the cast), and to note whether any additional cutting tools were used during cast removal (Figure 2).

The study protocol was approved by local ethics committee (Date, 16 February 2017; number65, Metin Sabancı Baltalimanı Bone Diseases Training and Research Hospital, Ethical Committee for Clinical Investigations).

Statistical analysis: A summary of the data was presented as mean, standard deviation and percentage. Comparisons of the categorical characteristics were analysed by using the Chi-square test and the Mann-Whitney test. IBM-SPSS 20 program was used for analysis.In all tests, the level of significance was adjusted to 0.05.

RESULTS

Forty extremitiesof 29idiopathic clubfoot patients were included in the study. Thirteen (44.8%) of the patients were male and 16 (55.2%) were female. While the mean time to start treatment for the 15 patients in the modified group was 3.46 (2-7) days, mean time for the 14 patients in the classical group was 3.78 (2-10) days. No statistically significant difference was found between the two groups in terms of the age of treatment (p:0.502) (Table 2). Cast removal time for each extremity was recorded in minutes from cast soaking to complete removal of the cast.

While the mean cast removal time of the 20 extremities of 15 patients in the modifying group was 10.9 minutes (8-14.3 min); for the 20 extremities of 14 patients in the classical group, it was 22.2 minutes (17.1-29.5 min). Cast removal time was found significantly short in the group treated with marked technique (p<0.001) (Table 3).

While no additional cutting tools were used during cast removal for any patient in the modified group, additional cutting tools were used for 75% (15/20) of the patients in the classical group during cast removal, and statistically significant difference was found between the groups (p<0.001) (Table 1).

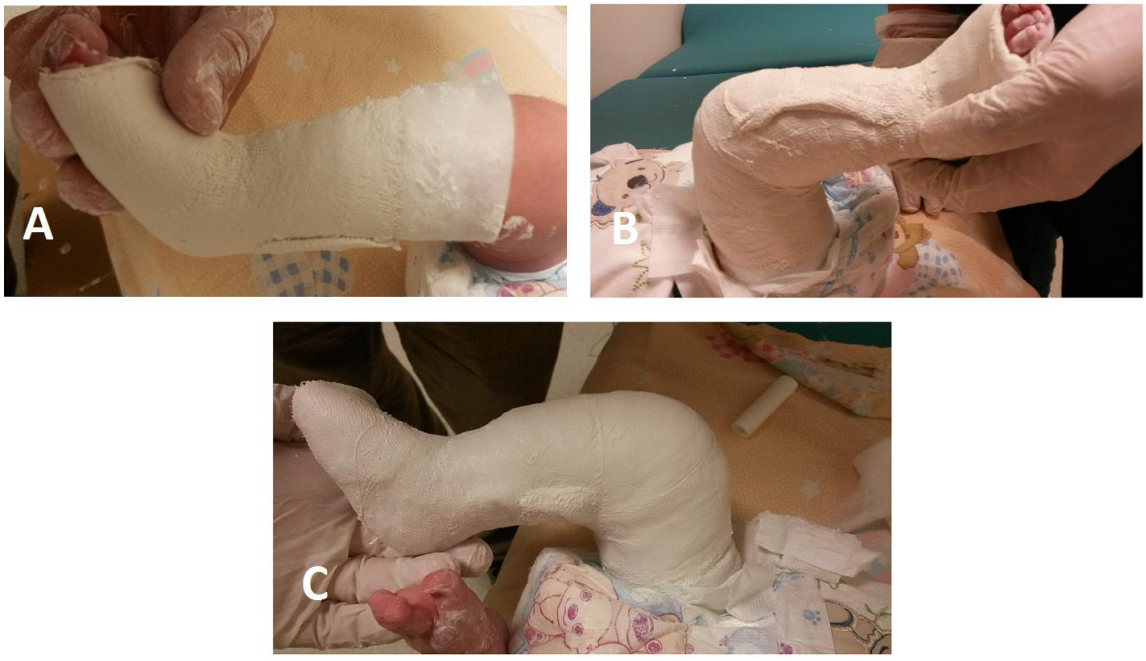

Figure 1: Marking by folding method of the cast ends of the idiopathic clubfoot patient treated with Ponseti technique. A) Marking of the first cast end. B) Marking of the second cast end. C) Marking of the third cast end.

http://dx.doi.org/10.36472/msd.v8i2.464

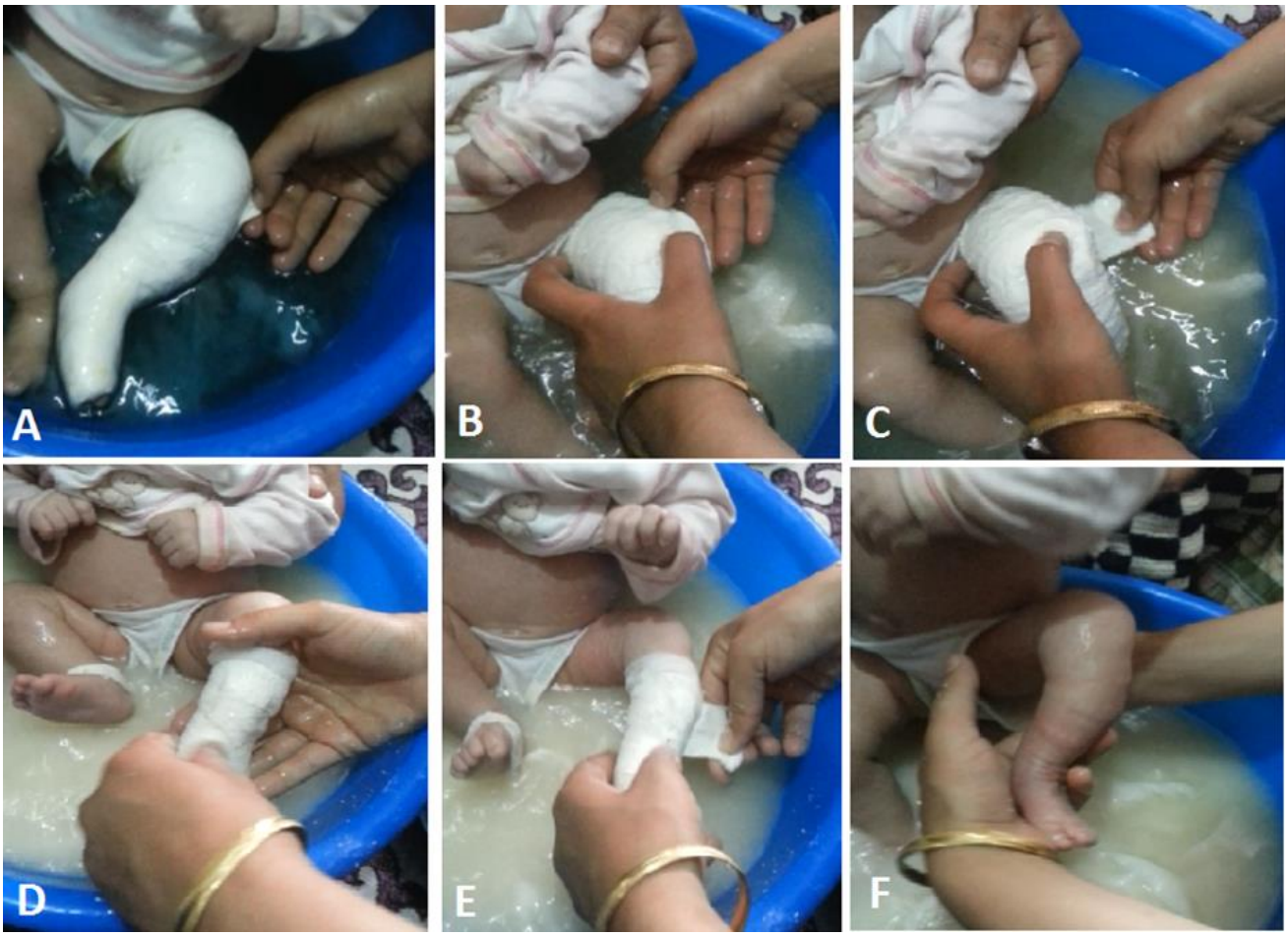

Figure 2: Ponseti cast removal steps. A) Finding the first marked cast end after being completely soaked in a bathtub. B) Removal of the first cast. C) Finding the second cast end and removing the cast. D) Finding the third cast end. E) Removal of the third cast. F) Complete cast removal in 7 minutes after being soaked.

Table 1. Statistical analysis of the use of the cutting tool between the two groups with *Pearson-chi square test

Technique		The use of the cutting tool		Total
		Yes	No	
Modified Group	n	0	20	20
	%	0,0%	100,0%	100,0%
Classical Group	n	15	5	20
	%	75,0%	25,0%	100,0%
Pearson chi-square		p<0.001		

Table 2. Statistical analysis of the age of treatment between the two groups with Mann-Whitney* test. No statistically significant difference was found between the two groups in terms of the age of treatment (p>0.502)

Technique	n	Mean (Age of treatment-day)	SD (Standart Deviations)	P *Mann-Whitney Test
Modified Technique Group	15	3,46 days	1.45	,502
Classical Technique Group	14	3,75 days	2.08	

Table 3. Statistical analysis of the cast removal time between the two groups with Mann-Whitney* test. Cast removal time was found significantly low in the modified group (p<0.001).

Technique	n	Mean (Cast removal time-min)	SD (Standart Deviations)	P *Mann-Whitney Test
Modified Technique Group	20	10,940 min	1.702	,000
Classical Technique Group	20	22,295 min	3.424	

DISCUSSION

Ponseti method is accepted as the gold standard in the treatment of idiopathic clubfoot. The safety and effectiveness of the method has been repeatedly shown in the literature, which has led to increased useof the method throughout the world in the last 20 years (7). Despite the detailed description of the cast application steps of this technique widely used in the treatment, little attention has been paid to castremoving (3-5).

During the Ponseti cast removal; it is suggested that the electrical castsaw should not be used as it may cause skin irritation and frighten the family and the baby. There are two options in the literature about cast removal. In the first option; it has been proposed to wrap the cast with a damp towel after 20 minutes of soaking of the cast, and first to remove above the knee and then below the knee with a plaster knife.The plaster knife should be used obliquely to avoid skin damages. In the other option, it is suggested to soak the cast completely in a bath tub and to unwrap it after being softened enough. It has also been reported that this method is effective but takes longer (4,8,9). In our study, we argued that the end points of the casting should be marked by folding during casting in the clubfoot patients treated with Ponseti technique, which will shorten cast removal time and remove the need of cutting tools for cast unwrapping. In this respect, we compared the cast removal times and the need to use of cutting tools for the patients treated with marking and without marking of the cast.

While the mean cast removal time of the 20 extremities of 15 patients in the marked group was 10.9 minutes (8-14.3 minutes); for the 20 extremities of 14 patients in the classical group, it was 22.2 (17.1-29.5 minutes). Statistically, cast removal time in the treatment group with the marked method was found significantly low ($p<0.001$). While no additional cutting tool was used during cast removal in the marked group, additional cutting tools were used during removal of cast in 75% (15/20) of the patients in the classical group and statistically significant difference was found between two groups in terms of the use of cutting tools ($p<0.001$).

CONCLUSION

We found that the cast ends' being marked by folding during plastering in idiopathic clubfoot patients treated with Ponseti technique is costless, easy to apply, significantly shortens cast removal time, does not require the use of cutting tools, and thus is a notably safe method for these patients.

Author contributions: FD, ÖC; Design of the study Patient examinatins, therapy and data collection. **ÖC;** review of the literature, analyzes and writing of the manuscript.

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. This research did not receive and specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical issues: All authors declare originality of research.

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MSD

Medical Science and Discovery
ISSN: 2148-6832

Can Zonulin level be a new diagnosis and follow-up criterion in active ulcerative colitis?

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ABSTRACT

Objective: In this study, we compared the serum zonulin levels in patients diagnosed for the first time with active ulcerative colitis with those in healthy cases and attempted to determine whether serum zonulin levels were different in the active ulcerative colitis.

Material and Methods: A total of 53 naive patients admitted to our hospital between 2019 and 2020 and diagnosed with active ulcerative colitis by colonoscopy were included as a group of cases and 37 patients with no acute or chronic diseases whose colonoscopy was normal as the control group.

Results: The study was conducted on 90 cases, 65.5% male and 34.5% female. The patients with ulcerative colitis were compared with the control group in terms of serum zonulin levels. Average serum zonulin levels of the patients with ulcerative colitis (16.73 ± 5.49 ng/ml) were not significantly different than those in the control group 17.48 ± 8.31 ng/ml). Serum zonulin levels of the patients were also compared according to location and severity of disease and did not differ statistically significantly between the groups in terms of the Montreal Classification. When serum zonulin levels were grouped according to the Truelove and Witts criteria, there was no statistically significant difference between the patient groups themselves and the control group.

Conclusion: Serum zonulin levels were not greater in the patients with naive active ulcerative colitis compared to the healthy controls. Several previous studies have shown that serum zonulin levels are elevated in patients with ulcerative colitis, but more studies are needed on this subject.

Keywords: zonulin, inflammatory bowel diseases, ulcerative colitis

INTRODUCTION

The intestinal wall is composed of large, single-celled, thick epithelial cells surrounding the inner membrane of the intestine. Maintaining the integrity of this barrier and controlling its permeability is very important in terms of the regulation of the immune system and protection of it against pathogens. There are two ways in which elements pass through the intestinal lumen into the blood circulation. These two paths are the “transcellular pathway” with transporters along the brush border of enterocytes and the “paracellular pathway” through spaces between cells (**1, 2**). The paracellular pathway is controlled by gates with a protein structure called “tight junctions.” These dynamic structures are opened and closed in harmony with the nutritional status, physical activity, hormonal and neural signals, and inflammatory mediators (**3, 4**). Zonulin, a precursor to haptoglobins (HPs), which is called pre-haptoglobin-2 (pre-HP2), is a physiological and reversible modulator in a key position at these tight junctions. This protein is formed in the mucosa and directly controls the permeability of the intestine (**1, 5, 6**). In response to stimuli such as bacteria in the intestinal lumen or triggers in foods, zonulin is released into the lumen, binds to receptors on the apical surfaces of epithelial cells, and activates the pathways that cause deterioration of the integrity of tight junctions (**7, 8**). Genetic structure and environmental triggers may play a role in the pathogenesis of chronic inflammatory diseases (CIDs), which can be classified as allergic, autoimmune, and metabolic diseases. However, increased intestinal permeability is a common consequence of a number of complicated processes in such diseases (**3, 9, 10**).

Research Article

Received 17-01-2020

Accepted 07-02-2021

Available Online: 08-02-2021

Published 24-02-2021

http://dx.doi.org/10.36472/msd.v8i2.467

Long-term zonulin upregulation caused by environmental triggers leads to increased intestinal permeability and continuous passage of intestinal antigens to the submucosa. If intestinal permeability increases, a large amount of antigenic substances enter the systemic circulation. This can lead to chronic intestinal mucosal damage in the long term. Moreover, type 1 diabetes mellitus, multiple sclerosis, rheumatoid arthritis, celiac disease and many other autoimmune diseases may develop due to the increased intestinal permeability (**9, 11**). Excessive permeability from the intestine has also been associated with chronic diseases such as irritable bowel syndrome, whose etiology is not well known. In a recent clinical study, serum zonulin levels were found to be higher in patients with inflammatory bowel disease (IBD) — in patients with ulcerative colitis (UC) and Crohn's disease (CD) both — than in normal healthy controls (**12**). This reinforces the hypothesis that certain zonulin-mediated pathways cause UC. In our study, we assessed whether serum zonulin levels of patients with active UC were different from those of normal healthy controls.

MATERIAL AND METHODS

Before the study began, ethics committee approval for the study was obtained from the ethics committee of our institution. The study was conducted on 37 healthy people in the control group and a total of 53 patients consisting of 21 women and 32 men diagnosed with naive active UC by colonoscopy, biopsy and laboratory findings from among patients admitted to our clinic between 2019 and 2020. The case and control group patients were included in the study voluntarily. Each patient was given an informed consent form before participation and was asked to sign it. Blood samples were taken and kept at -80 °C before starting treatment from the patients diagnosed with UC. As a control group, a total of 37 patients including 12 women and 25 men, who underwent a colonoscopy and whose colon mucosa was found to be normal, who did not have a CID, and whose laboratory values were normal, were included in the study. Similarly, blood samples taken from these patients were kept at -80 °C. Serum zonulin levels of these samples were determined by using the Sunred B10 Human Zonulin kit at the end of the study and were studied by using the ELISA method.

Statistical analyses: Statistical analyses were conducted in the Number Cruncher Statistical System (NCSS) 2007 software (Kaysville, Utah, USA). Descriptive statistical methods (means, standard deviations, medians, frequencies, ratios, minimum and maximum values) as well as comparative statistical methods were used when assessing the data of the study.

Whether the quantitative data were normally distributed was tested by using the Kolmogorov-Smirnov test, Shapiro-Wilk test and graphical evaluations. The quantitative data of the two groups with normal distribution were compared through student t-tests, and the data that were not normally distributed were compared through Mann Whitney U tests. Data of three or more groups that were not normally distributed were compared through Kruskal Wallis Tests. Qualitative data were compared through Pearson chi-square tests. Significance was assessed at least at $p < .05$ level.

RESULTS

The study was conducted on a total of 90 cases — 65.5% (n = 59) male and 34.5% (n = 31) female — at the Gastroenterology Clinic of Education and Research Hospital of a university. The ages of the cases ranged from 19.6 to 78.4 years, and the mean age was 37.61 ± 12.66 years.

Of the cases included in the study, 41.1% (n = 37) constituted the control group, and 58.9% (n = 53) constituted the group of patients with UC. The demographic characteristics of the case and control group patients included in the study are shown below in Table 1.

After the patients with UC were compared with the control group patients overall, they were also compared with the control group separately depending on disease location and severity. Disease locations were classified according to the Montreal Classification. Disease severity was assessed according to the Truelove and Witts criteria. Table 2 presents demographic data of the patient and control group cases according to disease location and severity.

When the cases were examined for disease locations according to the Montreal Classification, 28.3% (n = 15) of the cases with UC had ulcerative proctitis, 34.0% (n = 18) left-sided colitis, and 37.7% (n = 20) had pancolitis.

According to the Truelove and Witts (severity) criteria, 30.2% (n = 16) of the cases were mild, 37.8% (n = 20) moderate, and 32.0% (n = 17) were cases involving severe colitis.

The patients with UC were compared with the control group in terms of serum zonulin levels. Serum zonulin levels of the patients in the UC group were 16.73 ± 5.49 ng/ml on average, while serum zonulin levels of the patients in the control group were 17.48 ± 8.31 ng/ml on average. There was no statistically significant difference between the patients and the control group in terms of zonulin levels ($p > .05$).

Serum zonulin levels of the patients were also compared with those of the control group according to disease location and severity. Serum zonulin levels of the patients did not differ statistically significantly between the classes/groups that were classified in terms of the Montreal Classification ($p > .05$).

Serum zonulin levels showed no statistically significant difference between the patient groups themselves with UC that were grouped according to the Truelove and Witts criteria, and neither between them and the control group ($p > .05$).

The distribution of the control group and patient data according to the Montreal Classification and Truelove Witts criteria is shown in Table 3.

The graphical expression of serum zonulin levels of the patients with UC according to the location and severity of the disease is shown in Figure 1 (Montreal Classification: p= .375, Truelove and Witts: p= .796).

http://dx.doi.org/10.36472/msd.v8i2.467

Table 1. Distribution of demographic characteristics of cases (n = 75)

	Total (n = 90)	Control Group (n = 37, 41.1%)	Ulcerative Colitis (n = 53, 58.9%)
Age (Years)			
Min–Max (Median)	19.6–78.4 (35.7)	20.5–64.3 (40.4)	19.6–78.4 (30.5)
Mean±SD	37.61±12.66	41.23±11.83	34.05±12.99
Gender, n (%)			
Male	59 (65.5)	25 (67.6)	32 (65.5)
Female	31 (34.5)	12 (32.4)	21 (34.5)

Table 2. Distribution of patient and control groups according to disease location and severity

	Total (n = 90)		Control Group (n = 37, 41.1%)		Ulcerative Colitis Group (n = 53, 58.9%)	
	n	(%)	n	(%)	n	(%)
Montreal Classification (Localization)						
Control Group	37	(41.1%)	37	(100.0%)	0	(0.0%)
Ulcerative proctitis	15	(16.7%)	0	(0.0%)	15	(28.3%)
Left-sided colitis	18	(20.0%)	0	(0.0%)	18	(34.0%)
Pancolitis	20	(22.2%)	0	(0.0%)	20	(37.7%)
Truelove and Witts (Severity)						
Control Group	37	(41.1%)	37	(100.0%)	0	(0.0%)
Mild	16	(17.8%)	0	(0.0%)	16	(30.2%)
Moderate	20	(22.2%)	0	(0.0%)	20	(37.8%)
Severe	17	(18.9%)	0	(0.0%)	17	(32.0%)

Table 3. Distribution of data by Montreal Classification and Truelove and Witts Criteria

	Montreal Classification				
	Control Group (n = 37)	Ulcerative proctitis (n = 15)	Left-sided colitis (n = 18)	Pancolitis (n = 20)	p
Zonulin (ng/ml)					
Min–Max	11.4–49.4	10.7–30.2	10.8–24	10.1–33.3	.375
(Median)	(14.7)	(17.7)	(13.2)	(17.3)	
Mean±SD	17.48±8.31	18.11±6.36	14.63±3.74	17.70±6.02	
	Truelove and Witts Criteria				
	Control Group (n = 37)	Mild (n = 16)	Moderate (n = 20)	Severe (n = 17)	p
Zonulin (ng/ml)					
Min–Max	11.4–49.4	10.7–30.2	10.1–25.3	11.2–33.3	.796
(Median)	(14.7)	(13.9)	(14.8)	(16.3)	
Mean±SD	17.48±8.31	16.91±6.54	15.98±4.49	17.60±6.06	

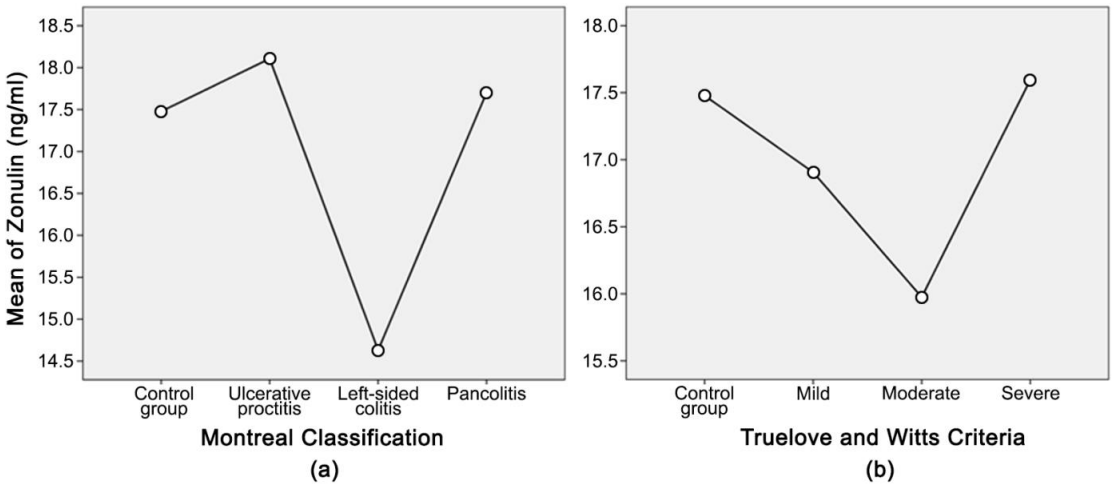

Figure 1. Distribution of Serum Zonulin levels according to **a.** Montreal Classification and **b.** Truelove and Witts Criteria

DISCUSSION

UC and CD are immune-mediated conditions characterized by chronic inflammation of the intestine, collectively called IBD. Their exact etiologies are unknown, but increased intestinal permeability has been shown to play a fundamental role in the pathogenesis of IBD. Zonulin is one of the little-known physiological mediators of paracellular intestinal permeability. Mature human HPs are heterodimeric plasma glycoproteins. Zonulin is a pre-HP2 isolated from human serum through proteomics studies (13). Most of the studies on the role of zonulin in IBDs are experimental animal model studies (14-17). There are not enough clinical trial studies conducted on humans (11, 13, 18). In the study of Caviglia et al. comparing UC and CD patients with healthy normal controls, serum zonulin levels were found to be significantly higher in UC and CD patients (12). In a study carried out by Vanuytsel et al., it has been shown that haptoglobin-2 (HP2) carries a higher frequency of risk allele in both UC and CD compared to healthy controls (13). In cirrhosis patients secondary to chronic hepatitis C, low serum zonulin levels were found, which were probably due to decreased production of this mediator in the liver (19). However, there is currently no study that reports serum zonulin levels of patients with UC to be normal or low compared to those of healthy controls.

In our study, the patients with naive active UC were compared with the healthy controls in terms of serum zonulin levels, but no significant difference was found between the patients and the control group. In addition, when the UC cases were grouped into different disease severities and locations, their serum zonulin levels were not different from those of the normal healthy controls. The results of our study suggest that serum zonulin levels cannot be a diagnostic and activity indicator of UC. As a matter of fact, it was shown in another clinical study that fecal zonulin in UC was a weaker marker than other parameters in assessing the response to infliximab (20). In a recent study by Wegh et al., serum zonulin was found to be a better indicator of intestinal permeability in patients with UC than fecal zonulin because of its compatibility with other intestinal permeability biomarkers (21). As can be seen, contradictory results have been obtained in a limited number of clinical studies on zonulin.

The sensitivity of the commercial ELISA kit, which was used to determine the level of serum zonulin, to the corresponding zonulin molecule may also be an important parameter determining the results. The development of methods that more precisely determine pre-HP2 levels may make zonulin a more sensitive parameter in IBD in the future (22).

However, the efficacy and diagnostic value of zonulin in UC is not a subject on which light has been fully shed. If HP2 is defined as a risk allele for IBD, the next question to answer is how HP affects the pathogenesis of the disease. This may be due to HP's possible immunomodulatory effect. Based on animal model studies, HP can be assumed to play a role in reducing the severity of inflammation and modulating the production of IL-17, which is a cytokine that is thought to be very important in the pathogenesis of IBD. However, clinical trials in humans are not yet available. In this respect, another hypothesis may be related to zonulin. Zonulin, described as pre-HP2, is defined as an important physiological instrument of paracellular intestinal permeability. Due to the permeability-enhancing effect of the carriers of the zonulin gene (genotype HP21 or HP22) on the intestinal barrier, it is likely that zonulin carries the risk of causing IBD. Further studies on the role of zonulin in IBD and its relationship to intestinal permeability will be important to find more answers for many interesting questions.

Author contributions: EA, GÜ; Both authors equally contributed to the conception and planning of the work that led to the paper and interpretation of the evidence it presents; and the writing of the paper/reviewing of successive versions.

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. This research did not receive and specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical issues: All authors declare originality of research.

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Medical Science and Discovery
ISSN: 2148-6832

The effect of attenuation correction on image quality in single photon emission computed tomography

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ABSTRACT

Objective: Attenuation has a significant influence on data and consequently on image quality. Attenuation correction corrects the weakening of the gamma photons in various depths. Non-diagnostic, low-dosage CT is usually used for attenuation correction when images are taken with a SPECT/CT. The purpose of the study was to determine the influence of attenuation correction in SPECT/CT on image quality in NEMA body phantom analysis in different background/sphere ratios.

Material and Methods: The NEMA IEC Body Phantom was filled with isotope technetium-99m (99mTc), with a different ratio between the phantom background and spheres. The images were reconstructed using filtered back projection (FBP), non-corrected iterative reconstruction (IR), and iterative reconstruction using computer tomography for attenuation correction (CT-AC). The average number of counts in the background and in all six spheres was measured. This was followed by a comparison of the contrast in images that were reconstructed using different methods.

Results: The average number of counts in sphere increased as we increased the activity concentration ratio between the background and sphere. Statistical analysis showed that contrast is significantly divergent between different methods of reconstruction.

Conclusion: The use of iterative reconstruction with CT-AC improves the contrast and image quality compared to iterative reconstruction and FBP.

Key words: SPECT/CT, iterative reconstruction, attenuation correction, filtered back projection, contrast

INTRODUCTION

Single photon emission computed tomography (SPECT) is a nuclear medicine tomographic imaging technique using gamma photons. In tomography, the camera rotates around the patient and a large number of images are taken at different angular projections (1). An issue in imaging is caused by the attenuation of photons, the resulting artefacts and inhomogeneity, and thus a deterioration in the quality of SPECT. To improve image quality, it is important that SPECT images are corrected for attenuation. There are different ways to correct attenuation. In some cases, a SPECT gamma scanner may be built to operate with a CT scanner (SPECT/CT). The function of CT is to ensure the improved localisation and definition of organs. In addition to anatomical data, CT images also serve to correct the attenuation of emission data (1-3). It is necessary to be aware that CT also causes additional radiation exposure to the patient. It is therefore necessary to carefully plan and optimise the SPECT/CT imaging protocols (1).

Attenuation has a significant impact on data and thus image quality. Photon attenuation means a decrease in the number of events from the body. It is a loss of photons due to interactions between photons and electrons. The energy of a photon is converted into the energy of an electron during absorption. Attenuation on reconstructed images causes artefacts and inhomogeneity, resulting in false positive results or negative results. It is therefore important that SPECT images are corrected for attenuation (1-3). In SPECT, attenuation depends on photon energy, tissue composition and density (1, 4).

Research Article

Received 18-01-2020

Accepted 03-02-2021

Available Online: 08-02-2021

Published 24-02-2021

http://dx.doi.org/10.36472/msd.v8i2.470

Due to the lungs, there is less attenuation in the chest than in the abdomen. Bones have slightly greater attenuation than soft tissues. Due to attenuation effects, there is a minimum of accumulation of activity in the centre of the image. The result of greater attenuation from inside the body is reduced intensity in tomograms for these areas (**4**).

Non-diagnostic, low-dose CT (10-40 mA) is used to correct attenuation, making images of appropriate quality for their purpose (**1**). CT imaging follows immediately after SPECT acquisition (**5**). With CT imaging, we obtain transmission maps that are used to correct attenuation on SPECT data. The efficiency of AC depends on the quality of the transmission folders. In reconstructed images, artefacts resulting from inconsistencies in CT data and emission data are common. Artefacts are most often seen in areas where there is a great deal of movement (movement due to respiration) and where there are major changes in attenuation coefficients (**6, 7**). Poorer image quality is therefore caused by metal implants and patient movement during CT acquisition. In addition to movement and respiration, the cause of discrepancy between emission and transmission imaging the table which is bent when it is driven into the gantry (**8**). CT imaging takes much less time than SPECT, resulting into a time mismatch. This poses a problem, especially when imaging the chest because the heart and lungs are moving organs. This temporal mismatch can lead to unwanted artefacts on attenuation-corrected images, which can lead to the misinterpretation of results (**3**). To exclude artefacts due to AC, it is important to always check both corrected and uncorrected images (**1, 3**). The anatomical accuracy of image fusion should be checked before interpreting corrected scintigrams (**5**).

The effect of attenuation correction on image quality in SPECT/CT have already been studied by Yong-Soon et al. who evaluated phantom scans (**9**), Sung et al. who explored the effect on different phantoms (**10**) and Schulz et al. who also performed a patient study (**11**) and various studies evaluating the image quality in myocardial perfusion scintigraphy (**12-14**).

The purpose of our study was to systematically perform SPECT/CT imaging of NEMA body phantom with eight different background/sphere activity concentration ratios, which has not been done by previous authors, and to determine the effect of attenuation correction on image contrast. With our research we aimed to confirm that the use of CT-AC improves the visualization of smaller spheres at different background/sphere ratios.

MATERIAL AND METHODS

We used an experimental method, phantom imaging and research with image processing on SPECT/CT. Imaging was performed on a Siemens Symbia T2 gamma camera and included dual-slice spiral computed tomography. We used NEMA IEC Body Phantom (NEMA 2012/IEC 2008) for imaging, which contains six spheres of different sizes. The phantom was filled with the isotope technetium-99m (99mTc). Phantom imaging was performed eight times, each with a different ratio of specific activity between the spheres and phantom background. The phantom background was filled each time with approximately 100 MBq of 99mTc. The ratio in specific activity between the background of the phantom and the spheres in the phantom was thus 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8 and 1:9 (Table 1). Imaging was performed immediately after the phantom was filled. The imaging protocol is shown in the Table 2. The images were processed using an Oasis hybrid reconstruction application. The quality of the images was then compared. Each image was reconstructed using FBP algorithm (Butterworth reconstruction filter, order 4 and cutoff 0.75) IR algorithm (4 iterations and 10 subsets) IR algorithm with CT-AC (4 iterations and 10 subsets). After reconstruction we marked regions of interest (ROI) around the spheres and in the background and measured the average number of counts as demonstrated in Figure 1. In each reconstructed image, we marked all six spheres with diameters of 10 mm, 12 mm, 16 mm, 22 mm, 28 mm and 36 mm, and the background in six different places. A circle diameter of 20 mm was used to measure number background counts. Contrast (C) was calculated as a relative difference between foreground and background by using the equation:

$$C = \frac{A - B}{B}$$

where C represents the calculated contrast, A represents the average number of counts in the spheres of the NEMA phantom and B represents the average number of counts in the selected background region of the NEMA phantom. To determine the difference in average number of counts in spheres and the contrast in all three reconstruction methods we performed repeated measures ANOVA. We tested the pair difference between image contrast in the Matlab program. Because the data were not normally distributed, we performed the Wilcoxon signed rank test. The data were statistically processed in Statistical Package for the Social Sciences (SPSS) program, version 22. A significance of $p < 0.05$ was used for all the tests.

Table 1: Activities of 99mTc expressed in MBq/L in spheres and background

Ratio	1:2	1:3	1:4	1:5	1:6	1:7	1:8	1:9
Background (MBq/L)	10,38	10,27	10,41	10,45	10,03	10,25	10,07	9,91
Spheres (MBq/L)	20,42	31,72	43,06	54,57	62,41	73,93	81,2	91,10

Table 2: The imaging protocol on SPECT/CT.

Number of Views	32
Time per view	20 sec
Zoom	1
Matrix size	128 X 128
Starting angle	0
Degrees of Rotation	180
Rotation Direction	CW
Detectors	Both Detectors
Detectors Configuration	180
Mode	Step and shoot
mAs	25
kV	130

http://dx.doi.org/10.36472/msd.v8i2.470

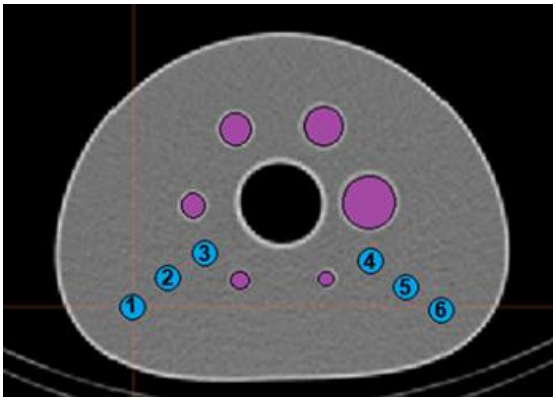

Figure 1: The CT image shown the areas where we marked the ROI to determine the number of average counts in spheres (red) and background (blue) on SPECT/CT.

RESULTS

Figure 2 shows the average number of counts in spheres in all three reconstruction methods. The average number of counts in spheres increases as we increase the activity concentration ratio between the background and spheres.

With repeated measures ANOVA the significant differences in the average number of counts ($p < 0.001$) between FBP reconstruction, IR and IR CT-AC reconstruction were confirmed.

Contrast of FBP, IR and IR CT-AC reconstructions in all six phantom spheres for all radioactivity concentration ratios are given in Figure 3.

When comparing the contrast between IR CT-AC, IR and FBP reconstructed images (Figure 4), we found that larger spheres (> 12 mm) were well visible in all reconstructions but the difference was observed in smaller spheres and in a low activity-to-sphere ratios. The smallest 10 mm sphere can be seen in IR CT-AC reconstructed images at the activity concentration ratio of 1:9, but is not visible in IR or FBP reconstructed images.

The 12 mm sphere is seen in IR CT-AC images at the ratios of $\geqslant$ 1:6, in IR images at ratio $\geqslant$ 1:7 and in FBP images at ratios of $\geqslant$ 1:8.

An analysis of the contrast between the phantom spheres and background with repeated measures ANOVA showed a statistically significant difference between different reconstruction methods ($p < 0.001$). We also compared two reconstructions and determined the significance of the difference using the Wilcoxon matched-pairs signed rank test. A statistical analysis between IR CT-AC vs FBP, IR CT-AC vs IR and IR vs FBP reconstruction showed $p < 0.001$, which means that the contrast and thus image quality are significantly different between the two reconstructions. Figure 5 shows the contrast for all reconstructions and the subtraction of images. The figures (A, B and C) represent the contrast of all the scans, with eight different ratios, as well as the contrasting of the six spheres. Black colour represents a negative contrast and a contrast of less than 10%, which means that such lesions cannot be seen. Lighter colour represents a positive contrast; the lighter or whiter the field, the better is contrast. White represents the contrast, higher than 65%.

The last figure (D) is the contrast subtraction of two different reconstructions, where the dark fields mean there is not much difference between the two reconstructions; the brighter the fields, the greater the difference in contrast.

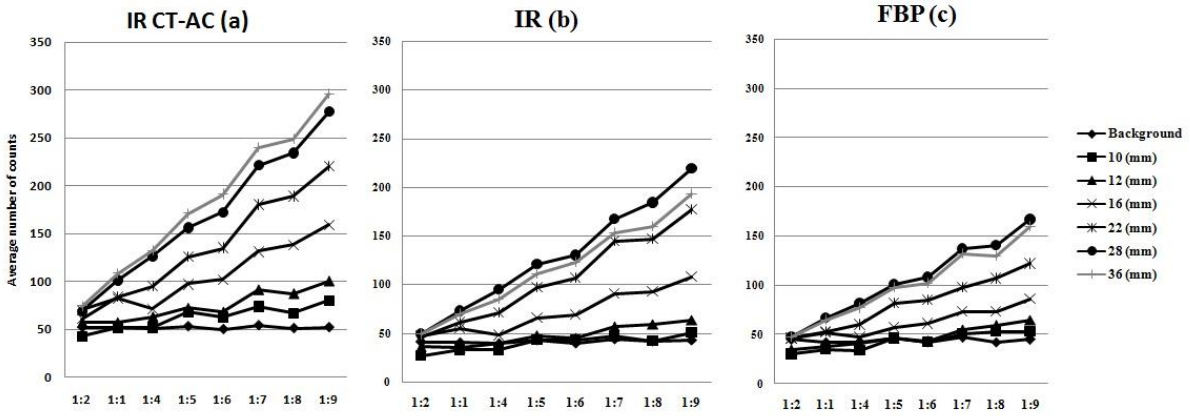

Figure 2: The effect of different reconstruction algorithms with and without AC on number of average counts in background and different spheres for eight radioactivity concentration ratios.

http://dx.doi.org/10.36472/msd.v8i2.470

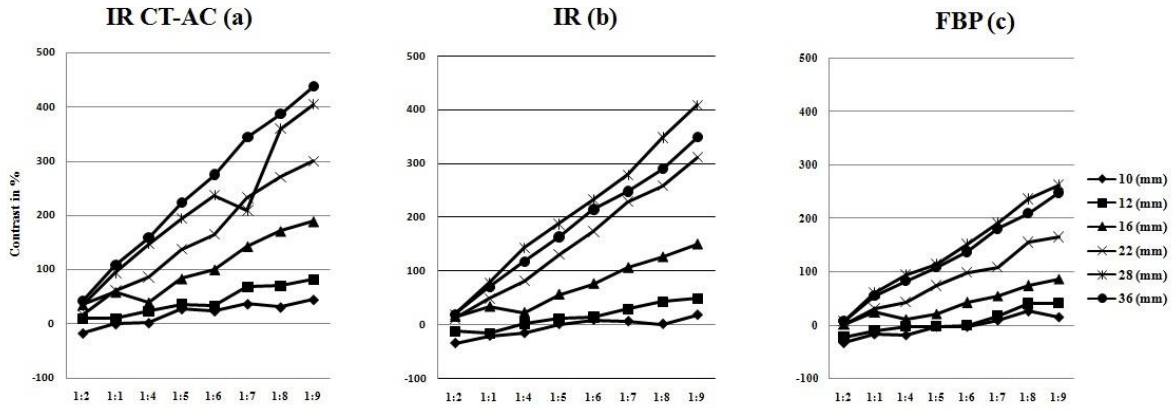

Figure 3: Measurements of contrast expressed in % in all six NEMA phantom spheres for all radioactivity ratios reconstructed with iterative reconstruction corrected with CT-AC (a), iterative reconstruction (b), FBP (c).

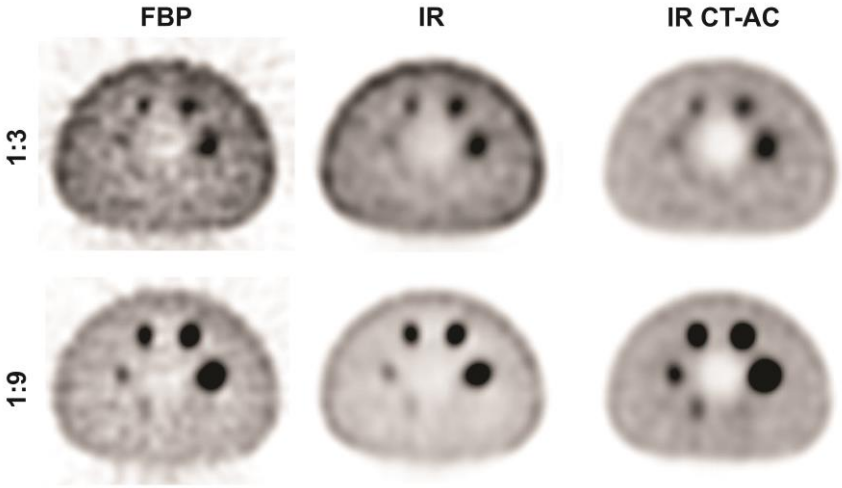

Figure 4: NEMA body phantom, filled in the radioactivity concentration ratio background/sphere1:3 (top row), 1:9 (bottom row) and reconstructed FBP, IR and IR CT-AC.

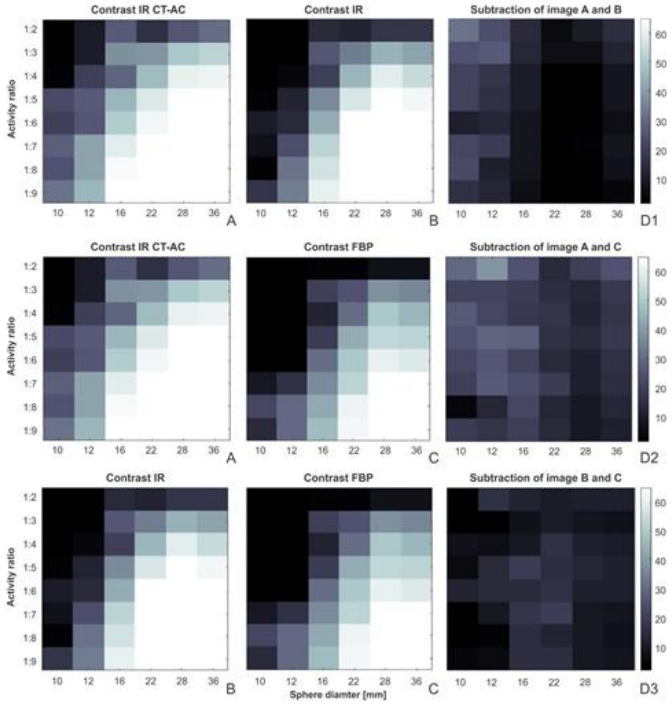

Figure 5: Contrast of iterative reconstruction with CT-AC (A) ,iterative reconstruction (B) and FBP (C). The subtraction of figure A vs B is figure D1, A vs C is figure D2 and B vs C is D3. Black colour on figure A, B and C represents a negative contrast and a contrast of less than 10%, which means that such lesions cannot be seen. Lighter colour represents a positive contrast. White colour represents the contrast, higher than 65%. The figure C represent the contrast subtraction of two different reconstructions, where the dark fields mean that there is not much difference between the two reconstructions; the brighter the fields, the greater the difference in contras

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DISCUSSION

The ratio of activity concentration between the background and sphere has no effect on the average number of counts in the background because we always filled the background with comparable activity. In FBP and IR due to the influence of attenuation, the average number of counts on the outside of the phantom is greater and falls towards the middle of the phantom. Due to attenuation, the background is inhomogeneous. CT-AC corrects the effect of attenuation, making the average number of counts more even over the entire background of the phantom and the line almost straight.

The size of the sphere and background-sphere activity concentration ratio affect the average number of counts and thus the contrast of the image. We showed that the average number of counts and thus the contrast of the image increases with the size of the sphere and with a larger ratio of activity between the background and the sphere in all three methods of reconstruction. A larger sphere means a larger average number of counts, i.e. better contrast and image quality . The SPECT/CT limitation is the relatively poor spatial resolution of the detector system, which makes it impossible to visualize small spheres ($\leqslant$ 12 mm) with FBP and IR at lower ratios ($\leqslant$ 1:6). With IR CT-AC, the visualization of the spheres is improved, but for small spheres at lower ratios it is not displayed ($\leqslant$ 1:7).

The reason for the negative contrast in smaller spheres is higher number of average counts in background obtained from ROIs placed over the entire diameter of the NEMA body phantom. Due to attenuation, the number of average counts in FBP and IR reconstructions was higher than the number of average counts in spheres, especially in spheres $\leqslant$ 12 mm and in the ratio $\leqslant$ 1:5.

When comparing the contrast between IR CT-AC and FBP reconstructed images, we found that the largest spheres were well visible in both reconstructions. The largest difference was seen in smaller spheres and in a low activity-to-sphere ratio. In clinical practice, this means that minor lesions on FBP reconstructed images can be overlooked. There is also a major difference in the contrast of the images between the two reconstructions at a background-sphere ratio of 1:2.

When comparing the contrast between IR CT-AC reconstructed images and IR, we found that the most significant difference in contrast is at small spheres. With large spheres and with a high background-sphere ratio, there is not much difference in the contrast of the image between the two reconstructions, which means that we will see the spheres well in both images.

FBP and IR have a similar contrast in small lesions at a low ratio and in large lesions at a high ratio. We will not see the smallest sphere on any reconstruction, while we will see large spheres on both. A difference occurs in the central part, in medium-sized spheres. The contrast of the image is better in IR.

Based on all the results, we can conclude that the images using IR CT-AC reconstruction have better image quality than uncorrected images. Several studies have been conducted where similar results have been obtained. One such study was conducted by Yong-Soon et al. who came to the same conclusions (**9**). They concluded that image quality was improved using CT-AC reconstruction and that CT dose had no significant effect on image quality. It is thus not necessary for the CT dose to be higher if the CT serves us only for localisation and AC. When CT is needed for diagnostic purposes, the dose may be increased.

Sung et al. came to similar conclusions in their study (**10**). They compared the contrast before and after the use of CT-AC on the NEMA IEC Body PhantomTM and the Jaszczak phantom, and the spatial resolution using the NEMA SPECT Triple Line Source PhantomTM. Contrast was improved by using IR CT-AC on both phantoms, as well as spatial resolution. They concluded that SPECT/CT provides significantly better image quality than SPECT, while contrast and spatial resolution were improved.

Schulz et al. found that CT-AC corrects distribution inhomogeneity due to attenuation, but that inconsistencies between SPECT and CT images can lead to erroneous results. It is thus very important to check the accuracy of image fusion in each patient (**11**). They conclude that further research would be needed to fully investigate the impact of CT-AC.

Most researchers have observed the effect of attenuation correction using CT on myocardial perfusion scintigraphy and all have come to similar conclusions.

Malkerneker et al. described that CT-AC in myocardial perfusion scintigraphy improves image quality and increases diagnostic accuracy (**12**). Pazhenkottil et al. found that CT-AC adds prognostic value and provides higher left ventricular imaging homogeneity in healthy subjects and increases diagnostic accuracy (**13**). The results of this study showed that correction of attenuation successfully reduces the number of false-positive results, especially with CT.

Research conducted by Fricke et al. showed that myocardial perfusion scintigraphy using CT-AC provides more accurate images than an examination without the use of correction (**14**).

According to some data, attenuation-uncorrected images are also of better quality when using IR compared to FBP reconstruction (**15**). Our results are comparable; the image quality was improved IR relative to FBP reconstruction. Also, a study conducted by Narayanan et al. confirms that IR provides better efficacy for localising perfusion defects and detecting coronary artery disease (CAD) than FBP reconstruction (**16**). It is best to use IR in combination with AC for the best CAD detection efficiency.

In perfusion myocardial scintigraphy, an incorrect result can lead to an invasive examination (coronary angiography), which is unacceptable. Thus, the correction of attenuation is extremely important and its use is recommended by the American Society of Nuclear Cardiology and the Society of Nuclear Medicine (**17**).

CONCLUSION

Based on our results, the use of iterative reconstruction with CT-AC improves the contrast and image quality relative to iterative reconstruction and FBP. In clinical practice, tumours and various lesions are of all possible shapes and sizes. Thus,

the use of CT-AC reconstruction is recommended for all examinations. Because the use of CT to correct attenuation may increase the radiation exposure of patients, imaging protocols should be carefully designed.

Author contributions: NF, LL, JZ, SR; Literature search and study design, experimental aplications **SR;** Writing article and revisions

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. This research did not receive and specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical issues: All authors declare originality of research.

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Medical Science and Discovery
ISSN: 2148-6832

The relationship between serum Vitamin D level and type 2 diabetes mellitus

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ABSTRACT

Objective: Vitamin D (VD) could play a role in pathogenesis of Type 2 Diabetes Mellitus (T2DM) by affecting either insulin sensitivity or pancreatic β-cell function. This article is about the relationship between T2DM and VD levels.

Material and Methods: The 4678 individuals were included in the study. Of these, 1764 were T2DM patients and 2914 were healthy individuals. Correlation analysis was carried out between VD, age, Body Mass Index (BMI), Hemoglobin A1c (HbA1c), and duration of illness in the T2DM patients. Logistic regression analysis was used to determine the independent predictors.

Results: VD levels were significantly lower in the T2DM patients compared to the control group. The VD level of T2DM patients with HbA1c >7% was lower than those with HbA1c <7%. The VD level of T2DM patients using insulin was found to be significantly lower compared to those not using insulin. Among the T2DM patients, VD level was found to be the highest in those without complications and the lowest in those with nephropathy. The cut-off value for VD was calculated as 16.95 ng/mL. According to the logistic regression test, low serum VD levels were found to be an independent risk factor for the development of T2DM and its complications.

Conclusion: VD deficiency may be a risk factor for the development of T2DM. In our study, VD levels were significantly lower in the T2DM patients and those having complications of T2DM than the healthy individuals.

Keywords: Vitamin D, Type 2 Diabetes Mellitus, Nephropathy

INTRODUCTION

The role of vitamin D (VD) in maintaining body health is well known (1). VD level is evaluated by serum 25(OH)D levels. 25(OH)D indicates VD uptake and endogenous production. VD levels less than 20 ng/mL refer to a VD deficiency, those between 21 and 29 ng/mL to a VD insufficiency, those higher than 30 ng/mL to a normal VD level, and those higher than 150 ng/mL to a VD intoxication (2).

There are many risk factors for VD insufficiency or deficiency, including lack of sun exposure, inadequate dietary intake, darker skin color, age, obesity, and use of various medications (3). Low VD levels increase the risk for rickets and fractures and are also associated with hypertension, cancer, cardiovascular disease, Type 2 Diabetes Mellitus (T2DM), and chronic kidney disease (4).

In recent years, some studies have been carried out on the role of VD in the development of T2DM. VD could play a role in the pathogenesis of T2DM by affecting either insulin sensitivity or β-cell function, or both. 25(OH)D concentration has a positive relationship with insulin sensitivity. Low VD levels in elderly men were reported to be associated with glucose intolerance (5).

Observational studies and clinical trials show evidence that normal VD levels reduce the risk for T2DM. VD deficiency is related to insulin secretion, insulin resistance, and β-cell dysfunction in the pancreas (6). VD receptors (VDR) in pancreatic β-cells play an important role in the progression of T2DM (7).

Research Article

Received 21-01-2020

Accepted 06-02-2021

Available Online: 08-02-2021

Published 24-02-2021

http://dx.doi.org/10.36472/msd.v8i2.472

1alpha,25 Dihydroxyvitamin D3 (1,25(OH)2D3) binds to specific VDR and regulates hormone secretions, cell proliferation and differentiation. Detection of VDR in the pancreas and adipose tissue, skeletal muscles, and immune cells were reported to imply the antidiabetic role of VD by enhancing insulin synthesis and exocytosis, increasing the expression of the insulin receptor, and modulating immune cells function (**8**). VD can decrease the effects of systemic inflammation and protect against β-cell cytokine-induced apoptosis by directly modulating the expression and activity of cytokines, as shown in animal models (**9**). VD deficiency can affect the development of T2DM complications, insulin secretion, insulin sensitivity, inflammation, immunosuppression, microvascular and macrovascular events, and angiogenesis in several ways (**10**). Hyperglycemia is the main risk factor for diabetic microvascular complications, retinopathy, neuropathy, and nephropathy (**11**). In this study, we aimed to examine the relationship between VD levels and the development of T2DM and its complications.

MATERIAL AND METHODS

Participants

4678 people were included in the study. Of these individuals, 1764 were T2DM patients and 2914 were healthy individuals. The individuals were grouped according to age, gender, Body Mass Index (BMI), Hemoglobin A1c (HbA1c), T2DM complications, and diabetes drugs. VD levels were compared between these groups.

Socio-demographic and clinic data were obtained retrospectively from the hospital automation system. 30 ng/mL was accepted as the lower limit for normal VD.

The VD values were evaluated according to age, gender, BMI, HbA1c, T2DM complications, and diabetes drugs in the control group and the T2DM patients.

The T2DM patients and healthy individuals over 18 years old and those with VD test results were included in the study. Those with active infection or pregnancy, and those having a VD replacement therapy were excluded from the study (Figure 1).

Statistical analysis: Statistical analysis of the data was carried out using IBM SPSS (v.22.0). Chi-square test, one-sample t test, independent sample t-test, and ANOVA test were used to make comparison for parametric data, and Mann-Whitney U test for non-parametric data.

Correlation analysis was carried out between VD, age, BMI, HbA1c, and duration of illness in the T2DM patients.

Logistic regression analysis was carried out to determine the independent predictive factors for T2DM development. Statistical significance was set at $p< 0.05$.

Ethics committee approval: Approval was obtained for this study from the local clinical studies ethics committee (Decision Number: 2020/254).

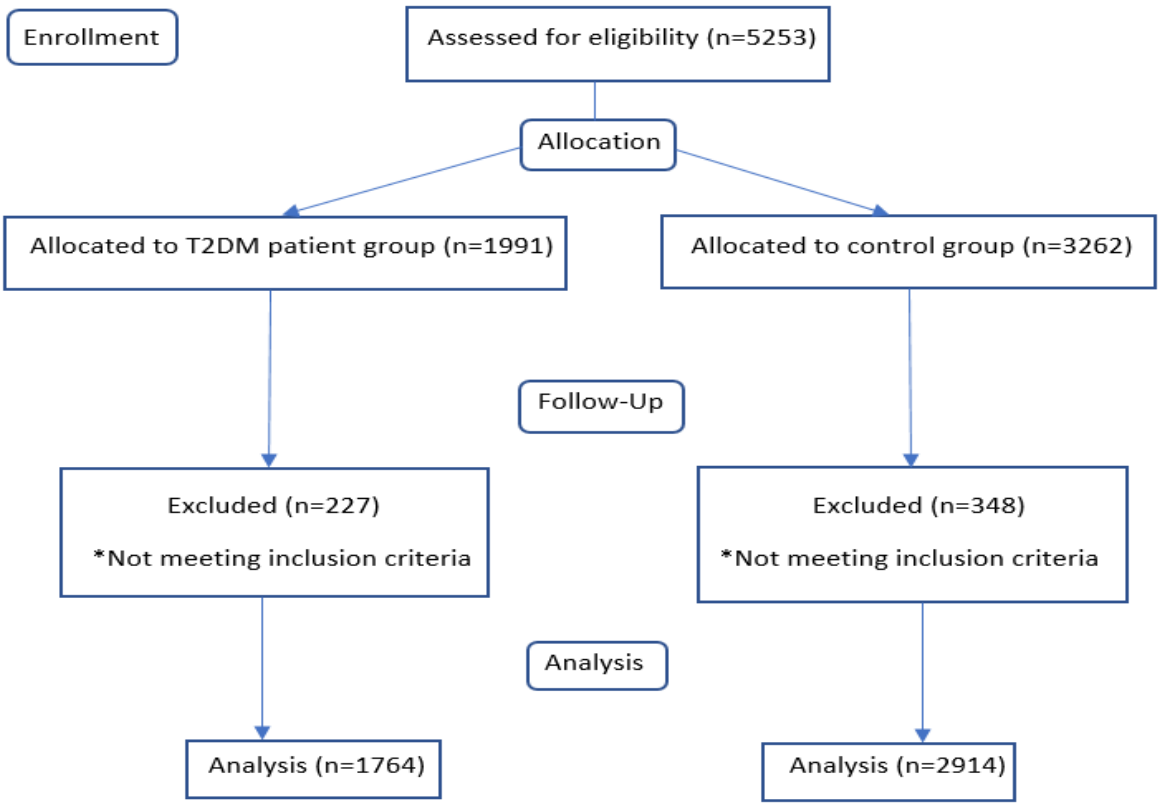

Figure 1. Flow diagram of the study. For our study, the data of 5253 individuals were retrospectively scanned. 1991 of these individuals were from the T2DM patient group and 3262 from the control group. 227 individuals from the T2DM group and 348 from the control group were excluded from the study because they did not meet the inclusion criteria. As a result, data of 1764 T2DM patients and 2914 control groups were analyzed.

http://dx.doi.org/10.36472/msd.v8i2.472

RESULTS

Of the 4678 individuals who participated in the study, 1764 (37.7 %) were T2DM patients and 2914 (62.3 %) were healthy individuals. The mean age (p=0.638) and gender characteristics (p=0.445) of the control group and diabetes mellitus patients were similar. In addition, there was no statistically significant difference between the control group and the group of T2DM patients in terms of profession, education, place of residence, smoking, and alcohol use (p > 0.05). The T2DM patients' mean BMI (28.7±6.5 kg/m2) was higher than the control group (p< 0.001). In the T2DM patients, the mean duration of illness was 7.2±9.4 years, and the mean HbA1c level was 8.3±1.7 % (Table 1). In our study, the mean serum VD level was found to be below the normal limit in both groups (< 30 ng/mL). The mean serum VD level in patients with T2DM was significantly lower than the control group (14.2±11.2 ng/mL versus 21.7±10.1 ng/mL, p< 0.001).

The T2DM patients over the age of 65 had a significantly lower mean serum VD level than the control group (12.1±10.9 ng/mL vs 20.4±10.2 ng/mL, p< 0.001). The mean serum VD level of the T2DM patients under 65 years of age was significantly lower than the control group (14.6±11.3 ng/mL vs 22.4±9.9 ng/mL, p< 0.001). The T2DM patients older than 65 years had lower VD levels than those younger than 65 years (14.6±11.3 ng/mL vs 12.1±10.9 ng/mL, p< 0.001). In the control group, those over 65 years of age had lower VD levels than those under 65 years of age (p< 0.001). The mean serum VD level in the female patients with T2DM was significantly lower than in the females in the control group (13.9±11.9 ng/mL vs 21.4±10.2 ng/mL, (p< 0.001). The mean serum VD level in the male patients with T2DM was significantly lower than in the males in the control group (14.6±10.1 ng/mL vs 21.9±9.8 ng/mL, p< 0.001). The T2DM patients' VD levels were found to decrease with increasing BMI (p< 0.001) (Table 2).

Table 1. Sociodemographic and clinical characteristics of all patients

Variables	Total (n = 4678)	T2DM patients (n = 1764)	Control group (n = 2914)	p
Gender, n (%)				
Female	2818 (60.2)	1055 (59.8)	1763 (60.5)	0.638*
Male	1860 (39.8)	709 (40.2)	1151 (39.5)	
Age (years), n (%), mean ± SD	60.0 ± 18.3	60.3 ± 13.0	59.9 ± 20.9	
< 65	2704 (57.8)	1066 (60.4)	1638 (56.2)	0.445**
> 65	1974 (42.2)	698 (39.6)	1276 (43.8)	0.075*
BMI (kg / m^2), n (%), mean ± SD	27.3 ± 8.5	28.7 ± 6.5	26.3 ± 6.8	
< 25	1672 (35.8)	558 (31.6)	1114 (38.2)	0.000**
25-30	1661 (35.5)	617 (35.1)	1044 (35.8)	0.000*
>30	1345 (28.7)	589 (33.3)	756 (26.0)	
Occupation, n (%)				
Not working	2412 (51.5)	899 (50.9)	1513 (51.9)	0.415*
Working	2266 (48.5)	865 (49.1)	1401 (48.1)	
Education, n (%)				
Primary school	2447 (52.3)	915 (51.8)	1562 (53.6)	0.093*
High school	2231 (47.7)	849 (48.2)	1352 (46.4)	
Residence, n (%)				
Town	1770 (37.8)	712 (40.3)	1058 (36.3)	0.069*
City center	2908 (62.2)	1052 (59.7)	1856 (63.7)	
Smoking, n (%)				
Yes	1836 (39.2)	698 (39.5)	1138 (39.0)	0.723*
No	2842 (60.8)	1066 (60.5)	1776 (61.0)	
Alcohol Use, n (%)				
Yes	253 (5.5)	91 (5.4)	162 (5.6)	0.663*
No	4425 (94.5)	1673 (94.6)	1752 (94.4)	
Duration of T2DM (years), mean ± SD	-	7.2 ± 9.4	-	-
HbA1c (%), mean ± SD	-	8.3 ± 1.7	-	-

p*, Chi square test; p**, Independent samples test; n, number; SD, Standard deviation; T2DM, Type 2 Diabetes Mellitus; VD, Vitamin D; BMI, Body Mass Index; HbA1c, Hemoglobin A1c.

Table 2. The comparisons of VD levels of all patients

Variable	VD (ng / mL) mean ± SD			
	Total (n = 4678)	T2DM patients (n = 1764)	Control group (n = 2914)	p
Gender				
Female	18.8 ± 10.5	13.9 ± 11.9	21.4 ± 10.2	0.000
Male	18.9 ± 11.5	14.6 ± 10.1	21.9 ± 9.8	0.000
p	0.816	0.184	0.202	-
Age (years)				
< 65	19.0 ± 11.2	14.6 ± 11.3	22.4 ± 9.9	0.000
> 65	16.9 ± 10.8	12.1 ± 10.9	20.4 ± 10.2	0.000
p	0.000	0.000	0.000	-
BMI (kg / m^2)				0.000
< 25	19.0 ± 11.2	15.6 ± 9.4	22.6 ± 10.1	
25-30	18. 6 ± 9.9	12.3 ± 7.9	21.8 ± 8.4	
> 30	16.9 ± 10.8	9.3 ± 8.8	20.1 ± 9.3	
p	0.057	0.038	0.065	-
Total	18.9 ± 11.1	14.2 ± 11.2	21.7 ± 10.1	0.000

p value, Independent samples test; n, number; SD, Standard deviation; T2DM, Type 2 Diabetes Mellitus; VD, Vitamin D; BMI, Body Mass Index.

http://dx.doi.org/10.36472/msd.v8i2.472

It was found that 55.9 % of the T2DM patients had an HbA1c level of >7 %. The T2DM patients with HbA1c >7 % had lower VD levels than those with HbA1c< 7 % (10.5±6.8 ng/mL vs. 14.1±7.2 ng/mL, p< 0.001). 1372 (77.7 %) of the T2DM patients had no complications whereas 392 (22.3 %) of them had complications. Nephropathy was present in 57 (3.2 %) of the T2DM patients, peripheral vascular disease in 72 (4.0 %), polyneuropathy in 154 (8.7 %), and retinopathy in 109 (6.1 %).

When the T2DM patients were grouped according to their complications, the mean VD levels of all groups were found to be statistically significantly lower than that of the control group (DM patients without complications, 14.7±11.6 ng/mL; nephropathy, 9.7±6.9 ng/mL; peripheral vascular diseases, 12.1±8.1 ng/mL; polyneuropathy, 14.3±10.7 ng/mL; retinopathy, 10.2±9.5 ng/mL) (p< 0.001, all).

In the T2DM patients, the VD levels were found to be the highest in those without complications and the lowest in those with nephropathy. 5.3 % of the T2DM patients were using insulin and 41.7 % were using Oral Anti Diabetics (OAD) + insulin. The most common diabetes drug they used was OAD (53.0 %).

The mean serum VD level (8.4±6.7 ng/mL) of the T2DM patients using insulin was significantly lower than that of the other T2DM patients (p< 0.001) (Table 3). According to the correlation analysis results between VD, age, BMI, HbA1c, and duration of illness in T2DM patients, a statistically significant negative correlation was observed between VD and age (r: -0.008, 95 % Confidence Interval (CI): -0.026, 0.013, p=0.041), BMI (r: -0.010, 95 % CI: -0.038, 0.042, p=0.039), HbA1c (r: -0.012, 95 % CI: -0.029, -0.005, p=0.021), and duration of illness (r: -0.004, 95 % CI: -0.015, 0.033, p=0.047). There was a positive correlation between HbA1c and BMI (r: 0.809, 95 % CI: 0.689, 0.779, p< 0.001), age (r: 0.257, 95 % CI: 0.028, 0.499, p=0.029), and disease duration (r: 0.321, 95 % CI: 0.034, 0.150, p=0.026) (Table 4).

According to the logistic regression test, low serum VD levels were found to be an independent risk factor for the development of T2DM (Odds Ratio (OR): 1.085, 95 % Confidence Interval (CI): 1.078, 1.093, p< 0.001) and its complications (OR: 1.027, 95 % CI: 1.014, 1.040, p< 0.001). It was seen that every 1-unit decrease from the VD value of 16.95 ng/mL increased the risk for T2DM (OR: 1.079, 95 % CI: 1.071, 1.086, p< 0.001) (Table 5).

Table 3. The comparisons of VD levels of T2DM patients

Variables of T2DM patients	N (%)	VD (ng / mL) mean ± SD	p
HbA1c (%)			
< 7	778 (44.1)	14.1 ± 7.2	0.000
> 7	986 (55.9)	10.5 ± 6.8	
Complications of T2DM, n (%)			
Nephropathy	57 (3.2)	9.7 ± 6.9	
Retinopathy	109 (6.1)	10.2 ± 9.5	
Peripheral vessel disease	72 (4.0)	12.1 ± 8.1	0.000
Polyneuropathy	154 (8.7)	14.3 ± 10.7	
No complication	1372 (77.7)	14.7 ± 11.6	
Diabetes drugs, n (%)			
Insulin	94 (5.3)	8.4 ± 6.7	0.000
OAD + Insulin	735 (41.7)	13.1 ± 9.8	
OAD	935 (53.0)	15.0 ± 11.5	

p value, Independent samples test; n, number; SD, Standard deviation; T2DM, Type 2 Diabetes Mellitus; VD, Vitamin D; HbA1c, Hemoglobin A1c; OAD, Oral Anti Diabetics.

Table 4. Correlation analysis between VD, age, BMI, HbA1c and duration of illness in T2DM patients

Variables		VD	Age	BMI	HbA1c	Duration of illness
VD	**r**	-	-0.082	-0.110	-0.102	-0.044
	95 % CI	-	-0.261, 0.132	-0.384, 0.423	-0.295, -0.056	-0.153, 0.334
	p	-	0.041	0.039	0.021	0.047
Age	**r**	-0.082	-	0.181	0.257	0.874
	95 % CI	-0.261, 0.132	-	-0.100, 0.513	0.028, 0.499	0.880, 0.976
	p	0.041	-	0.166	0.029	0.000
BMI	**r**	-0.110	0.181	-	0.809	0.129
	95 % CI	-0.384, 0.423	-0.100, 0.513	-	0.689, 0.779	-0.131, 0.391
	p	0.039	0.166	-	0.000	0.281
HbA1c	**r**	-0.102	0.257	0.809	-	0.321
	95 % CI	-0.295, -0.056	0.028, 0.499	0.689, 0.779	-	0.034, 0.150
	p	0.021	0.029	0.000	-	0.026
Duration of illness	**r**	-0.044	0.874	0.129	0.321	-
	95 % CI	-0.153, 0.334	0.880, 0.976	-0.131, 0.391	0.034, 0.150	-
	p	0.047	0.000	0.281	0.026	-

p value, Pearson Partial Correlation Test; r, Correlation Coefficient; CI, Confidence Interval; VD, Vitamin D; BMI, Body Mass Index; HbA1c, Hemoglobin A1c

http://dx.doi.org/10.36472/msd.v8i2.472

Table 5. Logistic regression analysis for the independent predictive factors of T2DM

Variables of T2DM patients	OR	95 % CI	*p*
VD			
< 16.95	1.079	1.071, 1.086	0.000

DISCUSSION

In our study, the VD level was found to be below the threshold level of 30 ng/mL in all groups. The mean serum VD level was significantly lower in the T2DM patients compared to the control group. A study reported that 25(OH)D concentration was lower in patients with T2DM than in the nondiabetic control individuals (**12**). A meta-analysis, including twenty observational studies involving 16515 individuals, revealed that maternal VD deficiency was associated with an increased risk for gestational diabetes (**13**).

In our study, 77.7 % of the T2DM patients had no complications. Nephropathy was observed in 3.2 % of them, peripheral vascular disease in 4 %, polyneuropathy in 8.7 %, and retinopathy in 6.1 %. In a study involving 842 diabetic patients, VD deficiency was found to be associated with severe diabetic retinopathy (**10**). Previous studies indicate that VD deficiency is associated with a significantly increased risk for diabetic retinopathy in T2DM patients (**8**). A study carried out on 1633 diabetic patients reported that the prevalence of peripheral neuropathy was 9.5 %, and VD deficiency was a risk factor for diabetic retinopathy (**11**).

A meta-analysis of data on the relationship between VD deficiency and the development of diabetes-induced neuropathy also revealed a strong correlation. Recovery of insulin secretion, increasing insulin sensitivity of target tissues, and reducing the inflammatory response have been proposed as potential mechanisms for improving the clinical manifestations of diabetic neuropathy following VD supplementation (**8**). In a study, 600,000 IU VD replacement provided a significant reduction in symptoms in patients with painful diabetic neuropathy (**14**). In the literature, various lower extremity complications (diabetic foot ulcer and peripheral arterial disease) have been reported to be associated with low serum VD in T2DM patients (**15**).

Our study found that the mean serum VD level was significantly lower in the T2DM patients using insulin than those not using insulin. In their study involving 632 T2DM patients, Suzuki et al. showed that the patients with low VD levels had higher HbA1c levels, and those using insulin had lower VD levels than those using OAD or receiving diet therapy (**16**). It can be asserted that, in T2DM patients, the nephropathy which develops due to using insulin decreases endogenous VD synthesis. We observed that the VD level was the highest in the T2DM patients without complications, while it was the lowest in those with nephropathy. VD deficiency is common in patients with diabetic nephropathy, and its severity increases with the progression of diabetic nephropathy. The degree of kidney dysfunction may affect serum 25(OH)D levels, and studies have reported that low serum 25(OH)D levels occur in patients with chronic kidney disease (**17**).

It was reported in the literature that high VD intake was associated with lower incidence of diabetes nephropathy in patients with T2DM (**18**). Supplementation with VD or its active derivatives has been shown to improve endothelial cell damage, reduce proteinuria, alleviate kidney fibrosis, and consequently delay the progression of diabetic nephropathy (**19**).

Obesity, a metabolic disorder that frequently accompanies T2DM, is an important risk factor for the development of diabetes. Although T2DM does not develop in all obese patients, the majority of T2DM patients are obese (**20**). In our study, it was observed that T2DM patients' VD levels decreased as their BMIs increased ($p< 0.001$).

HbA1c is a routine marker showing the average 3-month glycemic level. It also predicts the risk for developing diabetic complications. A one-unit increase in HbA1c increases the risk for developing cardiovascular disease by about 18 % (**21**). In the study of Özdoğan et al. a direct proportion was found between HbA1c levels and age of diabetes (**20**). In our study, a positive correlation was found between HbA1c and BMI, age, and disease duration. Targher et al. found that, in patients with T2DM, HbA1c levels were high in those with 25(OH)D deficiency. This was explained by the effect of VD on the recovery of beta-cell function in T2DM (**22**). Studies have shown that insulin sensitivity can improve by as much as 60 % when the level of VD increase from 25 to 75 nmol/L (**23**). Tekin et al. found that the lower the VD level, the higher the HbA1c value in female patients with T2DM (**24**). Yıldırım et al. found that VD levels were significantly lower in those with HbA1c > 7 % than in those with HbA1c< 7 ($p< 0.001$) (**25**). In our study, the VD level of the T2DM patients with HbA1c > 7 % was lower than that of those with HbA1c< 7% (10.5±6.8 ng/mL vs 14.1±7.2 ng/mL, $p< 0.001$).

In our study, a statistically significant negative correlation was found between VD and age, BMI, HbA1c, and duration of illness in T2DM patients. Thus, we observed that low VD level was associated with increasing age, BMI, HbA1c, and duration of illness. According to the logistic regression test, the increase in the VD level reduced the risk for T2DM (OR: 1.085, 95 % CI: 1.078, 1.093, $p< 0.001$). The decrease in the VD level increased the risk for developing complications in T2DM patients (OR: 1.027, 95 % CI: 1.014, 1.040, $p< 0.001$). VD deficiency may be a risk factor for T2DM. Adequate VD levels can be achieved through UV rays, nutrients, and, if necessary, drug therapy. VD supplementation may have a role in modifying the metabolic and cardiovascular derangements that accompany T2DM, including hypertension and endothelial dysfunction (**26**).

In a meta-analysis involving a total of nine randomized controlled trials and 43,559 participants, it was reported that in patients with prediabetes, VD supplementation at moderate to high doses (> 1000 IU/day) significantly reduced the risk for T2DM compared to placebo (27). In another meta-analysis, short-term VD support was not found effective in a population with T2DM. However, VD normalization has a positive effect on fasting glucose in patients with poorly controlled T2DM (28). A study on patients with type 1 diabetes mellitus showed that HbA1c levels decreased significantly after 12 weeks of serum VD treatment (29). In a meta-analysis, it was reported that there was insufficient evidence of beneficial effect to recommend VD supplementation as a means of improving glycemia or insulin resistance in patients with diabetes, normal fasting glucose or impaired glucose tolerance (30).

Our study was conducted at 41.20 north latitude and 32.60 east longitude. In the literature, it has been suggested that sunlight is not sufficient for the production of VD in the skin between November and February on 42.20 north latitude (31). In our study, the reason for low VD levels in older age was thought to be sun avoidance due to high temperature. In a study that includes Mediterranean countries, similar results supporting ours were obtained (32). The critical role of sunlight exposure in VD synthesis is a very important factor for VD level.

One of the limitations of our study is that it is a retrospective single-center study. Also, the VD levels consisted of the results measured every month of the year. To obtain more accurate results, there is a need for prospective studies covering seasonal differences. Another limitation of our study is that the benefits of VD replacement for patients with prediabetes and diabetes could not be evaluated due to the study design. The significance of our study lies in that it compares the VD level according to the complications in T2DM patients.

CONCLUSION

VD deficiency may be a risk for the development of T2DM. In our study, the VD levels in the T2DM patients and those who developed T2DM-related retinopathy, nephropathy, neuropathy, and peripheral vascular disease were significantly lower than those of the healthy individuals. However, lower VD levels in insulin users may indicate that low VD levels play an important role in the etiopathogenesis of T2DM and its complications. Well-designed clinical trials are needed to further study the relationship between VD deficiency and clinical outcomes in patients with T2DM.

Author contributions: Hİ, Fİ; Literature search and study design, data collection and analyzes **Hİ;** Writing article and revisions

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. This research did not receive and specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical issues: All authors declare originality of research.

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MSD

Medical Science and Discovery
ISSN: 2148-6832

Efficacy of Tocilizumab in the treatment of severe COVID-19 patients with respiratory failure

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ABSTRACT

Objective: This study aimed to investigate the impact of tocilizumab, an IL6R inhibitor, on clinical features and laboratory tests of subjects admitted with severe COVID-19 and respiratory failure.

Material and Methods: A total of 30 patients with positive polymerase chain reaction for COVID-19 and respiratory failure were analyzed in a retrospective manner. All patients received 8mg/kg body weight tocilizumab i.v. once in addition to the standard COVID-19 treatment protocol, including Oseltamivir phosphate 75mg twice daily, hydroxychloroquine 200 mg twice daily, and azitromycine 250 mg once daily following a 500 mg loading dose. Demographic characteristics, and clinical features including oxygen saturation, the concentration of oxygen inhalation, body temperature, mean arterial pressure and heart rate, and SpO2, end-tidal CO2, and blood tests including complete blood count, procalcitonin, C-reactive protein (CRP), Troponin-I, D-dimer, and liver and kidney function tests were recorded before and after treatment with tocilizumab.

Results: A significant increase occurred in SaO2 on first and third days following treatment with tocilizumab (84.3 % and 90.3%, respectively, $p<0.001$ for both recordings compared to baseline). There was also a significant increase in end-tidal CO2. The increase in mean SaO2 after tocilizumab was followed by a decline in respiratory rate on the first and third days of treatment. A dramatic decline was observed in body temperature from the first day of treatment with tocilizumab. Lymphocyte count increased following tocilizumab and C-reactive protein and Troponin I levels were reduced.

Conclusion: Tocilizumab appears as an effective therapeutic option for improving oxygenation, symptoms and laboratory surrogates of ongoing inflammation in subjects with severe COVID-19.

Keywords: COVID-19, SARS-CoV-2, Tocilizumab, Cytokine storm

Research Article

Received 23-01-2020

Accepted 06-02-2021

Available Online: 10-02-2021

Published 28-02-2021

INTRODUCTION

Several cases of pneumonia with unknown etiology have emerged in Wuhan, Hubei Province, China towards the end of 2019 (1). Fewer and cough, which were before an acute respiratory distress syndrome were the most prominent initial symptoms (2). Following the identification of a novel coronavirus in the throat swab sample of one patient by the Chinese Center for Disease Control and Prevention (CDC), World Health Organization (WHO) named the novel coronavirus as 2019nCoV (3). The rapid spread of pneumonia to other regions of China and overseas led World Health Organization (WHO) to declare this outbreak as the public health emergency of international concern (PHEIC). In February 2020, the virus was renamed as severe acute respiratory syndrome coronaviruse-2(SARS-CoV-2) by the International Committee on Taxonomy of Viruses (4). Epidemic disease caused by SARS-CoV-2 was further announced by the WHO as coronavirus disease 2019 (COVID-19).

http://dx.doi.org/10.36472/msd.v8i2.473

Coronavirus disease 2019 was the third coronavirus disease within the last two decades following severe acute respiratory syndrome coronavirus (SARS-CoV) which resulted in more than 8000 infections and 774 deaths in 37 countries, and Middle East respiratory syndrome coronavirus (MERS-CoV) which resulted in 2494 infections 858 deaths (**5, 6**). As of May 25, 2020, COVID-19 has been reported to affect about 5.5 million individuals and caused over 340.000 fatalities globally (**5**). Although nonspecific treatment has been shown to promote dramatic improvement in critical COVID-19 patients, several agents including opinavir, ritonavir, remdesivir, macrolides, hydroxychloroquine and colchicines are currently used for the treatment of COVID-19 (**6, 7**). Tocilizumab, a recombinant humanized anti-human IL-6 receptor monoclonal antibody which is commonly used in the treatment of rheumatic disorders (**8**). We hypothesized that tocilizumab could alleviate the cytokine storm which is directly associated with increased mortality in COVID-19 patients with respiratory failure. This study aimed to investigate the impact of tocilizumab on clinical features and laboratory tests of subjects admitted with severe COVID-19 and respiratory failure.

MATERIAL AND METHODS

A total of 30 patients meeting the inclusion criteria were enrolled in this retrospective study. Inclusion criteria were as follows: Age ⩾ years, definite SARS-CoV2 in presence of symptoms and positive polymerase chain reaction (PCR), and signs of severe respiratory failure including ambient air SpO2 ⩽ 92%, need of ⩾ 6l O2/min, need for NIV (non-invasive ventilation) or IMV (invasive mechanical ventilation). Subjects with severe hepatic failure (AST/ALT ⩾ 5x ULN), white blood cell count <2000/mm3/ µl, platelet count <50000/ mm3/ µl, concomitant severe bacterial infection, immunosuppressive treatment other than prednisolone ⩽10mg/d, sulfasalazine or hydroxychloroquine, active/chronic tuberculsosis, active/chronic hepatitis, known allergic reactions to tocilizumab. Written informed consent was obtained from all subjects included in the study. The study was approved by Institutional Ethical Committee and was conducted in accordance with the Helsinki Declaration.

All patients received 8mg/kg body weight tocilizumab (Roche Pharma [Schweiz] Ltd.) i.v. once in addition to the standard COVID-19 treatment protocol recommended by Science Advisory Board of Turkish Ministry of Health, including Oseltamivir phosphate 75mg twice daily, hydroxychloroquine 200 mg twice daily, and azitromycine 250 mg once daily following a 500 mg loading dose. Demographic characteristics, and clinical features including oxygen saturation, the concentration of oxygen inhalation, body temperature, mean arterial pressure and heart rate, and SpO2, end-tidal CO2, and blood tests including complete blood count, procalcitonin, C-reactive protein (CRP), Troponin-I, D-dimer, and liver and kidney function tests were recorded before and after treatment with tocilizumab. The change in clinical features and laboratory parameters before and after treatment with tocilizumab was the primary outcome measure of this study.

Statistical analysis: All analyses were performed on SPSS v21 (SPSS Inc., Chicago, IL, USA). Shapiro-Wilk test was used to determine whether variables are normally distributed. Data are given as mean ± standard deviation for continuous variables and frequency (percentage) for categorical variables. Repeated measurements of normally distributed variables were analyzed with two-way repeated-measures analysis of variances (ANOVA). Two-tailed p-values of less than 0.05 were considered statistically significant.

RESULTS

The mean age of the study group was 50 ± 6 years and 63.3 % were male. Comorbid diseases are presented in **Table 1**. The mean APCHE and SOFA scores were 26.6 ± 6.6, and 4.2 ± 0.9, respectively. The mean time from symptom onset to admission was 3.8 ± 0.8 days.

Table 1. Demographic and clinical features of the study group

	n=30, %
Age, years	50 ± 6
Gender, male	19 (63.3%)
Comorbid diseases	
Diabetes, n	8 (26.7%)
Hypertension, n	10 (33.3%)
Current smoking, n	8 (26.6%)
CAD, n	7 (23.3%)
Obesity, n	1 (3.3%)
Asthma, n	3 (10%)
COPD, n	7 (23.3%)
Heart failure, n	2 (6.7%)
CKD, n	2 (6.7%)
CVD, n	1 (3.3%)
FMF, n	2 (6.7%)
APACHE score	26.6 ± 6.6
SOFA score	4.2 ± 0.9
Time from symptom onset to admission, days	3.8 ± 0.8

The mean SaO2 was 79.7% before treatment with tocilizumab. A significant increase occurred in SaO2 on the first and third days following treatment with tocilizumab (84.3 % and 90.3%, respectively, p<0.001 for both recordings compared to baseline). There was also a significant increase in end-tidal CO2 (**Table 2**).

A dramatic decline was observed in body temperature from the first day of treatment with tocilizumab. Consistent with the improvement in SaO2, the mean heart rate decreased from 105.2/min to 97.8 /min on the first day and to 90.3 /min on the third day of treatment with tocilizumab. Mean arterial pressure was also observed to increase discordant with the decrease in HR. The increase in mean SaO2 after tocilizumab was followed by a decline in respiratory rate on the first and third days of treatment.

Changes in laboratory parameters following the administration of tocilizumab is given in **Table 2**. Hemoglobin, leukocyte count, platelet count, and neutrophil count were similar before and after tocilizumab. However, a significant increase occurred in lymphocyte count after administration of tocilizumab and lymphopenia returned to normal range on day 3. Although there were no significant

http://dx.doi.org/10.36472/msd.v8i2.473

changes in procalcitonin concentration, CRP significantly decreased from 15.5 ng/mL to 12.3 ng/mL on day 1 and 8.9 ng/mL on day 3. Troponin I level also decreased from 0.156 ng/mL to 0.119 ng/mL on day 3. ±

The number of intubated patients decreased from 22 to 17 on the first day and to 11 o the third day of treatment with tocilizumab. 96.6 of the subject have been discharged.

Table 2. Clinical findings and laboratory tests before and after tocilizumab

Parameters of patients (**n:30**)	**Before tocilizumab Mean±SD**	**Day 1 Mean± SD**	**Day 3 Mean± SD**	**P value**
Body temperature, degrees	37.9±0.61	37.1±0.77	36.8±0.79	0.687
Mean arterial pressure,mmHg	90.2±9.1	96.9±9.96	98.1±9.22	**0.001**
Heart rate, beats/min	105.2±13.1	97.8±16.5	90.3±14.2	**<0.001**
SaO2	79.7±4.3	84.3±5.3	90.9±3.5	**<0.001**
End-tidal CO2	36.3±1.7	39.4±2.3	40.3±6.2	**0.001**
O2 intake, lt/min	11.4±1.09	11.0±1.27	9.7±4.29	**0.037**
Respiratory rate, n/min	36.3±3.9	28.8±4.99	18.3±4.57	**<0.001**
Hemoglobin, mg/dL	11.9±1.63	11.9±1.84	12.0±1.99	0.965
Hematocrite, %	36.2±5.23	36.3±5.17	36.4±5.35	0.943
Platelet count, x10^3/ µL	220±109	242±72.3	255±142.8	0.242
Leukocyte count, x10^3/ µL	8.2±2.8	8.7±3.0	9.5±4.15	0.229
Neutrophil count, x10^3/ µL	80.1±14.2	80.1±11.2	77.9±15.2	0.290
Mena platelet volume, fL	9.4±0.92	9.3± 0,85	9.4± 1.01	0.687
Red cell distribution width, %	13.6±1.57	13.8±1,51	13.8±1.72	0.413
Lymphocyte count, x10^3/ µL	0.60±048	0.79±0.46	1.19±0.53	**<0.001**
Procalcitonin, ng/mL	0.61±1.26	0.76±1.09	0.84±1.54	0.548
CRP, mg/L	15.5±6.1	12.3±6.7	8.9±6.4	**<0.001**
GFR, %	90.8±33.0	81.6±32.1	82.4±33.3	**0.048**
BUN, mg/dL	22.4±23.1	25.0±22.1	28.0±23.6	0.110
CR, mg/dL	1.2±1.59	1.1±1.72	1.4±1.62	0.255
Na, mEq/L	136±7.6	142±8.4	145±6.7	**<0.001**
K, mEq/L	4.0±0.51	3.8±0.47	3.7±0.52	0.068
Calcium, mEq/L	7.5±0.64	7.3±0.63	7.5±0.65	0.455
HCO3, mEq/L	21.6±5.01	23.0±4.43	23.9±5.82	0.133
ALB, g/dL	3.2±0.48	3.0±0.51	3.1±0.42	0.683
INR1	1.25±0.59	1.3±0.47	1.4±0.66	0.849
TRP1, ng/mL	0.156±661	0.147±763	0.119±411	**<0.001**
AST, U/L	60.7±60	73.6±58	71.3±56	0.478
ALT, U/L	61.8±61	69.3±67	64.5±57	0.312
LDH, U/L	586±481	626±323	691±493	0.077
Total bilirubin, mg/dL	0.68±0.33	0.66±0.37	0.62±0.35	0.374
D-dimer, ng/mL	5.8±0.4.98	6.5±5.99	6.4±4.90	0.411
Fibrinogen, mg/d	626±223	577±221	541±250	0.115
Ferritine, mg/L	1875±1001	1468±1021	1266±936	**<0.001**
Triglyceride, mg/dL	180±138	169±127	187±143	0.725

DISCUSSION

This study retrospectively observed tocilizumab, an IL6R inhibitor, in the treatment of 30 severe COVID-19 patients with respiratory failure. Our findings show improvement in oxygenation and blood pressure and in blood tests including CRP and troponin I following the administration of tocilizumab. Lymphocyte count displayed a dramatic increase after tocilizumab. Concordant with the improvement in oxygenation a prominent decline occurred in oxygen volume delivered per minute and in respiration rate. These findings suggest that tocilizumab can be a valid therapeutic option in the treatment of severe COVID-19 patients.

COVID-19 is a novel coronavirus infection that predominantly affects the lungs and leads to rapidly progressing respiratory failure in a substantial amount of subjects. CT findings include multifocal ground-glass pattern, thickened interlobular and intralobular lines, vascular dilatation, and subpleural bands (**9, 10, 11, 12**). Subjects with lung involvement almost always require oxygen therapy and assisted ventilation. About 14% of patients infected by SARS-CoV-2 develop severe COVID-19 characterized by dyspnea, respiratory frequency ⩾30/min, blood oxygen saturation ⩽93% and PaO2 /FiO2 ratio 50% of the lung field and approximately 6% develop respiratory failure, septic shock, and multiorgan dysfunction (**13**).

In this study, following the administration of tocilizumab a significant improvement was observed in oxygenation and signs of hypoxia. Oxygen intake flow was reduced concordant to the improvement in oxygenation.

Emerging evidence indicates that CRP and troponin-I concentrations have prognostic value in COVID-19 patients and both are associated with the intensity of the ongoing inflammation (**14, 15, 16**). Our findings show that a significant reduction occurs in CRP and troponin-I following the administration of tocilizumab. The number of lymphocytes is also considered as an important indicator for the severity of COVID-19 (**1**). Results of the present study show that lymphocyte count rapidly returns to normal after treatment with tocilizumab. During the treatment, no adverse drug reactions and subsequent pulmonary infections were reported. These findings are consistent with the preliminary data demonstrating the efficacy of tocilizumab in the treatment of severe COVID-19 patients. Xu and colleagues have shown in their study on 21 severe COVID-19 patients that tocilizumab is associated with remarkable improvement in symptoms and oxygenation a few days after administration (**17**). A prospective, randomized, double-blind placebo-controlled trial is currently enrolling patients to evaluate the efficacy and safety of tocilizumab in patients with severe COVID-19 pneumonia (**18**). The findings of that study will provide valuable data regarding the role of tocilizumab in severe COVID-19.

Our findings indicate that tocilizumab provides promising improvement not only in oxygenation of the patients with severe COVID-19 but also reduces the blood concentrations of the inflammatory markers which are indicative for the cytokine storm. The recognition of SARS-CoV-2 by endothelial cells and alveolar macrophages triggers the generation of pro-inflammatory cytokines and chemokines which further attract monocytes, macrophages and T cells to the site of infection and promote a cytokine storm, in particular, in subjects with a defective immune response (**19, 20**). Although the mechanism of how tocilizumab improves symptoms and oxygenation in COVID-19 is unclear, it is considered that this agent can repress the cytokine storm which is responsible for alveolar damage in severe COVID-19. Results of this study also indicate that tocilizumab appears as a safe therapeutic option without adverse drug reaction.

The retrospective nature and relatively small sample size are the main drawbacks of this study. The lack of monitoring IL-6 levels during treatment with tocilizumab is also another limitation for this study.

CONCLUSION

In conclusion, tocilizumab appears effective in improving oxygenation, symptoms and laboratory surrogates of ongoing inflammation in subjects with severe COVID-19. These findings suggest that tocilizumab should be considered to repress the deterioration of severe COVID-19 patients.

Take home message: In COVID-19 pneumonia, it was observed that tocilizumab suppressed cytokine storm.

Financial Disclosure: The authors declared that this case has received no financial support.

Author contributions: EA, NC, NB, SD, MC, NY; Literature search and study design, Patient examination and therapy, data collection and analyzes **EA;** Writing article and revisions

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. This research did not receive and specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical issues: All authors declare originality of research.

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Medical Science and Discovery
ISSN: 2148-6832

The effect of Captopril on electrogustometry thresholds, tongue tip vascularization, density and form of the fungiform papillae in humans

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ABSTRACT

Objective: Aim of the study was to study in parallel changes in electrogustometric thresholds (EGM), in morphology and density of the fungiform papillae (fPap) and in vessels' shape and density at the tip of the human tongue of patients receiving Captopril.

Material and Methods: In 18 female subjects receiving Captopril (50mg, once per day) as Monotherapy against Hypertension EGM thresholds at the chorda tympani, at the soft palate were recorded bilaterally. Morphology and density of the fungiform papillae (fPap) and blood vessels' density and morphology at the tip of the tongue were examined using contact endoscopy (CE). As control-group 18 healthy non-smokers female subjects of the same age group were also examined.

Results: The evaluation of subjects treated with Captopril showed a higher EGM-thresholds as the subjects of the control-group. The difference between the density of patients' fPap and that of the control-group was statistically significant. It is obvious that Captopril affects the shape and the vascularization of fPap. Captopril can affect seriously taste acuity.

Conclusion: The present study provides data concerning the simultaneous changes in both EGM-Thresholds and shape and vascularization of fPap. The use of captopril seems to have a negative effect on them.

Keywords: Captopril, contact endoscopy, electrogustometry, fungiform papillae, vascularization

INTRODUCTION

Taste perception plays a key role in systemic health, nutritional status and quality of life. Increasing age, smoking and a number of drugs are associated with a decline in the sense of taste and many gustatory disorders are secondary to a wide variety of diseases. Taste disturbance is caused by several endogenous and exogenous factors, such as drugs (1).

Antihypertensive drugs have been identified as potential causes of taste disturbance (1,2). One of these drugs is an angiotensin-converting enzyme, captopril. These drugs have been identified as potential causes of taste disturbance. The captopril molecule contains a thiol-group (–SH) and has been shown to form chelates with zinc (1). However, other ACE inhibitors without the thiol radical have been reported to cause taste disturbance.

Though the wide use of captopril there is a lack of data concerning the effects of captopril on tongue papillae. The majority of the references in the literature presents case-reports of patients who manifested severe dysgeusia and impaired quality of life attributed to the angiotensin-converting enzyme (ACE) inhibitor (3).

The present study had the following aims: first to determine the variations of electrogustometric thresholds in relationship to treatment with Captopril and second to examine the possible differences in densities, shape and vascularization of fPap.

Research Article

Received 24-01-2020

Accepted 06-02-2021

Available Online: 10-02-2021

Published 24-02-2021

http://dx.doi.org/10.36472/msd.v8i2.475

MATERIAL AND METHODS

The study was conducted according to the guidelines of the Declaration of Helsinki on biomedical research involving human subjects and was approved by the local ethics committee. All subjects provided spoken and written consent after they had been extensively informed about the study. Volunteers participated in the study only after they were informed of its background and purpose and after their written consent was obtained. To minimize variability in technique and interpretation of the findings, all examinations were carried out by the same examiner (PP).

Eighteen female subjects (age 51,2±2,5) receiving Captopril (50 mg, once per day) as Monotherapy against Hypertension participated in the study (long term treatment, mean±SE 2.6±0.2 yr). Another 18 healthy female (age 50,6±2,54) subjects participated in the study as control-group. Patient medical histories solicited information regarding current medical care, recent hospitalizations, significant medical histories such as hepatitis or epilepsy), allergies, cardiac murmurs and/or valve disease, prosthetic hip replacements, and pregnancy. No-one of the patients suffered from retinopathy, nephropathy, polyneuropathy or macroangiopathy. Exclusion criterias were determined as treatment with medications such as antirheumatic, antidepressant, or antiepileptic drugs. Subsequently, a complete otorhinolaryngological examination was performed. Two of the subjects receiving Captopril reported a reduced acuity to sweet substances and another two a metallic taste.

Electrogustometry testing: Taste acuity was evaluated with EGM. Electrical stimuli were delivered with an electrogustometer (TR-06, Rion Co, Tokio, Japan) with a single, flat, circular stainless steel stimulus probe (5mm in diameter). The device produces low-amplitude stimuli of pre-determined duration (1 second). A feedback circuit controls the output current with an error of < 1% (1).

All subjects were instructed not to drink an hour before the beginning of the testing session. First, a 30dB-stimulus was administered to test whether the subject was in a position to recognize electrogustometric stimuli. Stimulation started at the lowest stimulus amplitude (-6 dB) and increasingly stronger stimuli were presented until the subject recognized the stimulus. If the threshold for stimulus perception was not clearly determined, the next higher- and lower-strength stimuli were presented to the individual.

The electric threshold scores were measured at six locations, namely para-medially on both sides of the tongue apex (each 2 cm away from the tip), an area innervated by the chorda tympani, at the area of the vallate papillae on both sides of the tongue (innervated by the glossopharyngeal nerve) and at the soft palate (area innervated by the major petrosal nerve) bilaterally.

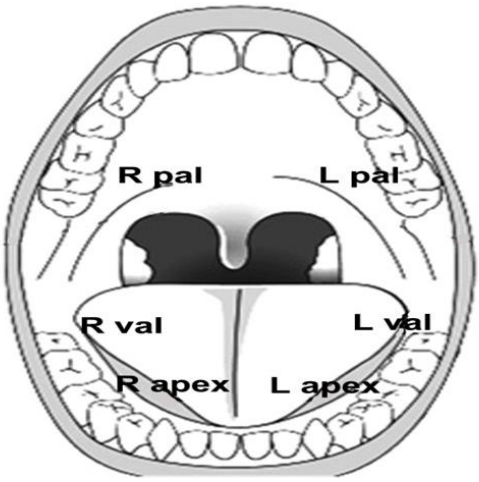

Figure I. The figure depicts the locations of threshold measurements. The sites have been chosen to evaluate the function of the chorda tympani, glossopharyngeal and greter petrosal nerves.

In healthy subjects, electric gustatory thresholds for the tongue apex, vallate papillae and soft palate are generally set at levels up to 8, 14 and 22 dB respectively. A 500-ms electric stimulus was applied, beginning at -6 dB and increasing up to +34 dB (3-400 μA) in 2dB-steps. Thresholds were first measured on the right side beginning at the soft palate (Rpal), then proceeding to the area of the vallate papillae (Rval) and finally at the tongue apex (Rapex). Electrogustometry thresholds were then recorded at the left side of the tongue first at the tongue apex (Lapex), then at the area of the vallate papillae (Lval) and finally at the soft palate area (Lpal).

The relationship between the logarithmic control settings and the output currents is shown on Table 1. All six areas were tested with the same stimulus duration before proceeding using stimulus duration. This procedure resulted in a 3–4 minutes' stimulus interval, therefore decreasing the possibility of the emergence of stimulus adaptation. The subjects had been instructed to discriminate between the perception of a sour/metallic taste (suggesting gustatory function - taste threshold) and the perception of an electrical sensation (suggesting trigeminal stimulation). We have measured the EGM-Thresholds as in previous studies (4). 2006). The subject was kept unaware of whether or not the current was applied (blind test) and no feedback was ever given to the subject. The test included successive applications of current in a staircase procedure 10-μA steps, then a 2-μA Up-and Down steps from the first "no" response. The EGM detection was taken as the lowest current intensity perceived by the subject for repeated trials (**4**).

Table 1: The relationship between the logarithmic control settings and the output current.The output current can be adjusted in 2-dB steps from -6 dB to 34 dB

Output Current dB Readings and Output Current																				
dB	-6	-4	-2	0	2	4	6	8	10	12	14	18	20	22	24	26	28	30	32	34
(μA)	4	5	6.4	8	10	13	16	20	25	32	50	64	80	100	130	160	200	250	320	400

http://dx.doi.org/10.36472/msd.v8i2.475

Contact endoscopy: Imaging was performed using a 30° contact endoscope (magnification × 60 and × 150; Karl Storz, Tuttlingen, Germany). Identification of fPap was first performed using a non-contact technique. Subjects were instructed to rinse their mouth with water before contact endoscopy. A contact technique was used first without staining for imaging of subepithelial vessels. After careful suctioning of the saliva, methylene-blue 1% solution was used to stain epithelia and taste pores. A filter paper strip delineating an area of 1 cm2 was placed in a paramedian position on the tongue tip as proposed in a previous study (5).To address the problem of instability of the tongue during endoscopy the subjects were advised to hold gently the tip of their tongue between their upper and lower teeth, to avoid venous congestion and hyperemia which could eventually confound contact endoscopic findings. The subjects were asked to seat in the examination chair with their head and neck supported by a pillow. The patients were asked to keep the tongue in a fixed position as much as possible. Examination time by CE was about 30 s. Anesthesia was not necessary. A cold light source was used to minimize any heat produced at the tip of the endoscope. No change (increase or decrease) in vascularization has been observed during examination by CE.

The form of the fungiform papillae was classified to one of four types according to a previously introduced classification paradigm as following: Type 1, (egg-shaped or long ellipse type – without surface thickness), Type 2 (slight thicker surface compared to type 1), Type 3 (thick and irregular surface) and Type 4 (remarkably flat and atrophic surface). Type 1 corresponds to the healthier state, while Type 4 shows a remarkably flat surface. It should be stressed that the mushroom-shaped papillae with horny tips were counted as filliform (and not as fungiform) papillae (5). For estimation of the density of fPap (number of papillae per cm2), the contact endoscopic image with the highest fPap density taken from each individual was used. Due to their very light staining, fungiform papillae could be readily distinguished from filiform papillae, which stained dark. The classification of the blood vessels' morphology at the tongue apex was also performed according to the previous classification (Negoro et al. 2004). Five types of vessels' morphology Type A (clear loop and wooden branch shape), Type B (unclear loop and wooden branch shape), Type C (elongated blood vessels), Type D (granular shape or dotted shape) and Type E (unclear blood vessels). Type A represents the "healthiest" morphology and Type E corresponds to the "unhealthiest".morphology.

All participants completed the study. The findings, and particularly the CE images and the classification of the fPap, have been checked by two persons (P.P and G.K) in order to achieve consensus and avoid any possible mistakes.

Statistical analysis: The null hypothesis was that there was no statistical difference in EGM-thresholds between age groups and between sexes. We used a quantile-quantile test (QQplot) to examine the distribution of our findings. A quantile-quantile plot (QQ plot), a graphical tool for assessing normality, is a plot of the sorted values from the data set against the expected values of the corresponding quantiles from the standard normal distribution. The QQ plot of the data did not show any normal distribution.

As a result, non-parametric tests were applied. The level of statistical significance was set at $p < 0.05$. On each occasion, the EGM-thresholds between the two groups were compared using Kruskal-Wallis and Mann-Whitney tests. The Bonferroni correction was used when necessary. Tukey's multiple comparison test was used to detect differences significant at the 0.05-level in mean thresholds for the various age categories.

For analysis of the regression between age, form, and vascularisation of fPap, the Kendall rank correlation coefficient was applied. The null hypothesis was that the two variables examined on each occasion were independent. The results were analyzed using SPSS software (Version 12 for Windows, SPSS Inc. Chicago, IL, USA)

RESULTS

EGM-thresholds

The EGM-Thresholds on the right and left side of the tongue of the patients after 1-second stimulation are depicted in **Table 2**. As it can be seen the EGM-Thresholds of the patients were higher than these of the healthy subjects. We have also found a statistic significant difference between the thresholds of the two groups, those of the patients were obviously higher (Threshold A: p=0.04, Threshold B: p=0.04, Threshold C: p=0.03, Threshold D: p=0.04, Threshold E: p=0.04, Threshold F: p=0.04).

Table 2: The mean EGM-Thresholds on the right and left side of the tongue after 1-second stimulation in patients receiving Captopril and healthy subjects of the control group. It is obvious that patients receiving Captopril produce a diminished taste acuity.

	Patients treated with Captopril (n=18)			Healthy Subjects (n=18)		
	Mean	Min.	Max	Mean	Min.	Max
Rpal	28.56	24	34	21.43	18	28
Rval	23.43	20	28	18.12	14	22
Rapex	14.46	12	18	8.34	8	12
Lapex	15.65	16	18	9.12	8	14
Lval	22.34	20	26	19.34	12	26
Lpal	27.65	24	34	23.23	22	28

Fungiform papillae structure

Changes in shape and density of fPap as well as in vascularization of the tip of the tongue were also detected by means of CE. It should be mentioned that no change in vascularization or shape has been observed during the examination due to pressure on the tongue's surface. The shape of fPap and vascularization of the tongue tip worsen significantly, as it can be observed with the use of Negoro's classification, as shown on **Table 3**.

We have also reported a difference in densities of fPap in patients compared to the healthy participants (Patients: Right Side: 20.4±2.2, Left Side: 19±4.6./Healthy Participants: Right Side: 24.4±3.7, Left Side: 25.3±4.3). There was also a high regression-analysis by application of Kendall's tau (τ) between taste thresholds and vascularization (τ=0.73), taste

http://dx.doi.org/10.36472/msd.v8i2.475

thresholds and shape (τ=0.81) and taste thresholds and density of fPap of women under treatment (τ=0.67).

Table 3: The table depicts the classification according to shape and vascularization of fungiform papillae on both sides at the tip of the tongue in patients and healthy subjects.

Types of form and vascularization in Patients (n=18)	3A	3C	4A	4D
	5	7	4	2
Types of form and vascularization In Healthy subjects (n=18)	**2C**	**2D**	**3A**	**3B**
	6	4	5	3

DISCUSSION

The results of the present study show a significant deterioration, both functional (EGM-Thresholds), and morphological (vascularization and shape of the fPap in patients receiving captopril.

Many gustatory disorders are induced by drugs. Frequently, patients are aware of this relationship and report on the close temporal relationship between the occurrence of the taste disorder and drug-intake. Numerous mechanisms of drug-induced gustatory dysfunction have been identified, including disposition of silver sulfate, altered influx of calcium and other ions, chelation or depletion of zinc, disturbed bradykinin catabolism, alteration of second messenger synthesis and altered prostaglandin synthesis (**6**).

Their taste disturbance disappeared within a few weeks after discontinuation of the drug. A variety of drugs are reported to cause taste disturbance, including thiamazol, D-penicillamine and captopril (**7**). Taste disturbance induced by captopril has been attributed to the adverse effect by chelation of zinc (**8**). Recent advances in molecular biology have identified receptors and ion channels on taste cells. Sweet and bitter taste receptors are the proteins that couple with G-proteins (**1,9**). Coupling and uncoupling to G-protein causes 'taste-on' and 'taste-off'(**1**). Angiotenisin II receptor, which is the target molecule of ARB, also belongs to the same category of the receptor (**10**). Zinc is an essential trace element playing an important role in many functions such as vision, taste and smell (**11, 12**). Previous studies suggested that taste impairment belongs to the symptoms of zinc deficiency (**2,11).** Takeda et al. (**11**) suggest that zinc deficiency is a predominant factor underlying hypogeusia even when zinc concentrations are within normal ranges in serum (**1**).

Because of the accessibility of the human tongue tip to examination by contact endoscopy (CE) and because of the established association between the fPap and the gustatory stimuli's thresholds we have chosen the fPap among the four different tongue papillae types to study structural variables that may be important for taste sensation as well as structure-function correlates (**13**). Similarly to the work of other investigators, we concentrated on the study of the fungiform papillae. In this way the results we obtained can be easily compared to those of previous studies and avoid any other measurements or observations coming from other areas of the tongue's surface. Seen from the patient's perspective, the CE procedure was simple because the gag-reflex or other complaints that could lead to the interruption of the evaluation were avoided.

CE has been used to assess the vascularization, shape and density of fPap as in previous studies (**13, 14, 15, 16**). The use of CE has the advantages that the procedure is not time-consuming (it lasts 10-15 minutes for each examined person) and that it is non-invasive. Of note, CE cannot detect taste pores. Even after methylene blue staining, reliably detecting of taste pores probably cannot be reached, as suggested by other authors (**13**). The previous findings concerning the effects of Captopril on the lingual mucosa were experimental. Chou et al. investigated the zinc-deficiency-induced morphologic changes in the vallate taste buds of weanling and young adult male Wistar rats. The authors concluded that the main effects of zinc deficiency were changes in the number and size of taste buds, and fine structure changes in the taste bud cells (**17**).

The use of CE provided the advantage to study the vascularization of the tongue tip and the shape of the fPap. The results of this study suggest that these two parameters are significantly associated with taste acuity as assessed by EGM. Nonetheless, it should be stressed again that not all fPap contain taste buds and therefore not all fPap produce taste sensation (**18, 19**) and that there are variations in sensitivity of fPap to chemical stimuli (**13**). It is obvious that patients receiving Captopril present worse vascularization and form of fPap than healthy patients. The above finding alone does not give the final explanation of taste disturbances caused by ABRs. Further study is needed to solve this issue.

CONCLUSION

In conclusion, the present study offers new data concerning the changes in fPap shape and density as well as the changes in vascularization of the tongue tip in correlation to EGM-thresholds in patients treated with Captopril. As far as we know this is the first time in which combined data such as the above are presented in the literature.

Author contributions: PP, GK, HG; Literature search and study design, patient examinations, data collection and analyzes **PP;** Writing article and revisions

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. This research did not receive and specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical issues: All authors declare originality of research.

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Medical Science and Discovery
ISSN: 2148-6832

The outcome of colposcopy in women attending with persistent postcoital bleeding and negative HPV-DNA test

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ABSTRACT

Objective: Women with postcoital bleeding (PCB) are recommended to be evaluated by colposcopy even if cytology is negative. Human papillomavirus (HPV) testing increases the detection of cervical intraepithelial neoplasia or worse compared to the Pap smear test. We aimed to determine the incidence of cervical pathology among women with persistent PCB with a negative Pap smear or HPV-DNA test. Our study, therefore, questions the place of HPV DNA test in women with PCB.

Material and Methods: The clinical data of 212 women with persistent PCB and negative cytology or negative HPV DNA test referred to colposcopy, between January 2010 and June 2019, were retrospectively evaluated.

Results: Among the 212 PCB patients, 161 (75.9%) were cytology negative and 51 (24.1%) were HPV DNA test (n=40) or co-test (n=11) (negative for HPV DNA test and cytology) negative. There were no cases of invasive cancer. The women referred with negative cytology were more likely than those with negative HPV DNA to have CIN (21/161 (13%), 1/51 (1.9%) p=0.042. Seven women (4.3%) were diagnosed with high-grade cervical dysplasia in the negative cytology group. None of the patients in the HPV DNA negative group was diagnosed with high-grade cervical dysplasia.

Conclusion: Our data show that a normal Pap smear cytology in women with PCB does not rule out the possibility of HSIL. HPV DNA testing is a useful triage test to determine if colposcopy referral is required in the context of post-coital bleeding with negative smear test.

Keywords: Postcoital bleeding, colposcopy, HPV-DNA test, cervical smear

INTRODUCTION

Postcoital bleeding (PCB) refers to bleeding that occurs during or immediately after intercourse. The significance of PCB as a potential symptom of cervical cancer has always been emphasized, particularly if it is persistent. On the other hand, it is a common gynecological symptom as the prevalence of PCB is 6% in menstruating women and it is mainly caused by a benign lesion of the cervicovagina such as infection, cervical polyps, ectropion (1). The prevalence of cervical intraepithelial neoplasia (CIN) in women presenting with PCB varied in different studies between 6.8% and 17.8% (2). The management of PCB is inconsistent. Currently, there are no guidelines or evidence from randomized clinical trials to base recommendations on the management of PCB. There is no controversy that women with abnormal high-risk human papillomavirus (HPV) DNA, pap smear test or visible lesion that is highly suspicious for underlying cancer should be referred for colposcopy. However, there is a debate on whether colposcopy should be performed on women who have PCB with a negative pap smear or negative HPV-DNA test. Multiple studies questioned the place of pap smear alone in women with persistent PCB. They reported that a normal pap smear alone would not be regarded as reassuring in a woman with PCB (3). In a retrospective study of 166 women with PCB and negative previous smear history, the rate of cervical cancer and CIN was found to be 3.6% and 9%, respectively (4). The false-negative rate of Pap smears in the presence of invasive cancer is as high as approximately 40-50% (5).

Research Article

Received 20-01-2020

Accepted 08-02-2021

Available Online: 09-02-2021

Published 24-02-2021

http://dx.doi.org/10.36472/msd.v8i2.471

Limitations of the Pap smear test have led to the development of HPV tests for cervical cancer screening programmes (6) . HPV DNA testing is cost-effective as a screening strategy compared to Pap smear (7). On the other hand, HR-HPV testing has a higher false-positive rate than cytology resulting in more women initially being referred for colposcopy (8).

This study was to determine the risk of finding cervical cancer and its precursors among women referred to the colposcopy primarily because of postcoital bleeding with negative cytology or HPV DNA. We aimed to answer whether a negative HPV DNA test excludes significant pathology in women with postcoital bleeding.

MATERIAL AND METHODS

We performed a retrospective study of patients referred to the colposcopy examination with persistent postcoital bleeding at the Gynecologic Oncology Unit, Uludag University Hospital between January 2010 to June 2019. The study protocol was approved by the Uludag University Institutional Ethics Board (2019-17/13).

Inclusion criteria are women with persistent PCB and negative cervical cytology or negative high-risk HPV DNA test. Women excluded from the study were those who presented with PCB and previous abnormal smears or positive high-risk HPV DNA test results, and patients with an obvious cervical lesion were excluded from the study. Persistent PCB is defined as the duration of symptoms for more than six weeks. Identification of these patients was taken from the case notes, and details were extracted from computer records.

National cervical cancer screening program using primary HPV with genotyping and reflex liquid-based cytology (LBC) triage started in 2014 in Turkey. In our institute HPV based testing has been using since 2017, before that primarily Pap smear test was used for follow–up and screening of cervical pre-invasive lesions. In our centre, we use colposcopy to evaluate women with persistent PCB, regardless of cytology or HPV DNA testing results.

Histopathology findings were classified according to the LAST Standardization. High-grade squamous intraepithelial lesion (HSIL) is used to define a group of histologically proven CIN2, CIN3 or carcinoma in situ (9). Patients with positive colposcopic findings underwent a directed biopsy. Standard colposcopic techniques were used, including the application of 3% acetic acid and biopsies were taken from suspicious-looking areas.

All calculations were performed using IBM SPSS 23.0 (IBM SPSS Inc., Armonk, NY, IBM Corp.). A descriptive analysis of the data was undertaken. Statistical significance for differences was analyzed using the t-test and $\chi 2$ test as appropriate. All P-values were tested as two-tailed and considered significant at <0.05.

RESULTS

A total of 212 women with PCB and negative cytology or negative HPV DNA testing were examined with colposcopy during the study period. Among the 212 PCB patients, 161 (75.9%) were cytology negative and 51 (24.1%) were HPV DNA (n=40) or co-test (n=11) negative (negative for HPV and cytology). The characteristics of the 212 women included are shown in Table 1.

Of the 161 women who had PCB and negative cytology, 78 (48.4%) had normal colposcopic appearances, hence had not histology. The remaining 83 (51.6%) had a punch biopsy. Of these, 62 (74.7%) had normal histology. Of the 51 women who had PCB and negative HPV DNA testing, only one woman had cervical pathology. Table 2 presents the details of management. The women referred with negative cytology were more likely than those with negative HPV DNA to have CIN (21/161 (13%), 1/51 (1.9%) p=0.042). There were no cases of invasive cancer.

In total, seven women (4.3%) were diagnosed with HSIL in the negative cytology group. However, none of the patients in the HPV DNA negative group was diagnosed with HSIL, but this finding did not reach statistical sig¬nificance. (p=0.199).

Table 1. Main characteristics of the study population

	Negative cytology group (n=161)	Negative HPV-DNA group (n=51)	p
Age (Median, min-max)	38 (20-66)	41 (25-60)	0.126
Parity			
Nuliparous	15 (9.3%)	5 (9.8%)	0.927
Primiparous	33 (20.5%)	8 (15.7%)	0.544
Multiparous	113(70.2 %)	38 (74.5%)	0.599

Table 2. Colposcopic and histopathologic findings of patients

	Negative cytology group (n=161)	Negative HPV-DNA group (n=51)	p
Macroscobic appearance of cervix			
Normal	106 (65.8%)	31 (60.8%)	0.507
Cervical ectopy	35 (21.7%)	16 (31.4%)	0.189
Cervical polyp	20 (12.4%)	4 (7.8%)	0.455
Colposcopy findings			
Unsatisfactory	38 (23.6%)	14 (27.4%)	0.579
Normal appearance	78 (48.4%)	31 (60.8%)	0.149
Abnormal	45 (28%)	6 (11.8%)	**0.023**
Histopathology			
LSIL	14 (8.7%)	1 (1.9%)	**0.042**
HSIL (CIN2/CIN3)	7 (4.3%)	0	0.199

DISCUSSION

PCB is a concern for women because of its association with cervical cancer risk. Pap smear is the cornerstone of the screening and prevention of cervical cancer; however, due to high false negativity, the reliability of negative smear remains a topic of debate in the management of PCB. Although patients referred with negative cytology, have a lower incidence of CIN than those with no cytology (10). Women with PCB are recommended to be evaluated by colposcopy even if cytology is negative (**11**). HPV testing has been replacing cytology in many regions as a primary screening tool for cervical cancer in recently years. HPV testing increases the detection of CIN3 or worse by approximately 40% compared to cytology (**12**). HPV testing is cost-effective as a screening strategy compared to conventional cytology. On the other hand, it has a higher false-positive rate than pap smear resulting in more women initially being referred for colposcopy (**8**). Our study, therefore, questions the place of HPV DNA testing in women with PCB.

There were no cases of invasive cervical cancer in our study. This result is a contrast to previous studies, which showed an incidence of 0.6 and 3% (**8, 13**). This can be due to the exclusion of patients who have suspected mass in our study group.

In the current study, 4.3% of women with postcoital bleeding had HSIL even though they had normal smears. These rates are consistent with other studies. Khattab et al. analyzed the data of 166 women referred with PCB and negative cytology history and reported that the rate of cervical cancer and CIN to be 3.6% and 9%, respectively (**4**). Sahu et al. reported that women with negative cytology and PCB had a 3.45% risk of severe than CIN2 histopathology (**11**).

In the last ASCCP Risk-Based Management Consensus Guidelines stated that when patients have an estimated immediate risk of a diagnosis of CIN 3+ of 4.0% or greater based on history and current results, referral to colposcopy is recommended (**14**). The immediate risk of HSIL as 4.3 %, which our study reported would lead to colposcopy.

None of the patients who had PCB with negative HPV DNA testing was diagnosed with HSIL. This consistent with a previous single study that reported no cancer or HSIL in 83 women with PCB and negative HPV testing (**15**).

The major limitation of the current study is the inherent drawbacks from its retrospective design. Also, the number of HPV negative patients was small because HPV testing had not been considered beforehand for all cases. Despite these limitations, our study does add supportive evidence to the management of PCB.

CONCLUSION

Our data show that a normal pap smear in women with postcoital bleeding does not rule out the possibility of HSIL. Consequently, HPV DNA testing is a useful triage test to determine if colposcopy referral is required in the context of postcoital bleeding with negative cytology. A negative HPV DNA testing record is able to be regarded as reassuring in a woman with PCB. In situations where the HPV test cannot be performed, women with PCB should be evaluated with colposcopy whether or not they have negative cervical cytology.

Author contributions: MB, HO; Literature search and study design, patient examinations, data collection and analyzes **MB;** Writing article and revisions

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. This research did not receive and specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical issues: All authors declare originality of research.

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MSD

Medical Science and Discovery
ISSN: 2148-6832

D-dimer levels and lymphocyte counts as prognostic and predictive factors in patients with COVID-19

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ABSTRACT

Objective: Coronavirus Disease 2019 (COVID-19) is characterized by high fever, sudden developing respiratory distress, and radiological findings failing to respond to conventional treatments. The purpose of this study is to identify the association of D-dimer levels and lymphocyte counts with poor prognosis and to predict the clinical course in patients with COVID-19.

Methods: A total of 118 hospitalized adult patients diagnosed with COVID-19 were included in the study. According to the National Institutes of Health (NIH) COVID-19 treatment guidelines, patients were divided into two groups with severe disease (n= 26) and non-severe (n= 92) disease. Detected at the time of diagnosis, D-dimer levels and lymphocyte counts were compared between severe and non-severe COVID-19 patient groups. Distinctive performance analysis of these values was performed, and cut-off values were determined.

Results: The mean age of patients was 63±7 years (range 42-80 years), and 63 (53.4 %) were female. The lymphocyte count was lower ($p < 0.001$), and D-dimer was higher in patients with severe COVID-19 compared to non-severe patients ($p < 0.001$). D-dimer's cut-off point when the sum of specificity and sensitivity is maximized was 2 mg/L (sensitivity, 0.731; specificity, 0.913), and 1500/mm3 was for lymphocyte count (sensitivity, 0.692; specificity, 0.609). Lymphocyte count and D-dimer had a significant discrimination power (AUC: 0.745 [95 CI: 0.644 - 0.846], AUC: 0.928 [95% CI: 0.879 - 0.978] respectively, $p < 0.0001$).

Conclusion: The lymphocyte value of $\leqslant$ 1500/mm3 and D-dimer value of $\geqslant$ 2 mg/L can be used in the early determination of patients with poor prognosis in COVID-19. Using these cut-off values for D-dimer and lymphocyte count will help predict prognosis and make rapid treatment decisions in patients with COVID-19.

Keywords: COVID-19, prognosis, D-dimer, lymphocyte

Research Article

Received 31-01-2020

Accepted 12-02-2021

Available Online: 16-02-2021

Published 24-02-2021

INTRODUCTION

In December 2019, it was revealed that a novel coronavirus-related pneumonia cases were observed in Wuhan, China. It resulted in an epidemic spreading all over China, followed by an increasing number of cases in other countries (1). The International Virus Taxonomy Committee Coronavirus Working Group suggested name this virus as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (2). On January 30, 2020, the World Health Organization (WHO) declared the epidemic as a global emergency (3). In February 2020, the WHO designated the disease as COVID-19, which refers to Coronavirus Disease 2019 (4). The disease is characterized by high fever, normal or decreased white blood cells, lymphopenia, sudden developing respiratory distress, and radiological findings (1). In the early stages of the disease, severe symptoms of acute respiratory failure occur. Some patients develop acute respiratory distress syndrome (ARDS) and other serious complications, followed by multiple organ failure (5).

http://dx.doi.org/10.36472/msd.v8i2.478

Severe COVID-19 is often complicated by coagulopathy, and disseminated intravascular coagulation (DIC) is detected in most deaths (**6**). The SARS-CoV-2 has not been shown to have specific procoagulant effects. However, it is considered that coagulation test abnormalities in patients infected with SARS-CoV-2 are likely to develop as a result of the severe inflammatory response (**7**). COVID-19 has been reported to be associated with hemostatic abnormalities and significantly higher D-dimer levels in patients (**8**). Excessive inflammatory response, increased proinflammatory cytokines and acute-phase responses cause a life-threatening condition called cytokine storm characterized by persistent fever. Abnormalities in some laboratory values (such as lymphocyte count) are associated with this profile (**9**). In this study, the association between D-dimer and lymphocyte count of patients with COVID-19 and poor prognosis has been investigated.

MATERIAL AND METHODS

The objective of this study is to identify factors related to thromboembolism and poor prognosis in patients with COVID-19, predict the clinical course and develop treatment strategies to prevent poor prognosis.

Study design: A total of 118 hospitalized adult patients (63 female, 55 male) diagnosed with COVID-19 were included in the study (laboratory-confirmed cases, SARS-CoV-2 RNA detected by molecular method). Patients under 18 years of age, patients with comorbid diseases (including those with autoimmune and hematological diseases), those who are pregnant, a history of thromboembolic events, those using anticoagulant and/or antiaggregant treatment for any reason, and those receiving corticosteroid treatment were excluded from the study **(Figure1)**. According to the National Institutes of Health (NIH) COVID-19 Treatment Guidelines, patients with COVID-19 were divided into two groups as patients with severe disease (1- Hypoxia presence: Oxygen saturation $\leqslant$ 93% on room air or PaO_2 / FiO_2 <300. 2- Tachypnea (respiratory rate> 30 breaths per minute) or respiratory distress. 3- lung infiltrates> 50% on chest imaging) and with non-severe disease (mild or moderate) (10). In consideration of this classification, 26 patients were included in the severe COVID-19 group, while 92 were included in the non-severe group. D-dimer level and lymphocyte count values were compared between severe and non-severe COVID-19 patient groups. Distinctive performance analysis of the values that were significantly different between the groups was performed, and cut-off values were determined.

Laboratory Analysis: Hemogram parameters and D-dimer levels were reviewed during the hospitalization process. Hemogram analysis was performed with Sysmex XN-1000 (Sysmex, Kobe, Japan). Reference range for lymphocyte count was 1260-3350 cell/mm3. D-dimer analyzes were performed with Siemens BCS XP (Siemens, Marburg, Germany). The reference range for D-dimer was 0-2 mg/L.

Settings: The study was conducted in patients with COVID-19 hospitalized in the University of Health Sciences, Konya Training and Research Hospital, Infectious Diseases and Clinical Microbiology Service and General Intensive Care Unit, located in an area with a high prevalence of COVID-19, is accepted as a reference center for COVID-19 care.

Ethical Approval: The study was approved by the Local Ethics Committee of University of Health Sciences, Konya Training and Research Hospital (decision no: 08.05.2020/38-09) and the study was conducted according to the Declaration of Helsinki 1975.

Statistical Analysis

SPSS version 22.0 statistical package software (IBM Corp., Armonk, NY, United States) was used for statistical analyses. Continuous variables are demonstrated as mean±standard deviation, median (min-max), and categorical variables as numbers and percentages. Kolmogorov–Smirnov test was used for evaluating the normality of distribution. When parametric test assumptions are provided, Independent-Samples T-Test and when parametric test assumptions are not provided, Mann-Whitney U test were used to compare independent group differences. The chi-square test was performed to compare the study groups in terms of categorical variables. ROC analysis method was used for diagnostic performance analysis of variables. The threshold for significance was defined at $p < 0.05$.

RESULTS

A total of 118 hospitalized patients with COVID-19 were included in the final analysis. The mean age was 63±7 years and 63 (53.4 %) were female. There was no statistically significant difference between severe COVID-19 patient group (n= 26, 22.1 %) and non-severe COVID-19 patient group (n=92, 77.9 %) in terms of age (p= 0.862) (**Table 1**).

Lymphocyte count was found as statistically significantly low (p <0.001) in the severe COVID-19 group while D-dimer level was statistically significantly higher in the group with severe disease (p <0.001) (**Table2**). As for the effectiveness of lymphocyte count in distinguishing severe and non-severe patients with COVID-19 when cut-off score 1500/mm3 was taken, sensitivity was 69.2% and specificity was 60.2%. Lymphocyte count was found to have a significant discrimination power (AUC = 0.745, p <0.0001, 95% CI (lower bound – upper bound) = 0.644 - 0.846) (**Table3**)(**Figure2**).

As for the effectiveness of D-dimer level in distinguishing severe and non-severe patients with COVID-19 when cut-off score 2 mg/L was taken, sensitivity was 73.1% and specificity was 91.3%. D-dimer level was found to have a significant discrimination power (AUC = 0.928, p <0.0001, 95% CI (lower bound – upper bound) = 0.879 - 0.978) (**Table 3**) (**Figure3**).

Table 1. Distribution of the numbers and mean ages of the severe and non-severe COVID-19 patients between genders

	Mild	Severe	p
Female : n (%)	50 (% 54.3)	13 (% 50)	0.728 *
Age (year)	61 ± 7	62 ± 8.5	
Male: n (%)	42 (%45.7)	13 (% 50)	0.240 *
Age (year)	66 ± 7	63 ± 5	
Total: n (%)	92 (%100)	26 (%100)	0.862 *
Age (year)	63 ± 7	63 ± 6	

*Independent-Samples T-Test (data were shown as mean ± standard deviation)

http://dx.doi.org/10.36472/msd.v8i2.478

Table 2. Comparison of severe and non-severe COVID-19 patients' findings

	(n=92)	Severe (n=26)	p
Lymphocyte count(cell/mm3)	1729 ± 698	1129 ± 540	**< 0.001** *
D -Dimer (mg/L)	0.6 (0.2 – 21.3)	10 (0.7 – 35)	**< 0.001**†

*Independent-Samples T-Test (data were shown as mean ± standard deviation), †Mann-Whitney U test (data were shown as median (min-max))

Table 3. Determination of the ability of lymphocyte count and D-dimer level to predict severe and non-severe COVID-19 patients through ROC curve

Variables	AUC (% 95 CI)	Cut-off	p	Sensitivity (%)	Specifity (%)
Lymphocyte count	0.745 (0.644 – 0.846)	1500	< 0.001*	69.2	60.9
D-dimer level	0.928 (0.879 – 0.978)	2	< 0.001*	73.1	91.3

* ROC analysis of lymphocyte and D-dimer baseline values; AUC: Area under the curve; CI: Confidence interval; COVID-19: Coronavirus disease 2019; ROC: Receiver operating characteristic

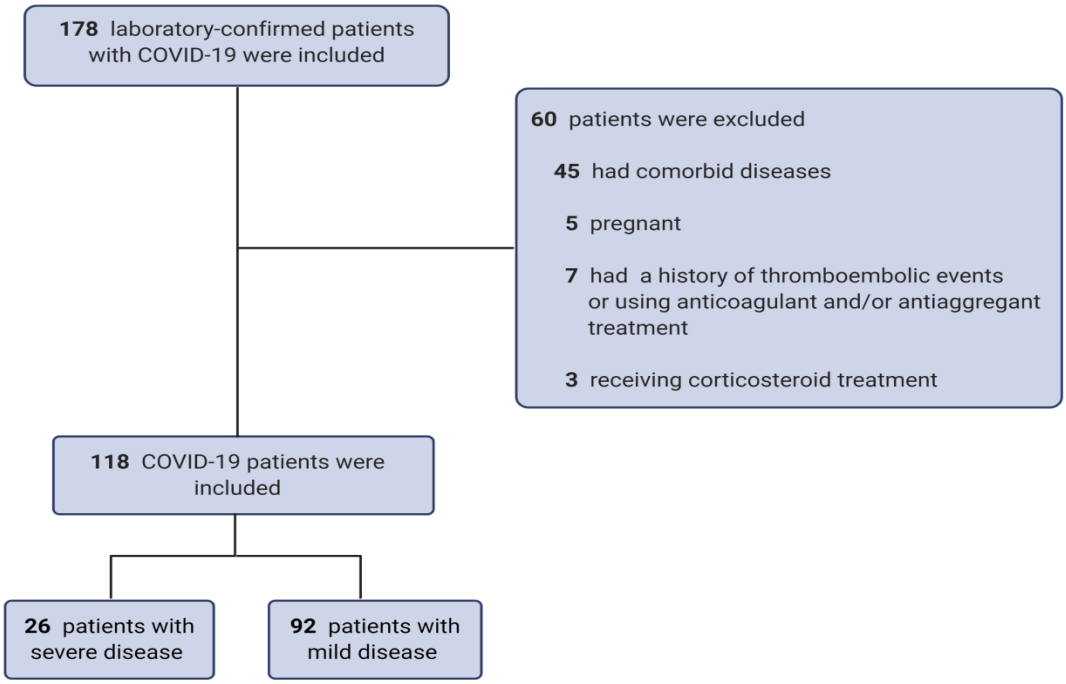

Figure 1. Flow chart of patients screening. Inclusion criteria and exclusion criteria were strictly applied throughout the screening process.

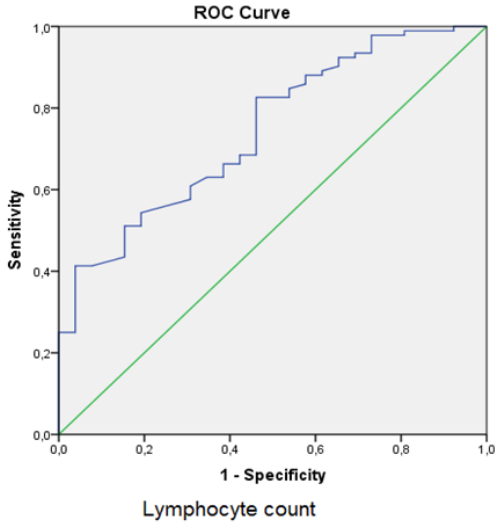

Figure 2. ROC analysis of lymphocyte baseline values. Notes: Lymphocyte count was set to a positive influence, and specificity and sensitivity of lymphocyte baseline values were plotted. Thecut-off point of lymphocyte count when the sum of specificity and sensitivity is maximized was 1500 cell/mm3 (sensitivity, 0.692; specificity, 0.609). Areaunderthecurve: 0.745 and 95% CI: 0.644-0.846. Abbreviations: CI, confidence interval; ROC, receiver operating characteristic.

http://dx.doi.org/10.36472/msd.v8i2.478

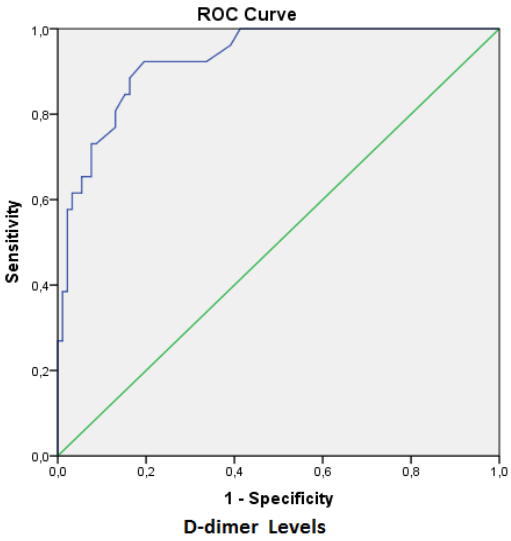

Figure 3. ROC analysis of D-dimer baseline values. Notes: D-dimer was set to a negative influence, and specificity and sensitivity of D-dimer baseline values were plotted. The cut-off point of D-dimer when the sum of specificity and sensitivity is maximized was 2 mg/L (sensitivity, 0.731; specificity, 0.913). Area under the curve: 0.928 and 95% CI: 0.879-0.978. Abbreviations: CI, confidence interval; ROC, receiver operating characteristic.

DISCUSSION

In the current study, our results indicate that lymphocyte count was lower and the D-dimer level was higher in patients with severe COVID-19 than non-severe COVID-19 patients. It was concluded that serum D-dimer levels and lymphocyte count had a significant discrimination power in predicting the prognosis of COVID-19 patients.

D-dimer is a product of the enzymatic breakdown of cross-linked fibrin by plasmin. However, high D-dimer levels are common in patients with a range of acute infectious and inflammatory diseases (**11**). In patients with COVID-19, the predominant coagulation abnormalities progress with clinical conditions that progress with hypercoagulation, and the risk of venous thromboembolism increases uncontrollably. This condition has been called by some experts as thrombo-inflammation or COVID-19-associated coagulopathy (CAC). Disseminated intravascular coagulation (DIC) has been reported in severely affected patients (**12**). Recent studies have shown that high D-dimer levels correlate with the severity of COVID-19 (**13**).

In severe COVID-19 patients, proinflammatory cytokines (IL-1, IL-6, and TNF-α) and chemokines (IL-8) were higher than those in non-severe cases. Although there is no direct evidence that cytokines and chemokines are responsible for lung pathology due to COVID-19, hyperinflammatory responses are considered to play a role in laboratory parameters (elevated serum chemokine and cytokine levels, increased neutrophil counts) in those with severe disease (**14**). In COVID-19, lymphopenia have been shown to be a severe disease predictor (**15**).

The study conducted by Wang et al. in 339 patients with COVID-19 was divided into four groups as mild, moderate, severe, and critical. Lymphopenia was detected in approximately 60% of all patients and 81.5% in patients who deceased.

It was concluded that the degree of lymphopenia might indicate the severity of the SARS-CoV-2 invasion or the state of antiviral immunity, thereby predicting the prognosis (**16**). In another study in this regard, it has been demonstrated that the lymphocyte count was found to be significantly lower in COVID-19 patients who were followed up in the intensive care unit compared to those followed in other clinical services (**17**). Our study shows that the lymphocyte level is significantly lower in the severe disease group than the non-severe group. Lymphocyte count 1500/mm3 and below were found to be reliable in predicting poor prognosis in COVID-19 patients. In COVID-19 patients, a decrease in CD4 and CD8 T cell numbers has been found, and this is considered to contribute to the progression of the disease with impaired immunity (**18**). The mechanism of reduced lymphocyte count in severe disease remains uncertain. The explanation of this mechanism shall serve as a guide for the treatment of severe patients. Apoptosis of lymphocytes is regulated by pro and anti-apoptotic mechanisms through endogenous and exogenous factors (**19**).

In SARS-CoV and MERS-CoV infections, T lymphocytes apoptosis induced lymphopenia has been reported (**15**). Patients with SARS have been found to have higher plasma Fas-ligand levels associated with higher intracellular cleavage caspase-3 positive CD4 and CD8 lymphocytes in the acute phase of the disease (**20**). In SARS-CoV-2 infection, the cytopathic effect caused by direct infection of T cells is emphasized. Other predicted lymphopenia mechanisms are sequestration in the lungs during extensive bilateral pneumonia and bone marrow suppression during cytokine storm (**21**). Flow cytometric analyzes have shown that the percentage of CD4+ T cells (CD3+ CD4+ CD45RA+) in peripheral blood increase and memory helper T cells (CD3+ CD4+ CD45RO+) decrease (**14**).

In a study conducted with 60 patients with COVID-19, total lymphocyte, CD4+ and CD8+ T cells, B cells and natural killer (NK) cells numbers were decreased. The authors emphasized the independent predictive value of the decrease in CD8+ T cells for COVID-19 severity and treatment efficacy (22).

The authors have conducted that D-dimer levels were higher in deceased patients due to COVID-19 than survivors (23). In a study investigating the relationship between D-dimer levels and mortality in patients with COVID-19, it was concluded that D-dimer levels higher than 2.0 µg/mL were independent predictors of mortality at the initial admission (24). According to our data, D-dimer levels at the time of admission were significantly higher in severe COVID-19 patients than non-severe group (p <0.001). Levels of 2 mg/L and above for D-dimer have been shown to be a reliable indicator that can be used to identify patients with poor prognosis. It is considered that this elevation of D-dimer in patients with COVID-19 may be due to increased systemic pro-inflammatory activation triggering the prothrombotic process. In autopsy findings of deceased patients with COVID-19, microvascular thrombosis has been observed in the lungs. The mechanism of thrombosis remains uncertain. It is considered that hypercoagulability may be directly related to endothelial injury, complement activation, or other procedures (12, 25).

SARS-CoV2 penetrates human cells by binding to angiotensin-converting enzyme 2 (ACE-2), which is highly expressed primarily in alveolar lung cells, cardiac myocytes, and vascular endothelial cells. Renin-angiotensin-aldosterone system (RAAS) pathway is activated by binding of the virus to ACE-2 and decreasing enzyme expression. Theoretically, RAAS activation leads to platelet adhesion and aggregation, thus carrying a risk of pulmonary embolism, pulmonary hypertension, and fibrosis (26).

Moreover, the dysfunction of endothelial cells induced by infection results in excessive thrombin production and inhibition of fibrinolysis, indicating the condition of hypercoagulability in patients with COVID-19 (10, 11). However, hypoxia observed in patients with severe COVID-19 increases blood viscosity and stimulates thrombosis through a signal pathway linked to hypoxia-induced transcription factors (HIF) (27). Viral infections cause a systemic inflammatory response and disrupt the balance between procoagulant-anticoagulant homeostatic mechanisms (28). Endothelial dysfunction, increased von Willebrand factor, tissue factor pathway and Toll-like receptor activation are considered to play a role in pathogenesis.Activation and interactions of monocytes, macrophages, platelets, lymphocytes, and endothelial cells play an important role in the formation of thromboembolic events observed in viral infections (29). Thromboembolic events have been reported in patients with influenza virus, human immunodeficiency virus (HIV), cytomegalovirus (CMV), herpes simplex virus (HSV), and other viral infections (30).

SARS-CoV and MERS-CoV infections are also associated with thrombotic events, similar to SARS-CoV-2 infection (31, 32). The prothrombotic effect of SARS-CoV remains mainly on the pulmonary vessels (33). Mononuclear cells infected with SARS-CoV have been revealed to express the procoagulant gene panel, characterized by an increase in factor II-II-X, fibrinogen, and SERPINs (D1 and A3). The Toll-like receptor 9 (TLR9) and thromboxane synthase (TBXAS) gene have been reported to be the target of SARS-CoV. Increased thromboxane production results in endothelial dysfunction, vasoconstriction, and platelet aggregation. TLR9 receptor is expressed from platelets and provides platelet activation, aggregation, and degranulation (34). DIC is one of the major complications reported in fatal MERS-CoV infections. It has been further reported that the effect of MERS-CoV on the coagulation cascade is associated with human dipeptidyl peptidase 4 (hDPP4) (35).

This study has a few limitations. The first one is that only the values of the laboratory parameters examined during the application have been taken into consideration and the changes in the following days have not been not followed. The second one is that patients with high D-dimer had not been further investigated for pulmonary embolism.

CONCLUSION

The values of ⩽ 1500/mm3 lymphocyte and ⩾2 mg/L D-dimer are can be used in the early determination of patients with good and poor prognosis in COVID-19. Thanks to the precautions to be taken as a result of determining patients with poor prognosis in the early stages and rapid treatment decisions, the morbidity, and mortality of these patients will be reduced.We think that targeted therapies will replace standard treatment regimens in viral infections. The relationship between viral infections and thromboembolic complications will be clearly enlightened and prevention and treatment strategies will be developed for this. Immune-modulator treatment options will come to the fore in viral infections.

Acknowledgments:We would like to thank the entire healthcare professionals working in the COVID-19 pandemic process and the entire scientists who have contributed to the diagnosis, treatment, and management of the pandemic through clinical trials.

Author contributions: FÇ, ŞÖA, FY, AT, FK, MŞ, EE, FÇ; Literature search and study design, patient examinations, data collection and analyzes **FÇ;** Writing article and revisions

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. This research did not receive and specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical issues: All authors declare originality of research.

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MSD

Medical Science and Discovery
ISSN: 2148-6832

Prostate-Specific antigen value and micro RNAs as potential diagnostic biomarkers for prostate cancer

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ABSTRACT

Objective: It is necessary to provide PSA alternatives or methods that can be used in conjunction with PSA to regress complications rising from negative biopsies and to increase diagnostic value.

Patients and Methods: The study is consisting of 59 men as the sample group. Blood samples from the individuals are grouped as prostate cancer and BPH (benign prostatic hyperplasia) groups. 27 prostate cancer patients whom some of them also operated are assembled in the patients group and the other 32 individuals are grouped as BPH group. Micro RNA expression levels evaluated by RT-PCR.

Results: Prostate cancer group when compared with the control group, it is observed that expression levels of miRNA-221 and miRNA-432 increased while expression levels of miRNA-17-5p, miRNA-30c, miRNA-107, miRNA-141, miRNA-145, miRNA-181a-2, miRNA-331-3p, miRNA-574-3p decreased and expression levels of miRNA-21 and miRNA-375 are quite similar between the groups.

Conclusion: The prospect of strong and sensitive serum miRNA expression levels in prostate cancer cases which are easily detectable by non-invasive methods as biomarkers is a promising field of study. Nevertheless, it is currently necessary to work in conjunction with both tissue and serum to enhance both sensitivity and specificity of miRNAs as biomarkers. As such, expression levels of the same miRNAs in tissue and serum provide different expression values which in turn make it difficult to indicate a common biomarker.

Keywords: Biomarker; miRNA; cancer; BPH; prostate.

INTRODUCTION

Prostate cancer is the second most commonly diagnosed malignancy in men and is the second leading cause of cancer death (1). The clinical behavior of prostate cancer ranges from a microscopic, well-differentiated tumor that may never be clinically significant to an aggressive, high-grade cancer that ultimately causes metastases, morbidity, and death. Clinically, prostate cancer is diagnosed with a transrectal ultrasound-guided prostate biopsy (TRUS) because of a suspicious serum prostate-specific antigen (PSA) and /or abnormal digital rectal examination findings usually used biomarker for the detection of prostate cancer, is limited by its lack of sensitivity and specificity for prostate cancer because benign prostate hyperplasia (BPH), prostatic inflammation and infection may cause elevated PSA, therefore not considered an ideal biomarker. Additionally prostate cancer screening and prevention trials, such as The Prostate, Lung, Colorectal, and Ovarian cancer screening trial (PLCO), the European Randomised Study of Screening for Prostate cancer (ERSPC) trial, Prostate Cancer Prevention Trial (PCPT) and Reduction by Dutasteride of Prostate Cancer Events (REDUCE) trial have highlighted that despite an increase in the diagnosis of prostate cancer using PSA and DRE (digital rectal examination), there is still no clear improvement in mortality (2).

Research Article

Received 30-01-2020

Accepted 16-02-2021

Available Online: 17-02-2021

Published 24-02-2021

http://dx.doi.org/10.36472/msd.v8i2.479

To reduce unnecessary biopsies and to improve the effect of screening modalities on mortality of prostate cancer are major goals of researchers. As a result, a search for a novel, minimally invasive, clinically relevant biomarkers for the detection of prostate cancer is required. MiRNAs are ~20–22 nucleotide long noncoding RNAs, which regulate gene expression at the post-transcriptional level by mRNA repression and/or degradation. It is becoming clear that miRNAs represent a vast, previously unrecognized layer of molecular signaling in eukaryotes, and that miRNAs play an important role in the regulation of protein expression (**3**). Several studies have investigated miRNAs as possible diagnostic or prognostic biomarkers for malignancies and other diseases. The experimental over-expression or inhibition of specific miRNAs in cellular and animal models can result in tumorigenesis, or in more aggressive cancer phenotypes, indicating the potential of miRNAs to function as oncogenes and tumor suppressors (**4**). Many miRNAs are known to be dysregulated in prostate cancer (**5**). For instance, miRNAs are differentially expressed between benign prostate and prostate cancer tissues: in a genome-wide microarray-based microRNA expression study, 25 miRNAs were found to be deregulated (**6**). Some of the miRNAs are dysregulated in prostate cancer tissue and some of the miRNAs are dysregulated detectable in the circulation of patients with prostate cancer (**7**). Consequently miRNAs may be novel, useful, stable, non-invasive biomarkers. This study aimed to assess and compare the levels of specific miRNA between men with prostate cancer and BPH.

MATERIAL AND METHODS

In our study, 2 groups were formed, including the BPH group (control group) of 32 individuals without prostate cancer and the prostate cancer group of 27 individuals with prostate cancer patients whom some of them also operated. The descriptive data as PSA, prostate volume (PV), age, body mass index (BMI), and Gleason score are collected from the individuals in this study. All the individuals are diagnosed by Cumhuriyet University Medical School Application and Expertise Hospital Urology Department between dates June 2014 and November 2014. Ethical approval has been granted before study by Medical School Ethical Commission (Date of Approval: 24.12.2013, Approval Number: 2013-12/16).

Isolation of Serum Samples

Blood samples taken into 3cc biochemical tubes are centrifuged by 10 min. at 4000 rpm. Serum portions of samples are taken into sterile 2 ml Eppendorf tubes and centrifuged under 100C cooling by 5 min. centrifuge at 10000 rpm. Supernatant taken into sterile 2 ml Eppendorf tubes and second high-speed centrifugation is done to remove the remaining cell remnants. The resulting serum samples are taken into sterile micro centrifuge tubes and stored at -800C until RNA extraction (**8**).

RNA Isolation and Reverse Transcription (RT) Reaction

Total RNA extracts from serum samples were obtained by following the manufacturer's directives using miRNeasy Mini Kit (Qiagen, cat. no: 217004). Total RNA samples are converted to cDNA by using Qiagen MiScript reverse transcription kit (Qiagen, Cat. No: 218161). cDNA samples are stored under -800C until PCR. 125 to 250 ng total RNA is used as starter for miRNA panel. For inactivation of MiScript Reverse Transcriptase, hold at 950C for 5 min. and 200 µl water is added then protocol is followed (**8**).

Preamplification of cDNA and qPCR

Twelve different miRNAs (hsa-miR-17-5p, hsa-miR-21-3p, hsa-miR-30c-1-3p, hsa-miR-107, hsa-miR-141-5p, hsa-miR-145-3p, hsa-miR-181a-2-3p, hsa-miR-221-5p, hsa-miR-331-3p, hsa-miR-375, hsa-miR-432-3p, and hsa-miR-574-3p) are selected for the study. These sequences are formed into a panel by a commercial service and bought as qPCR kit (Qiagen). In addition to pre-emptive 12 miRNAs, 4 control miRNA wells are used in custom miScript miRNA PCR Array (Qiagen, cat. no: 218300). For preamplification purposes miScript SYBR Green PCR kit is used. 2 µl cDNA sample is transferred into a clean well on 96-well plate and 9 µl DNA suspension buffer is added onto the well which then reacts thoroughly mixed. The 1/5 diluted 2 µl RT sample is preamplified in a PCR tube using 2x quantiTec SYBR Green master mix and 10x miScript Universal Primers. Real-Time PCR plate formation includes selected 12 miRNAs with specific forward-reverse primers for each and 4 universal control primers (Applied Biosystems). All the conditions are optimized in accordance with manufacturer directives to cover all samples (**8**).

Statistical Analysis

Obtained data uploaded into SPSS v.22.0 and analyzed. When parametrical test defaults are met Kolmogorov–Simirnov significance test of the difference between the two average is concluded, whereas when parametrical test defaults are not met, Mann Whitney U Test and Correlation Analyses are concluded. The error rate for analyses is chosen as 0.05 (P value).

RESULTS

In this study, a total of 59 patients of 27 prostate cancer and 32 BPH patients were contrasted by both clinical and genetic data using 12 miRNA regions compiled from the literature. The mean age was 65.35± 9.01 for prostate cancer patients and 64.29±4.61 for BPH patients, of which age contrast between groups is insignificant (P>0.05). Gleason scores of patients with prostate cancer range between 6 to 9 and the respective average is 7.85±0.948. Also, some metastasis conditions were observed in the different individuals within the prostate cancer group. Indeed 19 patients with prostate cancer (70.4%) showed no metastasis whereas 8 patients (29.6%) have shown metastasis to the bone of which 6 patients (22.2%) with single point metastasis and 2 patients (7.4%) with multiple point metastasis. The expression level of miRNA-30c is found to be the lowest value in both groups. The highest expression levels are obtained from miRNA-221 (7.459±3.887) value in patients with prostate cancer group and miRNA-181a-2 value (9.711±14.604) in BPH patients group. There are statistically significant differences between the groups according to the expression levels of the different miRNAs except for miRNA-21 and miRNA-375 levels (**Table 1**). It is observed that the expression levels of miRNA-17-5p, miRNA-30c, miRNA-107, miRNA-141, miRNA-145, miRNA-181a-2, miRNA-331-3p, and miRNA-574-3p are found to be lower in the patients with prostate cancer than the individuals with BPH.

 http://dx.doi.org/10.36472/msd.v8i2.479

Table 1. Comparing the groups according to miRNA expression value (*: P<0.05, miR: miRNA: micro RNA)

	Prostate Cancer			Benign Prostatic Hyperplasia			Statistical Value
miRNA name	**Min**	**Max**	**Mean±SD**	**Min**	**Max**	**Mean±SD**	**P**
miR**17-5p**	0.404	15.616	2.281±2.866	0.125	17.267	3.418±3.601	**0.034***
miR**21**	0.062	4.723	1.608±1.167	0.059	7.412	1.632±1.397	0.922
miR**30c**	0.003	0.027	0.007±0.005	0.004	0.56	0.023±0.014	**0.001***
miR**107**	0.188	24.16	3.599±5.553	0.079	34.775	6.495±7.178	**0.008***
miR**141**	0.061	4.084	0.139±0.941	0.014	3.797	1.315±1.007	**0.027***
miR**145**	0.034	4.69	0.835±0.956	0.015	3.810	1.279±0.889	**0.007***
miR**181a-2**	0.142	11.004	2.896±2.762	0.126	68.119	9.711±14.604	**0.009***
miR**221**	0.208	16.564	7.459±3.887	0.003	22.471	3.109±4.405	**0.050***
miR**331-3p**	0.639	15.030	2.298±4.193	0.096	12.728	5.423±3.491	**0.047***
miR**375**	0.360	7.972	2.441±1.914	0.062	10.966	2.864±2.848	0.814
miR**432**	0.088	8.027	4.765±1.885	0.008	2.445	0.799±0.648	**0.046***
miR**574-3p**	0.213	4.469	1.035±1.098	0.049	11.004	3.508±2.575	**0.034***

Table 2. Comparing PSA value and prostate volume between the groups (* :P<0.05, PSA: prostate-specific antigen, PV: prostate volume)

	Prostate Cancer			Benign Prostatic Hyperplasia			Statistical value
	Min	**Max**	**Mean±SD**	**Min**	**Max**	**Mean±SD**	**P**
PSA (ng/ml)	5.43	1778.2	111.52±335.05	3.89	19.48	8.06±3.70	**0.001***
PV (ml)	17.00	136.0	52.77±34.80	20.00	138.00	65.61±30.42	**0.032***

However, expression levels of miRNA-221 and miRNA-432 are found to be higher in the individuals with prostate cancer. Nevertheless, these three miRNA (miRNA-221, miR-331-3p and miRNA-432) expression levels have a low significance (P=0.05; P=0.047 and P=0.046 respectively) compared to others (Table 1). The average of PSA values are found as 111.52 ± 335.05 ng/mL in prostate cancer patients and 8.06 ± 3.70 ng/mL in BPH group. PSA values in the prostate cancer group were statistically higher than the BPH (control) group values (**Table 2**).

The difference about PSA values between the two groups was found to be statistically significant (P<0.05) (Table 2). Prostate volume (PV) values are 52.77 ± 34.80 mL in the cancer patients group and 65.62 ± 30.42 mL in the BPH group. PV values of the BPH group were higher than the prostate cancer patients group (Table 2). In terms of PV comparison, the difference between both groups was found to be statistically significant(P<0.05) (Table 2). Prostate volume values and PSA values are usually used for the cancer diagnostic and these are the important data for determining the prostate cancer. Therefore, finding a relationship between PSA and PV values and any miRNA values will be important in terms of finding a marker that can be used practically in the diagnosis of prostate cancer (Table 3). There was a positive correlation between PSA and PV values in prostate cancer group (correlation coefficient: 0.393) and this correlation is found to be statistically significant (P<0.05, **Table 3**).

However, no correlation was found between PSA and PV values in the BPH group (Table 4). Also, a significant positive correlation was found between PSA values and miR-432 in the prostate cancer group (correlation coefficient: 0.435).

In addition a positive correlation was found between PV values and miR-30c expression level in this group (correlation score: 0.502). On the other hand, a negative correlation is observed between Gleason score and miRNA-30c with the correlation coefficient -0.387 and a positive correlation is observed between Gleason score and miRNA-574-3p with the 0.464 correlation coefficients (Table 3). Even though these correlations are statistically significant, their relationship values are found to be weak.

Additionally there are no general correlation between Gleason score, PSA and PV values and other miRNAs' expression levels (Table 3). Among the 12 miRNAs we investigated in our study, serious correlations were detected between some micro RNAs. These results have shown that no direct relation is present between PSA and respective miRNA expressions. There is a weak positive correlation observed between PV and miRNA-30c expression (correlation score: 0.502) but no correlation has been seen for other miRNAs. Detailed correlation analyses between miRNAs are shown in Table 3.

Two-way correlation analyses were conducted on the individuals consisting BPH using PSA, PV and miRNA expression and no correlation has been observed between PSA and PV in this group. While PSA and miRNA-145 provided a positive and weak correlation (correlation coefficient: 0.391). There is another positive and weak correlation found between PV and miRNA-30c (correlation coefficient: 0.481), but no correlation has been observed for other miRNAs. Detailed correlation analyses between miRNAs are shown in Table 4. Also when we made 2 groups according to PSA value under and over 10; just miRNA-30c is statistically significant but has a weak correlation (P: 0,092; P<0,10)

doi http://dx.doi.org/10.36472/msd.v8i2.479

Table 3. The correlation analysis between Gleason score, miRNA expression level, PSA, and prostate volume inside the prostate cancer group. (miR: miRNA: micro RNA, PSA: prostate-specific antigen, PV: prostate volume)

Correlation Value	Gleason	miR 17-5p	miR 21	MiR 181a-2	miR 30c	miR 145	miR 107	miR 141	miR 331-3p	miR 574-3p	miR 432	miR 221	miR 375	PSA	PV
Gleason	1	0.122	-0.25	-0	**-0.387***	0.309	0.101	-0.062	0.078	**0.464***	-0.08	0.023	0.052	0.25	0.351
miR**17-5p**	0.122	1	**0.401***	**0.648****	-0.031	-0.034	**0.840****	0.258	**0.618****	0.163	0.098	-0.09	-0.11	-0.05	-0.26
miR**21**	-0.254	**0.401***	1	0.289	0.359	0.025	**0.624****	**0.488***	**0.722****	0.082	**0.602****	-0.09	0.136	0.091	0.119
miR**181a-2**	-0.001	**0.648****	0.29	1	-0.043	0.008	**0.668****	0.243	**0.675****	0.069	-0.04	0.16	0.073	-0.04	-0.2
miR**30c**	**-0.387****	-0.03	0.36	-0.04	1	0.075	0.297	0.204	0.095	-0.14	0.029	-0.13	0.217	-0.06	**0.502****
miR**145**	0.309	-0.03	0.03	0.008	0.075	1	-0.1	0.328	0.014	0.349	-0.17	0.018	**0.703****	0.049	0.143
miR **107**	0.101	**0.840****	**0.624****	**0.668****	0.297	-0.103	1	0.292	**0.760****	0.024	0.093	-0.12	-0.21	-0.1	-0.1
miR**141**	-0.062	0.258	**0.488***	0.243	0.204	0.328	0.292	1	0.203	0.224	**0.408***	-0.04	**0.482***	-0.08	0.126
miR**331-3p**	0.078	**0.618****	**0.722****	**0.675****	0.095	0.014	**0.760****	0.203	1	0.219	0.031	-0.19	-0.24	-0.08	-0.3
miR**574-3p**	**0.464****	0.163	0.08	0.069	-0.136	0.349	0.024	0.224	0.219	1	0.087	0.008	**0.393***	0.046	-0.15
miR**432**	-0.08	0.98	**0.602****	-0.04	0.029	-0.165	0.093	**0.408***	0.031	0.087	1	-0.08	0.125	**0.435****	-0.06
miR**221**	0.023	-0.09	-0.09	0.16	-0.127	0.018	-0.12	-0.04	-0.194	0.008	-0.08	**1**	0.276	-0.11	0.252
miR**375**	0.052	-0.11	0.14	0.073	0.217	**0.703****	-0.214	**0.482***	-0.244	**0.393***	0.125	0.276	**1**	-0.01	0.323
PSA	0.25	-0.05	0.09	-0.04	-0.061	0.049	-0.1	-0.081	-0.08	0.046	**0.435***	-0.11	-0.01	**1**	**0.393***
PV	0.351	-0.26	0.12	-0.2	**0.502****	0.143	-0.1	0.126	-0.302	-0.15	-0.06	0.252	0.323	**0.393***	**1**

http://dx.doi.org/10.36472/msd.v8i2.479

DISCUSSION

It is known that various oncogenes and tumor-suppressing genes are taking place in prostate cancer and their respective expressions increase or decrease by epigenetic regulations (**8, 9**). Since the last decade, many studies have been conducted on miRNAs from the point that they may take place in regulations of cancer-related genes and many studies are still ongoing (**10**). Many studies on prostate cancer have shown that some of miRNAs exhibit increased expression whereas other miRNAs decreased in expression. It has been stated in some studies that certain miRNA expressions are correlated with Gleason scores and might be related to clinical recurrences (**11**). According to CAPRA scoring, patients with severe risk have provided increased expression levels for miRNA-20a and miRNA-21 while patients with mild to severe risks have provided increased expression levels for miRNA-21, miRNA-17-5p and miRNA-145 (**12, 13**). It is also pointed out that they can be used to differentiate patients with mild, medium and severe risks and to estimate the aggressiveness of prostate cancer (**12**). We have observed 35% depressed expression of miRNA-17-5p among prostate cancer patients compared to BPH patients. There is a negative correlation observed between PSA and miRNA-17-5p expression and no significant correlation with Gleason score. Also, it is showed increased expression of miRNA-181a-2 in both tumoral tissue and serum (**14**). It has been stated that many different miRNAs have shown increased expression in tumors with Gleason scores ranging 8 (4+4) to 9 (4+5) (17). Some of studies have pointed out that miRNA-181 is functioning in the differentiation of hemopoietic cells and might take place in the formation of leukemia and other solid tumors (**15, 16**). BPH patients in the present study have shown 60% increased expression for miRNA-181 which is in accordance with previous studies. For this miRNA, there is a negative and weak relationship observed. Nevertheless, some researchers have stated that miRNA-181a-2 expression has increased among patients with higher Gleason score (**14**) we have not observed such a significant correlation in the present study. A significant relationship has been reported between the expression of miRNA-375 and miRNA-141 in various studies conveying metastatic prostate cancer patients that exhibit lymph node metastasis and higher Gleason score (**17**). Expression of miRNA-375 is also increased in breast cancer and since these two cancer kinds are similar in endocrinological and physiopathological aspects it has been stated that this miRNA is an oncogene for both cancers (**18**). Expression levels of miRNA-375 in the present study are found similar to each other. Additionally, no significant relationship has been observed for this miRNA expression with PSA and Gleason score in our study. It has been shown that miRNA-331-3p in prostate cancer cells reduces PSA expression by inhibiting androgen-sensitive promoter region of PSA gene which in turn results in lower expression in cancer cells compared to normal cells (**19**). Expression of miRNA-331-3p has been observed 60% reduced in prostate cancer patients compared to BPH patients in our present study which is in accordance with previous studies. Also there is a negative weak correlation observed between miRNA-331-3p expression and PSA levels. Some studies have reported diffuse reduced regulation of miRNAs in prostate cancer tissues (**20**). Expression levels of miRNAs are quite variable in tumoral tissue and serum and therefore differential diagnosis of these values improves findings. Tissue expression levels of miRNA-21 have shown an increase in many kinds of cancer including prostate cancer (**21**). Expression levels of miRNA-21 in the present study for both prostate cancer patients and BPH patients groups are similar. Also, there is a positive but weak correlation observed between miRNA-21 and PSA (**Table 3).** The contrast observed to other studies might be due to the expression of miRNA-21 is evaluated only in serum in the present study. Increased expression of miRNA-221 in prostate cancer has been reported (**20**). In contrast, some other studies indicated that miRNAs might be oncomirs and progressive reduction in expression of miRNA-221 in aggressive prostate cancer is possibly due to Gleason score, progression level, metastasis, and clinical recurrence (**22**). In present study, expression of miRNA-221 in prostate cancer patients was observed 2 times increased compared to BPH patients, and the expression difference is found to be significant ($P<0.05$) (Table 1). Also, there is a negative and weak correlation observed between miRNA-221 and PSA. MiRNAs which secreted from tumoral tissue into the blood stream can be used as biomarkers both in serum and plasma samples. Increased regulation of miRNA-141 in metastatic prostate cancer patients is also reported (**23**). It has been reported that miRNA-141 and miRNA-375 can be used as circulatory markers for metastatic prostate cancer patients both in serum and plasma (**24**). Conversely, in the present study such increase is not present and 85% actual depression in the expression of miRNA-141 in prostate cancer patients observed compared to BPH patients ($P<0.05$). Expression of miRNA-145 in the present study for prostate cancer patients observed as 30% reduced compared to BPH patients ($P<0.05$; Table 1) and showed a positive yet weak correlation with PSA. In another study where the evaluation of serum/plasma levels of different miRNAs in prostate cancer patients has shown reduced regulation of miRNA-30c (**25**). Another study has indicated that one of the 34 miRNAs with increased expression in prostate cancer tissue is miRNA-30c (**26**). For prostate cancer patients in the present study miRNA-30c exhibit, 70% reduced expression and provide a negative weak correlation with PSA. Also, a negative correlation between miRNA- 30c and Gleason score and a positive correlation between miRNA-30c and PV are observed in our present study. Various studies have pointed out that miRNA-107 and miRNA-574-3p are being prominent among miRNAs with increased expression in prostate cancer (**27**). Also, it is reported that the expression of miRNA-574-3p is greatly depressed in prostate tumoral tissue and this depression is found to be correlated with both Gleason score and progressed tumoral state (**28**). In our study, the expression level of miRNA-107 is found to be 45% lower in prostate cancer patients compared to BPH patients, and also a negative and very weak correlation with PSA observed (correlation coefficient: -0.1). In additionally the expression level of miRNA-574-3p in prostate cancer patients is obtained with a depressed value with 70% ratio compared to BPH patients and provided a positive weak correlation with PSA (correlation coefficient: 0.046) and a strongly positive correlation with Gleason score (correlation coefficient: 0.464). On the other hand, the expression level of miRNA-432 in prostate cancer patients observed as 85% increase compared to BPH patients and provided a positive strong

correlation with PSA (correlation coefficient: 0.435). Notably there is not much report on the relationship of this miRNA with prostate cancer. Porkka et al. have conducted a study using 319 different miRNAs and have reported that 51 of these miRNAs exhibited either increased or decreased expression in cancerous tissue compared to normal tissue. Also indicated that reduced expression is observed for 22 of these 51 miRNAs in all prostate cancer cases while 15 of them only in hormone-resistant cancers. In another study, it has been reported that 8 miRNAs are found to be increased in expression for all prostate cancer cases while 6 miRNAs are found to be increased in expression only in hormone-resistant prostate cancer cases (9). Upregulation of miRNA expression levels in prostate cancer cases has been generally supported by different comparative studies. In the present study, it is observed that expression levels of miRNA-221 and miRNA-432 are increased whereas expression levels of miRNA-17-5p, miRNA-30c, miRNA-107, miRNA-141, miRNA-145, miRNA181-a2, miRNA-331-3p, miRNA-574-3p are decreased for prostate cancer patients contrasted to BPH patients. Expression levels of miRNA-21 and miRNA-375 are found to be similar for both groups. As a summary, it is observed that of 12 miRNAs selected for our present study expression levels of 2 miRNAs (17%) observed as similar, another 2 miRNAs (17%) observed as increased and the remaining 8 miRNAs (66%) observed as decreased for prostate cancer patients compared to BPH patients (Table 1).

CONCLUSION

The prospect of strong and sensitive serum miRNA expression levels in prostate cancer cases which are easily detectable by non-invasive methods as biomarkers is a promising field of study. Nevertheless, it is currently necessary to work in conjunction with both tissue and serum in order to enhance both sensitivity and specificity of miRNAs as biomarkers. As such, expression levels of the same miRNAs in tissue and serum provide different expression values which in turn makes it difficult to indicate a common biomarker. Within the scope of this research, it was possible to work with a small sample group and the expression level of only 12 miRNAs was investigated. Although these are restrictive, our results are still informative and guiding for scientists working in this field. Therefore studies conveying comparative analysis of miRNAs in prostate cancer cases, BPH patients, and healthy individuals would hopefully provide determinative results into the subject for the future.

Author contributions: AA, SDD, EK, TYA, HS, RC; Literature search and study design, patient examinations, data collection and analyzes **AA;** Writing article and revisions

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. This research did not receive and specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical issues: All authors declare originality of research.

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MSD

Medical Science and Discovery
ISSN: 2148-6832

Analysis of compound muscle action potential in patients with chronic inflammatory demyelinating polyneuropathy and Charcot-Marie-Tooth disease type 1A

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ABSTRACT

Objective: To provide an additional contribution to the differential diagnosis of Charcot-Marie-Tooth disease type 1A (CMT1A) and chronic inflammatory demyelinating polyneuropathy (CIDP) by analyzing distal duration and proximal/distal amplitude and duration ratios on different nerves in these diseases that show demyelinating peripheral neuropathy features.

Material and Methods: We retrospectively reviewed the electromyography (EMG) findings of patients aged 18-80 years who were followed up with a diagnosis of acquired and hereditary demyelinating type polyneuropathy in the neuromuscular diseases outpatient clinic in our center. We analyzed the distal CMAP duration and amplitude, proximal and distal compound muscle action potential, and duration ratios on each nerve in the patient groups, separately.

Results: The CIDP group had significantly longer Peroneal nerve distal duration than the CMT1A group (p=0.04). Median, ulnar, and tibial nerve distal durations were similar between the groups (p=0.84, p=0.86, and p=0.13, respectively). The median nerve, ulnar nerve, and peroneal nerve proximal/distal amplitude ratios were not different between the CMT1A and CIDP groups (p=0.99, p=0.38, and p=0.16, respectively). The tibial nerve proximal/distal amplitude ratio in the CIDP group was lower than in the CMT1A group (p=0.003). Median, ulnar, peroneal, and tibial nerve proximal/distal duration ratios were statistically similar among the groups (p=0.21, p=0.66, p=0.62, and p=0.46, respectively).

Conclusion: This study may help to improve the management of challenging patients where there is an overlap between hereditary and inflammatory neuropathies. The different electrodiagnostic models of various acquired and hereditary demyelinating polyneuropathies should be clinically recognized.

Keywords: Compound muscle action potential, Duration, Demyelinating polyneuropathy

Research Article

Received 31-01-2020

Accepted 15-02-2021

Available Online: 17-02-2021

Published 24-02-2021

INTRODUCTION

Chronic inflammatory demyelinating polyneuropathy (CIDP) is an autoimmune-induced, demyelinating polyneuropathy (PNP) with a chronic progressive nature, or relapses and remissions. The basic pathology is the removal of myelin from axons via macrophages, the most striking feature of which is multifocal demyelination. However, type, the number, and the location of demyelinating lesions vary between CIDP subgroups and patients (**1**). Charcot-Marie-Tooth (CMT) disease, i.e. hereditary sensory and motor neuropathies, includes genetically heterogeneous hereditary neuropathies among which CMT1A is the most common form with autosomal dominant inheritance, and duplication in 17 p11.2-12 regions encoded by the peripheral myelin protein 22 (PMP 22) genes cause the disease. The classic clinical picture is distal muscle weakness that begins in childhood or adolescence and progresses slowly. However, cases can occur in adulthood.

Despite the guidance of family history and clinical findings, difficulties may be experienced in the differential diagnosis of hereditary motor sensory neuropathies with CIDP (**2, 3**).

Electromyography (EMG) helps in the diagnosis of PNP by detecting findings specific to acquired demyelinating neuropathies, showing that nerves, and especially myelin sheaths, are affected in most cases.

However, 48-64% of patients with CIDP may not show typical signs such as segmental conduction slowdown, or severely prolonged terminal latency, or conduction blocks. CMT cases with nerve conduction blocks have also been reported, albeit rarely.

In this study, distal duration and proximal/distal amplitude ratios on different nerves were analyzed in nerve conduction studies in CIDP and CMT1A patient groups showing major demyelinating peripheral neuropathy features, and it was aimed to examine the electrophysiologic features that made an additional contribution to the differential diagnosis of these diseases.

For this purpose, we hypothesized that distal duration and proximal/distal amplitude ratios would differ from the hereditary polyneuropathy group in patients with acquired polyneuropathy.

MATERIAL AND METHODS

The EMG findings of those at age of 18-80 years who were followed up with a diagnosis of acquired and hereditary demyelinating type PNP, whose diagnoses were established through clinical, genetic, and advanced laboratory examinations in the neuromuscular diseases outpatient clinic in our center, were retrospectively analyzed. The diagnosis of all cases of CMT1A was genetically confirmed.

The diagnosis of CIDP was made according to nerve conduction studies, cerebrospinal fluid (CSF) examination, and clinical features after the clinical criteria of EFNS/PNS (**4**). Those with toxin exposure, vitamin B12 deficiency, and diabetic PNP, uremic PNP, and additional systemic diseases were not included in the study. Ethics committee approval was obtained before the study (Decision number: 2.04.2019 / 1230).

In the electrophysiologic examination, the motor responses obtained using standard supramaximal stimulation techniques and the superficial electrode of each participant were evaluated.

The proximal and distal CMAP, which were recorded using standard methods, of the median nerve with the abductor pollicis brevis, the ulnar nerve with the abductor digiti minimi, the tibial nerve with the abductor hallucis, and the peroneal nerve with the external digitorum brevis, were retrospectively reviewed.

The baseline to the negative peak value was defined as the amplitude value. Duration of CMAP was accepted as the negative peak duration at 500 mV sensitivity. Distal CMAP duration, proximal and distal CMAP ratios were analyzed separately in the examined nerves.

Ratios of distal and proximal amplitude were determined as the ratio of CMAP amplitude (mV) of elbow stimulation / CMAP amplitude (mV) of wrist stimulation for median and ulnar nerves, and the ratio of CMAP amplitude (mV) of knee stimulation / CMAP amplitude (mV) of ankle stimulation for peroneal and tibial nerves. Proximal and distal t duration ratios were similarly calculated by proportioning the proximal CMAP duration to the duration of distal CMAP in each nerve.

Statistical Analyses

In the study, the statistical analysis was done using SPSS 22.0 program (Chicago, IL). The Kolmogorov-Smirnov test was used to see if the data complied with normal distribution, and the analysis of correlations between numerical variables was evaluated using Pearson's correlation test. Descriptive results for variables with normal distribution are expressed as mean±standard deviation (SD).

The t-test and/or the Mann-Whitney U test were used to evaluate differences between groups. Categorical variables are expressed as ratios and percentages. The results were compared using the Chi-square test. $p<0.05$ showed statistically significance in all tests.

RESULTS

A total of 22 patients with CMT1A and 26 with CIDP participated in the study. The 22 patients with CMT1A were aged between 18 and 80 years, 13 were female and nine were male. The mean disease duration was 7.5±6.2 (range, 1-26) years, and the meantime from the onset of symptoms to diagnosis was 5.72±6.67 (range, 1-28) years. The 24 patients CIDP were aged between 19 and 78 years, eight were female and 16 were male. The mean disease duration was 8.6□6.1 (range, 1-26) years, the mean age at disease diagnosis was 49.8±15.4 (range, 18-76) years, and the meantime from the onset of symptoms to diagnosis was 4.8±4.6 (range, 1-15) years.

When the sex distribution, age, age during diagnosis, duration of the disease, and the interval between the start of symptoms and the diagnosis were compared, the CMT1A and CIDP groups showed no statistically significant difference ($p>0.05$) (**Table 1**).

the CMT1A and CIDP groups did not show a difference in the ulnar, median, and peroneal nerve proximal/distal amplitude ratios, but the tibial nerve proximal/distal amplitude ratio was statistically lower in the CIDP group compared with the CMT1A group ($p=0.99$, $p=0.38$ $p=0.16$, and $p=0.003$, respectively).

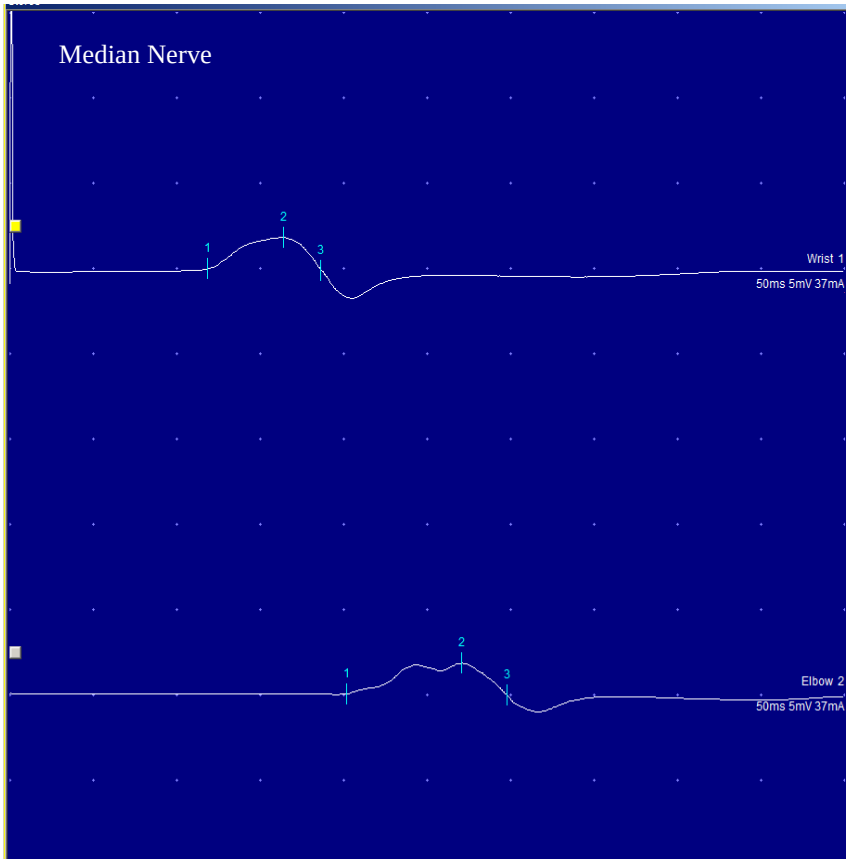
Ulnar, median, tibial, peroneal and nerve proximal/distal duration ratios were statistically similar among the patient groups ($p=0.21$, $p=0.66$, $p=0.62$, and $p=0.46$, respectively). Motor response examples of the ulnar nerve, the median nerve, peroneal nerve, and tibial nerve in patients with CMT1A and CIDP are shown in **Figures 1 and 2**.

http://dx.doi.org/10.36472/msd.v8i2.480

Table 1: Demographic features and EMG measurements of the CMT1A and CIDP groups

Parameter	CMT1A (n=22)	CIDP (n=26)	P
Age (years)	47.9±16.4	55±15.2	**0.11**
Sex			**0.08**
Female	13	8	
Male	9	18	
The time passed between symptom onset and diagnosis (years)	5.72±6.7	4.84±4.6	**0.65**
Age during diagnosis (years)	44.4±17.5	49.8±15.4	**0.26**
Disease duration (years)	7.5±6.3	8.9±6.2	**0.39**
Distal duration (mean)			
Median nerve	6.6 ±1.66	6.71±2.01	**0.84**
Ulnar nerve	7.21±1.51	7.23±1.02	**0.86**
Tibial nerve	4.85±2.94	7.99±4.86	**0.13**
Peroneal nerve	4.28±2.2	7.03±1.44	**0.04**
Proximal/distal Amplitude ratio			
Median nerve	0.80±0.17	0.80±0.19	**0.99**
Ulnar nerve	0.80±0.11	0.74±0.17	**0.38**
Tibial nerve	0.78±0.18	0.52±0.24	**0.003**
Peroneal nerve	0.80±0.17	0.66±0.27	**0.16**
Proximal/distal duration ratio			
Median nerve	1.42±0.61	1.20±0.31	**0.21**
Ulnar nerve	1.19±0.16	1.16±0.17	**0.66**
Tibial nerve	1.49±0.58	1.24±0.38	**0.62**
Peroneal nerve	1.20±0.23	1.13±0.21	**0.46**

Figure 1: Examples of the median nerve, ulnar nerve, peroneal nerve, and tibial nerve motor responses in a patient with CMT1A



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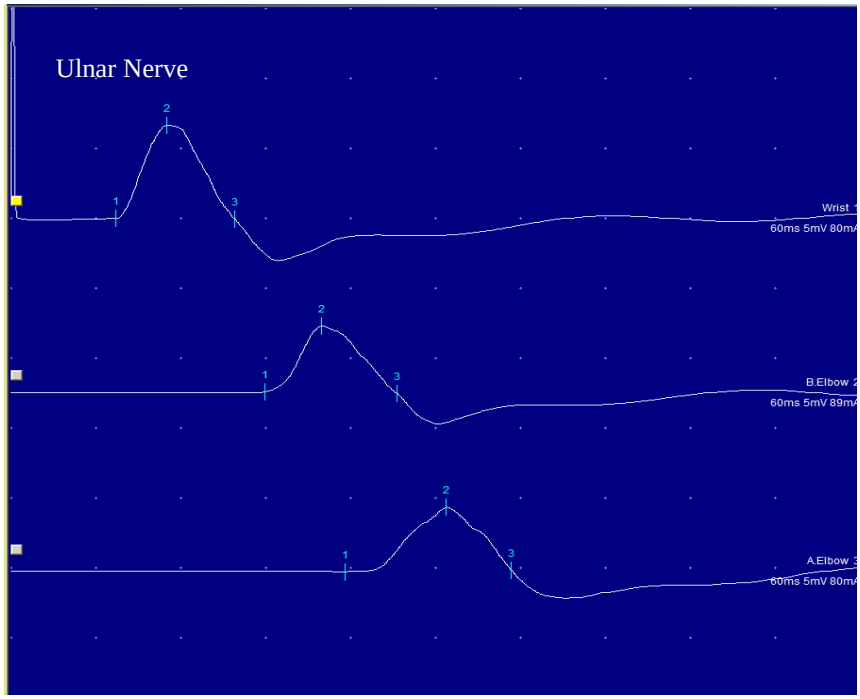

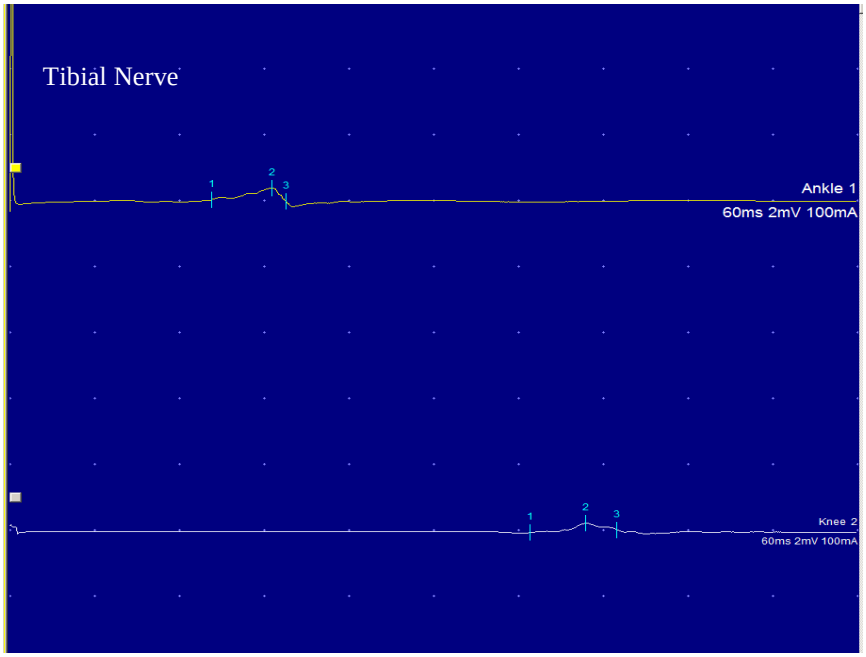

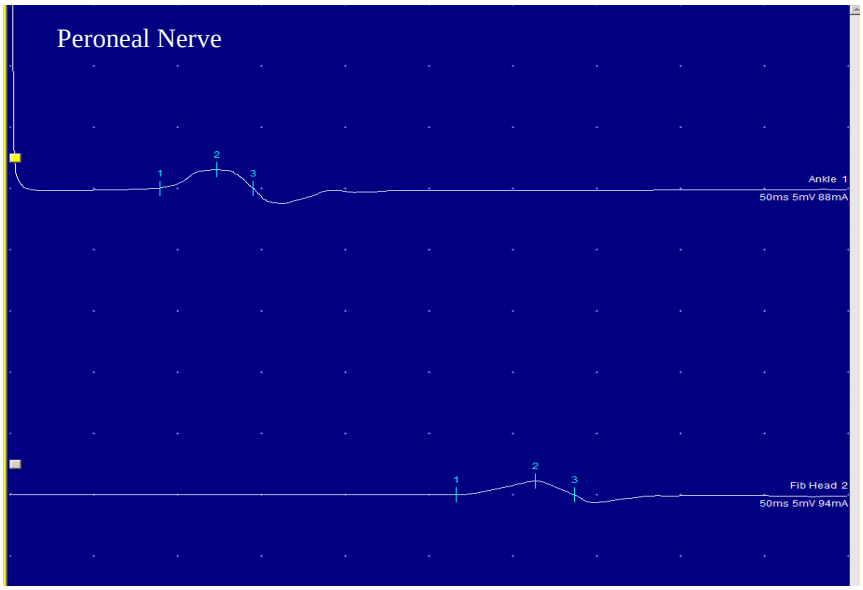

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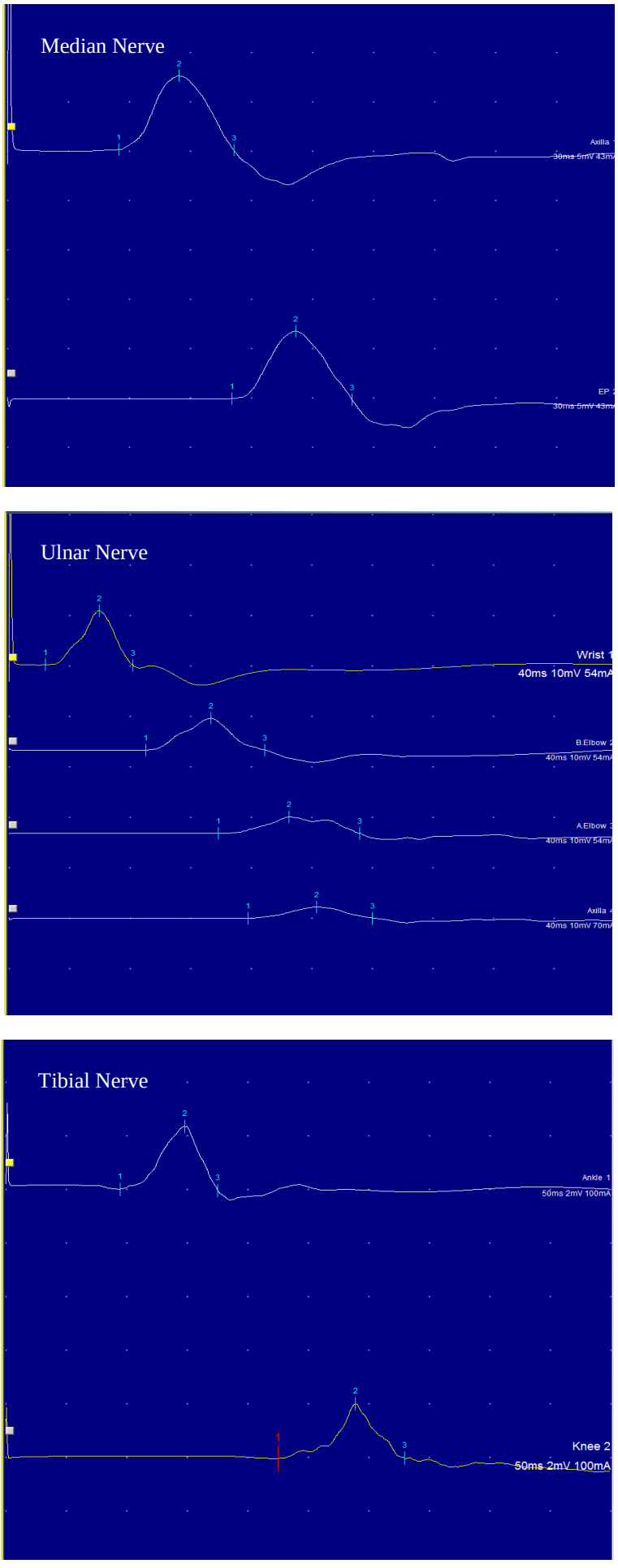
Figure 2: median nerve, ulnar nerve, peroneal nerve, and tibial nerve motor response examples in a patient with CIDP



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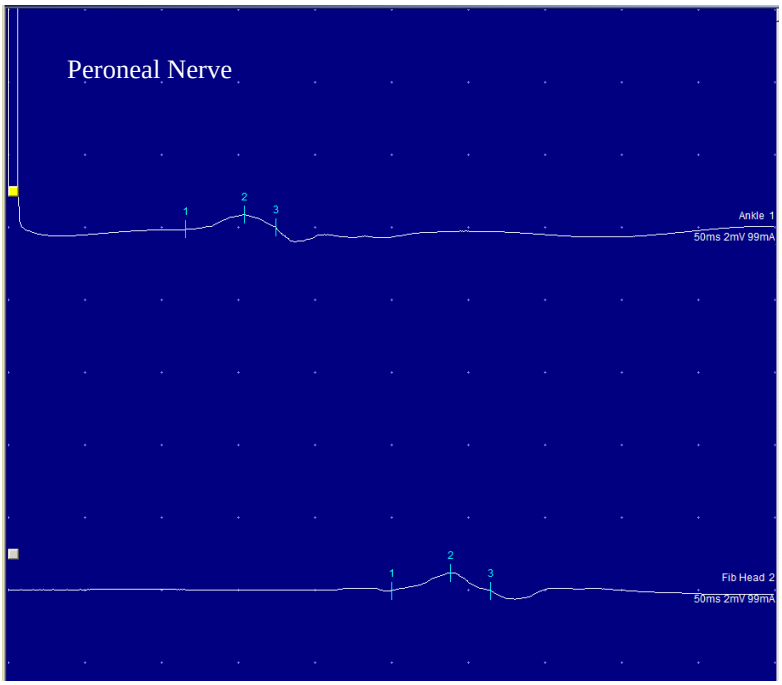

DISCUSSION

This study aimed to investigate hereditary and acquired demyelinating polyneuropathies as the different electrodiagnostic parameters. In our study, we found that the mean distal duration of the peroneal nerve was statistically significantly longer in the CIDP group compared with the CMT1A group. The groups had similar mean distal durations for the ulnar, tibial nerves, and median. The CIDP group had statistically lower tibial nerve proximal/distal amplitude ratio in the CIDP group than the CMT1A group had.

Tankisi et al. compared CMAP amplitude and durations in 132 patients with demyelinating polyneuropathy and 53 patients with axonal polyneuropathy and found the CMAP duration longer in demyelinating PNPs than the axonal PNPs (**5**). They emphasized that distal CMAP duration was a useful marker for reflecting distal demyelination. In this study, we compared the negative peak durations in demyelinating polyneuropathy subgroups instead of axonal/demyelinating polyneuropathies. We demonstrated that the CIDP group had longer peroneal motor nerve distal CMAP than the hereditary polyneuropathies. Likewise, our findings may indicate that CIDP has distal demyelination.

Our study supports previous studies suggesting elongated CMAP duration in CIDP. This study only found that the patient groups showed a difference in terms of distal duration of the peroneal nerve, and longer duration of the CIDP group. We found no difference in distal CMAP duration between the CMT1A and CIDP groups for the median, ulnar, and tibial nerves. One reason is that amplitude due to phase cancellation decreases. Another possibility may be inaccurate measurement due to low CMAP amplitude. If we had studied total distal CMAP duration instead of negative peak duration, perhaps a difference could be found.

Although both disease groups cause demyelinating polyneuropathy, there are differences in transmission characteristics.

Some patients with CMT1A underwent histopathologic examinations showing that all fiber sizes decreased as the most prominent ones in large fibers (**6**). There is evidence of axonal atrophy, as well as significant demyelination in nerve biopsies. Conduction velocities may be apparently slowed down due to significant demyelination all over the peripheral nervous system. In CMT1A, wave morphology is well preserved without evidence of temporal dispersion or conduction block by stimulating the distal and proximal parts (**6**). Various segments of each nerve and similar nerves are slowed down uniformly. However, nerve fiber of the peripheral nerves in acquired polyneuropathies such as CIDP may be impacted in various segments. Segmental demyelination and remyelination are the most important histopathologic changes in CIDP (**6**). The pathologic process is performed resulting in disruption of myelin (paranodal and segmental demyelination), impairing the salutatory conduction (**7**). Findings about asymmetric nerve conduction may be often shown in CIDP Patients, despite no clinically significant asymmetry. Besides, multifocal conduction blocks and excessive temporal dispersion in non-entrapment regions are typical for acquired demyelinating polyneuropathies (**7**). However, increased temporal dispersion can be seen in very rare hereditary polyneuropathies such as Charcot-Marie-Tooth disease type X (CMTX). In addition, very rarely, acquired demyelinating polyneuropathy findings such as newly developed Guillain Barré syndrome can be added to CMT1A cases. Therefore, such distinction may be relatively difficult.

Thaisetthawatkul et al. performed receiver operating characteristic (ROC) analysis of the tibial, median, ulnar, and peroneal nerve distal CMAP duration of 23 patients with CIDP, 54 patients who had non-neuropathic syndrome of musculoskeletal pain, 34 with diabetic polyneuropathy, 34 with amyotrophic lateral sclerosis (ALS), and (**8**), the mean distal CMAP duration in CIDP was longer than in the other groups.

They reported sensitivity and specificity of the distal CMAP dispersion as CIDP electrophysiologic tool. Although a different disease group was studied in our study, distal CMAP duration was found to be longer in the CIDP group. Our findings support the view that distal CMAP duration is a preferable measure of distal demyelination in CIDP.

A review of the electrophysiologic data of 471 participants (61 with ALS, 145 normal controls, 205 with other axonal neuropathies, and 60 patients with CIDP) by Isose et al. found the duration of distal CMAP to be a useful index for detection of distal demyelination (**9**). Distal CMAP duration was especially prolonged especially in the lower extremities, as the most prominent findings in the peroneal nerve. Our study showed significant prolongation of distal CMAP in the peroneal nerve in the CIDP group. Due to the effect of CIDP on the slow and fast conducting fibers at different rates and non-involvement of motor fibers in the demyelinated areas during the same period, different effects may occur in different nerves. Nodera et al. found prolonged CMAP duration in 34% of patients with CIDP in their study on 35 patients with CIDP and 30 normal controls in CMAP (T) durations recorded from the tibialis anterior muscle. They also found the longevity of CMAP duration which was recorded from the tibialis anterior muscle in 42% of patients with normal duration of CMAP which was recorded from the extensor digitorum brevis and 28% of patients who had normal CMAP duration which was recorded from abductor hallucis (**10**). They emphasized the usefulness of determining the duration of tibialis anterior CMAP due to a significant axonal loss.

Few studies reviewed the literature on proximal-distal CMAP dispersion and distal CMAP in patients who had hereditary polyneuropathies. Stanton et al. evaluated 33 CIDP patients and 91 patients who had hereditary neuropathies (17 HNPP, 31 CMT1A, and 10 CMTX). They calculated the percentage decreases in CMAP amplitude and percentage increases in CMAP duration between the distal and proximal stimulation zones for each nerve in the forearm and foreleg segments to detect conduction block or temporal dispersion. It has been demonstrated that dispersion of distal CMAP is more common in CIDP than in inherited neuropathies (**11**). In addition, they found the distal CMAP dispersion was almost more prevalent in CMT1A compared with other hereditary neuropathies. They found that the CIDP group had significantly longer mean distal CMAP duration than the group with hereditary neuropathies. Our study also supports this finding.

Normal individuals showed reduced amplitude of motor response in proximal stimulations. Typically, the CMAP amplitude decreases slightly as the stimulus point moves proximally. With increasing the distance of transmission, the slow-conveying fibers were slower than the fast-conveying fibers. CMAP amplitude decreases due to the phase cancellation and temporal dispersion (**7**). Conduction blocks and dispersion are common in acquired forms of demyelinating polyneuropathy. More proximal stimulation reduces amplitudes of CMAP due to higher conduction blocks and temporal dispersion along some fibers (**7**). Temporal dispersion in demyelination is caused by abnormal conduction velocity disruptions between individual axons of a nerve. Long distance of the transmission reduces amplitude of CMAP. While the elongation in the latency reflects the reduced speed of the fastest transmitting fibers, the duration of distal CMAP reflects the temporal dispersion between the slow and fast transmitting distal motor fibers.

Distal and proximal muscle recordings are compared to identify primary demyelination to effectively contribute to routine electrophysiologic studies to evaluate the polyneuropathy (**12**). In our study, we found that the tibial nerve proximal/distal amplitude ratio was lower in the CIDP group than the CMT1A group. The CIDP and CMT1A patient groups did not show any difference in terms of ulnar nerve, median nerve, and peroneal nerve proximal/distal amplitude ratio. In addition, our study found the similarity of the proximal/distal ratios for all nerves in the CMT1A group, while showing a difference of the rates in the CIDP group whose values between nerves had a wide range. Therefore, the previous studies support our findings that CMT1A patients showed homogeneous characteristics of polyneuropathy and CIDP patients show partial and focal decreases in nerve conduction and blocks of velocities conduction.

A retrospective analysis of NCS results of 30 CMT1 patients, 35 CIDP patients, and 77 healthy controls by Kang et al. In the qualitative analysis of proximal-distal CMAP amplitude ratios, showed lower values for the CIDP group in all tested nerves compared with the group with CMT1 (**13**). They stated that values close to 1 in proximal/distal ratios showed smaller differences in amplitudes between proximal and distal segments. Amplitude in all peripheral nerves was relatively equally reduced in the CMT1 group, while the CIDP group's findings showed the conduction blocks of which amplitude was significantly reduced in the proximal segments. Amplitude in all peripheral nerves was reduced relatively equally in the CMT1 group while the CIDP group's findings indicated that conduction blocks may have significantly reduced amplitude in the proximal segments as compared with the distal segments.

In this study, proximal and distal CMAP duration ratios were analyzed for the first time. We expect this ratio to be high in the acquired group, assuming that the speeds of fast and slow transmitting fibers would be significantly affected differently with increasing the transmission distance. However, we found no significant differences between the patient groups in terms of duration ratios. One reason may be that we calculated the negative peak duration. CMAP amplitude may be possibly lost since the secondary axonal damage accompanies the disease.

This study has some limitations. The first limitation is the relatively small number of subjects and the retrospective nature of our study. Prospective studies involving larger CIDP and CMT1A patient groups are needed. Perhaps, one can investigate the guiding role of analyses before and after immunotherapy in a larger sample. The strength of the study is the use of distal CMAP duration and proximal/distal amplitude ratio to distinguish between acquired and hereditary demyelinating polyneuropathies. In the future, studies that provide optimum integration with a larger patient group to existing electrophysiologic criteria are needed.

The data in our study support new efforts aimed to improve the performance of CIDP electrodiagnostic criteria. It cannot be used alone to distinguish between the hereditary demyelinating polyneuropathy and acquired demyelinating polyneuropathy though it can sometimes electrophysiologically differentiate from CMT1A. In this way, an approach to increasing the diagnostic capacity can be provided in patients with demyelinating polyneuropathy with severe secondary axonal involvement and few inducible motor responses, who have difficulty in the distinction between hereditary/acquired, severe secondary axonal involvement and few excitable motor responses can be recorded.

CONCLUSION

This study may benefit the diagnosis of patients with hereditary and inflammatory polyneuropathy with common features. Different electrodiagnostic models of hereditary and acquired demyelinating polyneuropathies should be clinically recognized, contributing to the diagnosis and treatment of these patients.

Author contributions: OA, CE; Literature search and study design, patient examinations, data collection and analyzes **OA;** Writing article and revisions

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. This research did not receive and specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical issues: All authors declare originality of research.

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Medical Science and Discovery
ISSN: 2148-6832

Effectiveness of neutrophil/lymphocyte, lymphocyte/MPV and platelet/MPV ratios in the classification and mortality prediction of acute stroke

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ABSTRACT

Objective: The aim of this study is to investigate whether Neutrophil / lymphocyte (NLR), Lymphocyte / MPV (mean platelet volume) (LMR) and thrombocyte / MPV (PMR) ratios obtained from the complete blood count, can be used as an effective marker in acute stroke for determining the prognosis and subtype of stroke.

Material and methods: Patients admitted to the emergency department with acute stroke symptoms between January 1, 2020 and December 31, 2020 were evaluated retrospectively. The patients were divided into two groups as hemorrhagic or ischemic cerebrovascular disease (CVD) according to the radiological findings. NLR, LMR and PMR ratios were calculated. The last diagnosis and hospitalization information were recorded and their 28-day mortality status was evaluated.

Results: A total of 764 patients were included in the study. The median age of the patients included in the study was 68 (IQR 25-75: 59-78) and 404 (52.9%) of the patients were male. In the analysis performed; it was observed that the LMR, NLR and PMR levels were significantly different in those who developed mortality on the 28th day ($p = 0.009$), ($p = 0.002$), ($p = 0.026$). In addition, only the NLR level was found to be significantly different in the ischemic group ($p < 0.001$).

Conclusion: We think that in cases with stroke, NLR, LMR and PMR levels can be used in predicting the prognosis of this disease. Also, NLR is significantly higher in ischemic stroke, and also significant in terms of showing that CVD type is hemorrhagic or ischemic.

Keywords: Emergency department, cerebrovascular disease, hematological parameters, stroke

Research Article

Received 31-01-2020

Accepted 15-02-2021

Available Online: 17-02-2021

Published 24-02-2021

INTRODUCTION

Stroke is an important cause of morbidity and mortality worldwide (1). Despite all urgent approaches and treatments, mortality still ranges between 20-30% (2). Prevention of mortality is associated with rapid diagnosis, effective use of imaging methods, and availability of vital interventions such as thrombolytic or interventional procedures, especially in emergency services, which are the first application area.

In the presence of acute cerebrovascular disease (CVD), it is only possible to distinguish the current clinic from ischemic or hemorrhagic cerebrovascular disease after the availability of imaging methods. Every patient who admitted with acute stroke clinic are being evaluated as a thrombolytic or thrombectomy candidate and effective diagnostic procedures are completed within the time when the decision for treatment is required.

Blood parameters such as complete blood count, biochemistry and coagulation tests obtained at the first evaluation in the emergency department are important in the presence of cerebrovascular disease in terms of treatment decision, supportive treatment or determining the existing additional pathology.

http://dx.doi.org/10.36472/msd.v8i2.482

However, a detailed examination of complete blood count parameters can make important contributions to the emergency physician in the evaluation process of the patient. The usage of hemogram parameters in the differential diagnosis of CVD as ischemic or hemorrhagic type can accelerate the functioning of emergency services and may allow appropriate triage for patients, especially at the decision point of stroke procedures like thrombolytic therapy or surgical intervention.

There are publications stating that neutrophil functions and neutrophil infiltration, which are an important part of the systemic inflammatory response, affect the results of ischemia (3). Studies have shown that neutrophil migration and systemic inflammatory response play a role in the repair of the blood-brain barrier regardless of the size of the infarct site (4). In the presence of strokes, a decrease in the total neutrophil count is observed (4). Neutrophil//lymphocyte ratio (NLR) is an important systemic marker of inflammation (5-7). Due to it is easy access, it can be easily used in patients who are suspected of stroke. Increased NLR can be used to predict prognosis in patients with stroke (8, 9).

Mean platelet volume (MPV) indicates the size of platelets and is associated with platelet function and activation. There are many studies related to its role in thrombosis and inflammation (10). In blood count results, the two most featured indicators regarding platelets are thrombocyte count and MPV. Platelet average volume is 7.8-11.0 fl. High MPV is considered in terms of coronary heart disease and stroke risk (10).

Our study aims to evaluate the effectiveness of NLR, lymphocyte/MPV ratio (LMR) and thrombocyte/MPV ratio (PMR) in the classification of acute CVD diagnosis and its place in mortality prediction.

MATERIAL AND METHODS

Patients who were admitted to the Health Sciences University, Bursa Yuksek Ihtisas Training and Research Hospital Emergency Department with acute stroke symptoms and diagnosed with CVD within a 1-year period between January 1, 2020 and December 31, 2020 were retrospectively analyzed. Written approval was obtained from the ethics committee of our hospital during the planning phase of our study (2011-KAEK-25 2021/01-16).

Patient data were obtained by scanning patient cards and patient epicrisis registered in the hospital automation system. Age, gender, chronic disease history, anticoagulant or antiaggregant drug use status of the patients were recorded. Patients were divided into two groups as hemorrhagic or ischemic cerebrovascular disease according to brain computed tomography and brain magnetic resonance imaging findings obtained after the first evaluation.

The neutrophil/lymphocyte ratio, lymphocyte/MPV ratio and thrombocyte/MPV ratios were calculated based on the hemogram examinations taken at the initial evaluation in the emergency service.

The last diagnosis and hospitalization information of the patients were recorded and their 28-day mortality status was evaluated. Patients under 18 years of age, patients with a history of previous cerebrovascular disease, presence of trauma, active malignancy were excluded from the study. Patients who presented with symptoms of the stroke but were evaluated as a transient ischemic attack due to no evidence of ischemic or hemorrhagic cerebrovascular disease on brain imaging were excluded from the study.

Statistical analysis: The data of the study were analyzed using SPSS 22.0for Windows (SPSS Inc., Chicago, IL, USA) software. Descriptive statistics were expressed as mean ± standard deviation or median values while categorical variables were expressed as numbers and percentage (%).

Kolmogorov-Smirnov test was used for the normality distribution of the data. The significance of the difference between the groups in terms of continuous numerical variables where parametric test statistics assumptions were met was examined with Student's t test, while the significance of the difference in terms of continuous numerical variables in which parametric test statistics assumptions were not met was evaluated with the Mann Whitney U test. Variables that may be effective in mortality were evaluated using the "enter" method in logistic regression analysis. $p < 0.05$ was considered statistically significant. Results were presented at 95% confidence interval.

RESULTS

The files of 1452 patients who were admitted to the emergency department with a stroke clinic within 1 year were retrospectively scanned. Of the remaining 1452 patients, 688 were excluded from the study for various reasons (Figure 1). A total of 764 patients were included in the study.

While the median age of the patients included in the study was 68 (IQR 25-75: 59-78), 404 (52.9%) of the patients were male. Also, 621 (81.3%) of the patients had comorbid diseases, while the most common additional disease was hypertension in 514 (67.3%) patients. Ischemic CVD was detected in 651 patients (85.2%), while mortality developed in 28 days in 70 (9.2%) patients (Table 1).

The mean LMR value of the patients was found to be 0.22 ± 0.13, the mean NLR value as 4.40 ± 4.50, and the mean PMR value as 25.50 ± 9.90 (Table 2). Mann Whitney U test was performed to investigate whether there was a difference between the LMR, NLR and PMR levels of the patients and the mortality on the 28th day.

As a result of this test, it was seen that the LMR, NLR and PMR levels were significantly different in those who developed mortality on the 28th day, respectively [($p = 0.009$), ($p = 0.002$), ($p = 0.026$)] (Table 3).

In the Mann Whitney U test conducted to investigate whether there is a difference between the LMR, NLR and PMR levels of the patients and the SVO type, it was found that the NLR level was significantly different in the ischemic group ($p < 0.001$) (Table 4).

In the logistic regression analysis performed to evaluate whether the variables of gender, comorbidities, antiaggregant and anticoagulant use status were an independent risk factor for mortality, it was found that the variables were not an independent risk factor. (Table 5)

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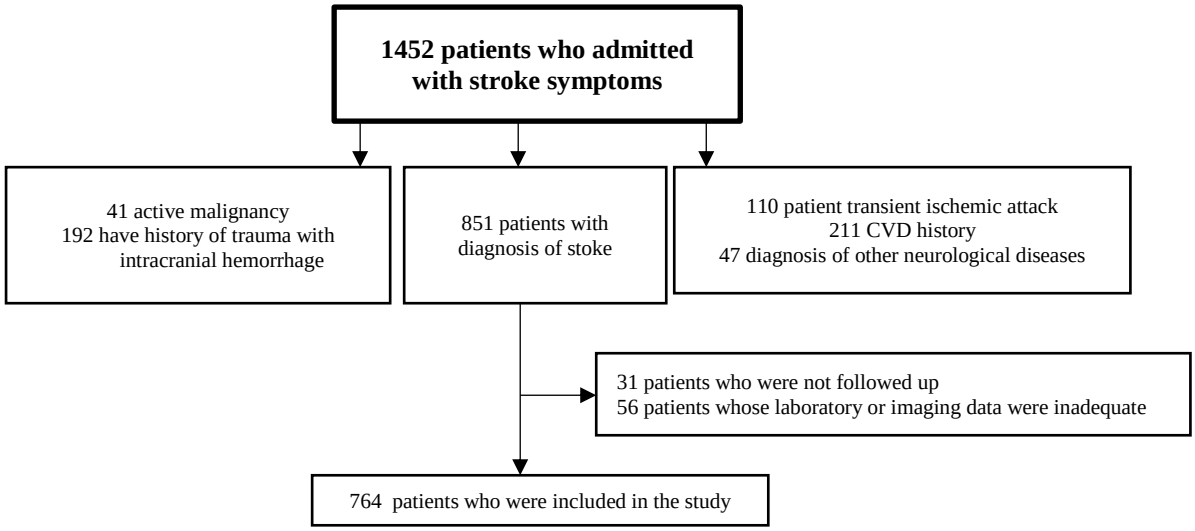

Figure 1. Identifying the cohort.

Table 1: Demographic characteristics of patients

		Total		Ischemic CVD		Hemorrhagic CVD	
		Frequency n	Percent %	Frequency n	Percent %	Frequency n	Percent %
Gender	Female	360	47,1	305	46,9	55	48,7
	Male	404	52,9	346	53,1	58	51,3
Additional Disease History	Yes	621	81,3	542	83,3	79	69,9
	No	143	18,7	109	16,7	34	30,1
HT	Yes	514	67,3	441	67,7	73	64,6
	No	250	32,7	210	32,3	40	35,4
DM	Yes	191	25,0	175	26,9	16	14,2
	No	573	75,0	476	73,1	97	85,8
CAD	Yes	167	21,9	149	22,9	18	15,9
	No	596	78,0	502	77,1	95	84,1
CRF	Yes	16	2,1	16	2,5	0	0
	No	748	97,9	635	97,5	113	100,0
Others	Yes	34	4,5	31	4,8	3	2,7
	No	730	95,5	620	95,2	110	97,3
Antiaggregant use	Yes	187	24,5	173	26,6	14	12,4
	No	577	75,5	478	73,4	99	87,6
Anticoagulant use	Yes	38	5,0	32	4,9	6	5,3
	No	726	95,0	619	95,1	107	94,7
Final status	Neurology clinic admissions	538	70,4	510	78,3	28	24,8
	Neurosurgery clinic admissions	65	8,5	0	0,0	65	57,5
	İntensive care unit admissions	29	3,8	11	1,7	18	15,9
	Stroke Unit admissions	132	17,3	130	20,0	2	1,8
28-Day mortality	Exitus	70	9,2	44	6,8	26	23,0
	Alive	694	90,8	607	93,2	87	77,0
Total		764	100,0	651	100,0	113	100,0

CVD: Cerebrovascular disease, **HT:** Hypertension, **DM:** Diabetes mellitus, **CAD:** Coronary artery disease **CRF:** Chronic renal failure

http://dx.doi.org/10.36472/msd.v8i2.482

Table 2: Laboratory values of patients

	Total			Ischemic CVD			Hemorrhagic CVD		
	n	Mean	Std. Dvt	n	Mean	Std. Dvt	n	Mean	Std. Dvt
Wbc	764	9,87	4,24	651	9,61	4,13	113	11,35	4,57
Neutrophil	764	6,85	3,33	651	6,57	3,03	113	8,45	4,39
Lymphocyte	764	2,17	1,12	651	2,18	1,04	113	2,13	1,52
Platelet	764	247,41	76,55	651	246,62	76,25	113	251,98	78,46
MPV	764	9,98	1,23	651	10,01	1,24	113	9,81	1,14
Lymphocyte/MPV	764	0,22	0,13	651	0,22	0,13	113	0,22	0,16
Neutrophil/MPV	764	4,40	4,50	651	4,04	3,80	113	6,48	7,00
Platelet/MPV	764	25,50	9,89	651	25,36	9,94	113	26,32	9,64

CVD: Cerebrovascular disease, **MPV:** Mean platelet volume

Table 3: Analysis of variables with 28-day mortality with Mann- Whitney U Test

	28-day Mortality	n	median (IQR: 25th-75th percentiles)	p value
LMR	Survival	694	0.20(0.14-0.29)	p<0.05
	Mortality	70	0.18(0.11-0.23)	
	Total	764	0.20(0.14-0.28)	
NLR	Survival	694	2.85(1.89-4.73)	p<0.05
	Mortality	70	4.24(2.16-8.28)	
	Total	764	2.92(1.90-5.10)	
PMR	Survival	694	19.13(24.70-30.50)	p<0.05
	Mortality	70	17.29(21.75-28.28)	
	Total	764	24.30(18.95-30.39)	

LMR: Lymphocyte/Mean Platelet Volume Ratio, **NLR:** Neutrophil/ Lymphocyte Ratio **PMR:** Platelet/ Mean Platelet Volume Ratio

Table 4: Analysis of variables with cerebrovascular disease type with Mann-Whitney U Test

	CVD	n	median (IQR: 25th-75th percentiles)	p value
LMR	Ischemic	651	0.20(0.14-0.28)	p>0.05
	Hemorrhagic	113	0.18(0.12-0.27)	
	Total	764	0.20(0.14-0.28)	
NLR	Ischemic	651	2.81(1.89-4.61)	p=0.000
	Hemorrhagic	113	4.17(1.98-8.45)	
	Total	764	2.92(1.90-5.10)	
PMR	Ischemic	651	24.09(18.84-30.13)	p>0.05
	Hemorrhagic	113	25.21(19.71-32.51)	
	Total	764	24.30(18.95-30.39)	

CVD: Cerebrovascular diseae **LMR:** Lymphocyte/Mean Platelet Volume Ratio, **NLR:** Neutrophil/ Lymphocyte Ratio **PMR:** Platelet/ Mean Platelet Volume Ratio

Table 5: Analysis of variables with logistic regression

	p value	Exp(B)	95% C.I.for EXP(B)	
			Lower	Upper
Gender	>0.05	0,712	0,430	1,177
Additional disease history	>0.05	0,469	0,175	1,258
Antiaggregant use	>0.05	1,231	0,623	2,433
Anticoagulant use	>0.05	3,639	0,784	16,905

http://dx.doi.org/10.36472/msd.v8i2.482

DISCUSSION

Stroke is still an important cause of mortality and long-term morbidity, despite the rapid development of diagnostic methods and the development of aggressive treatment methods. Ischemic stroke is approximately 80% of cases in acute CVD (**11, 12**). In our study, the rate of ischemic stroke is similar to the literature, and similar to the studies, male gender was predominantly affected (**12, 13**). Hypertension, smoking, diabetes, obesity, physical inactivity and atrial fibrillation have been identified as important risk factors in the development of strokes and recurrent stroke (**14**). In our study, 81.3% of our patient group had comorbid diseases, and especially 67.3% of hypertension was found, and its importance as the main risk factor is demonstrated. While comorbidities, anticoagulant or antiaggregant drugs used, or sex are important risk factors for the development of stroke, in line with the data in our study, they were not considered as independent risk factors in the prediction of stroke mortality.

It is known that leukocytes play a role in the development of tissue damage in acute ischemic stroke. This inflammatory response, which is characterized by an increase in neutrophil count and a decrease in lymphocyte ratio, can be easily evaluated with the N/L ratio. NLR has been found to be associated with severe and widespread coronary artery disease and stroke (**10**).

It was emphasized that in cases with high NLR at the first admissions of stroke patients cardioembolic causes among the etiological factors should be investigated in more detail (**15, 16**). NLR can be used as a simple, easy and independent marker for predicting mortality in patients with acute ischemic stroke (**17, 18**).

In our study, NLR was found to be significant in determining stroke prognosis in accordance with the literature. Especially when evaluated together with the etiology of CVD, NLR is significantly higher in ischemic stroke, and it can be a guide in terms of whether CVD is hemorrhagic or ischemic. In the study of Kakhi et al. Investigating the change of NLR towards the etiology of stroke, similar to our study, it is emphasized that ischemic stroke is higher than hemorrhagic stroke (**19**). In hemorrhagic stroke, high levels of NLR have been found to be particularly effective in predicting 90-day mortality (**20, 21**).

MPV is an important indicator showing platelet function and activation. Large platalets can synthesize more prothrombic factors, so high MPV values have been detected in stroke and cardiovascular diseases and have been associated with poor outcomes and mortality (**22, 23**). MPV, which we evaluated with thrombocyte/MPV and lymphocyte/MPV, was found to be significant in predicting prognosis when evaluated proportionally, but did not differ when cerebrovascular disease was ischemic or hemorrhagic.

CONCLUSION

As a result, NLR is widely used in predicting prognosis of various diseases today. However, we first emphasized that LMR and PMR can be used in predicting the prognosis of acute stroke diseases.

As a result of these data we have obtained, we think that the NLR, LMR and PMR levels obtained from the hemogram, which is a simple and easy to obtain parameter simultaneously with radiological imaging methods, can be used in the prognosis prediction of this disease. At the same time, NLR is significantly higher in ischemic stroke and it is significant in terms of showing that SVH is ischemic or hemorrhagic.

Limitations: Our study was conducted retrospectively and in a single center. For this reason, there may be a loss of patient data at the point of access. Due to the intensity of the Covid-19 pandemic, insufficient data in the patient files increased the number of patients excluded from the study relatively. It is known that the thrombocytes gain volume while the blood taken for MPV value measurement we used in our study is kept in EDTA tubes. The blood samples taken in our study were measured in as little as four hours, but as in other studies, no time-dependent correction was made.

Author contributions: SE, MY; Literature search and study design, patient examinations, data collection and analyzes **SE;** Writing article and revisions

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. This research did not receive and specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical issues: All authors declare originality of research.

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Medical Science and Discovery
ISSN: 2148-6832

Evaluation of Brucella Coombs Gel Test with Blood Culture

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ABSTRACT

Objective: To evaluate the correlation between Brucella spp. blood culture, which is accepted as the gold standard in the diagnosis of brucellosis, and the Brucella coombs gel test (BCGT), a new and rapid test developed in our country.

Material and method: Brucellosis is suspected in 100 patients from various clinics of our hospital microbiology laboratories, simultaneous blood culture results (Bact/allert 3D bioMerieux, France) and serum standard tube agglutination test (STA Seromed, Turkey), Brucella Coombs gel test (BCGT, Islab, Turkey) It was evaluated retrospectively.

Results: Serology and blood culture growth were negative in 82/100 of the patients. STA/BCGT results were consistent in 18/100, and ⩾ 1/320 was positive. Brucella melitensis was isolated from blood cultures of seven patients and concurrently taken BCGT was positive (100%). There are 11 patients with positive serology with culture negative.

Conclusion: Although blood culture is accepted as gold standard, it is not always possible to catch blood culture positivity. Serology tests, especially BCGT, should be preferred routinely because it gives early results, is cheap and safe.

Keywords: Brucellosis, Blood cultur, Serology

INTRODUCTION

Brucellosis is the most common zoonotic disease in the world. Brucella species (B.abortus, B.melitensis and B.suis) that cause infection in humans and animals are intracellular, aerobic, gram-negative, small bacilli (**1**, **2**). Brucella involves many tissues and organs and progresses with nonspecific symptoms. In cases with fever of unknown cause and nonspecific symptoms, clinicians are recommended to investigate brucellosis in diagnosis. The incubation period of the disease is approximately 2-3 weeks, and nonspecific findings such as fever (often corrugating), night sweats, muscle pain, back pain and loss of appetite occur. It is also known that various complications such as osteomyelitis, hepatomegaly, splenomegaly, meningitis, endocarditis and epididymo-orchitis can develop during Brucellosis (**3**, **4**). One of the two important criteria in the diagnosis of brucellosis is Brucella spp. isolation; the other is the detection of the presence of high titer specific antibodies in the serum accompanied by clinical findings and the demonstration of seroconversion. The specificity and sensitivity of serological tests differ, so it is recommended that the tests be combined. For example, since the standard tube agglutination (STA) test may produce false negative results due to the presence of blocking antibodies, this test should be validated with Coombs anti-Brucella (CAB) or capture agglutination (ICA) tests.

BCGT (ODAK Brucella Coombs Gel Test, Toprak Medikal, Istanbul), which has been developed in our country in recent years, has been introduced as a new and rapid method based on agglutination. This test is performed in wells with a gel matrix containing Coombs antibodies (anti-human IgG). During the test, the blocking antibodies are bound by centrifugation at high speed and Coombs serum, and the results are evaluated visually within 2 hours. In the present study, it is aimed to determine the efficiency of BCGT in serological diagnosis of brucellosis by comparing it with blood culture results.

Research Article

Received 28-01-2020

Accepted 23-02-2021

Available Online: 24-02-2021

Published 24-02-2021

http://dx.doi.org/10.36472/msd.v8i2.477

MATERIAL AND METHODS

A total of 100 patients, from whom STA and BCGT were simultaneously requested from the patient serum with blood culture, were selected and the growth and results were evaluated retrospectively. Blood culture bottles (Bact/allert 3D BioMerieux, France) samples that signaled in the blood culture device were inoculated in blood, chocolate and EMB medium.

Growths were evaluated after 24.48 hours 37 °C incubation. Colonies with gram negative cocci on gram stain were identified by VITEK 2 (BioMeriux, France) when catalase and oxidase were positive. STA (Seromed, Turkey) method and BCGT (Islab Turkey) were studied in serum samples.

BCGT: In this method, first serum dilutions were made in the wells reserved for each patient in the dilution plate. After adding 100 µl dilution fluid to the first well and 50 µl dilution fluid to the other wells, 5 µl of each patient's serum was added to the first wells and mixed; Serial dilutions (1 / 40-1 / 5120) were prepared by passing 50 µl to the other wells; 50 µl was taken from the last well and discarded. Then, 50 µl of Brucella antigen suspension was added to all wells, mixed and incubated at 37 °C by covering the plate. The gel matrix microtubes to be used at this time were marked with their respective serum numbers.

After the incubation, after shaking the plate well, 50 µl of the mixture in the relevant well was taken and pipetted into the relevant microtube in the gel matrix. Microtubes were incubated for 20 minutes at 37 °C, and then centrifuged for 20 minutes at the appropriate speed recommended by the manufacturer.

Results were evaluated visually. In the evaluation; In the absence of antibody, the precipitation of pink brucella antigens at the bottom of the tube was considered negative, and the presence of the pink antigen and antibody complex on the gel in the presence of antibodies was accepted as positive (**Figure 1**).

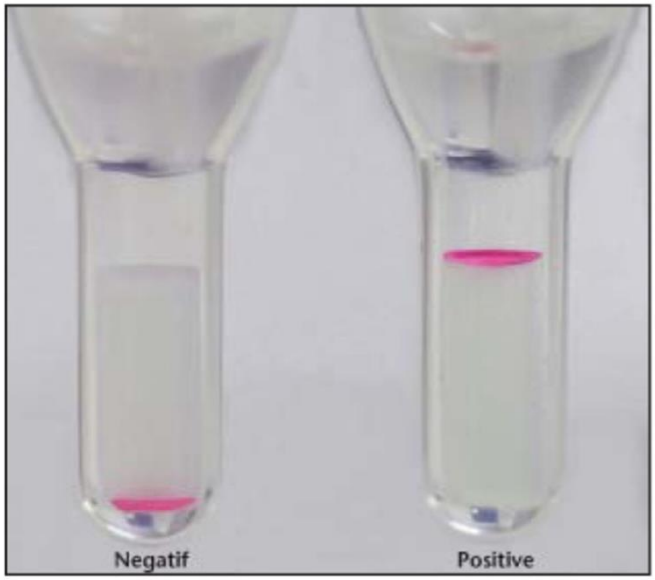

Figure 1:

RESULTS

The average age of the patients is 30.9 and it consists of 54 male and 46 famale. The results of Brucella STA and BCGT were found to be 100% compatible in 100 patients. Brucella melitensis grew in simultaneous blood culture of 7 out of 18 patient sera with Brucella STA / BCGT; 1/320 and above. When the titers are evaluated; Culture was negative in 1 patient with 1/320, and culture was negative in 3 patients with 1/640; Culture growth was positive in 4 patients with 1/1280, negative in 4 patients, positive in 1 patient with 1/2560; 1/5120 was positive in 2 patients and negative in 3 patients. Blood culture positivity 1-7. It has been detected between days. (**Table 1**)

Table 1. Correlation of BCGT / STA test with blood culture growth

Sample no	STA	BCGT	Blood culture signal	Positive signal day of blood culture
1	1/320	1/320	Negative	
3	1/640	1/640	Negatif	
4	1/1280	1/1280	Negatif	
4	1/1280	1/1280	Pozitive (*Brucella melitensis*)	5.day 6.day 6.day 7.day
1	1/2560	1/2560	Pozitive *(Brucella melitensis*)	5.day
2	1/5120	1/5120	Pozitive *(Brucella melitensis*)	1.day 7.day
3	1/5120	1/5120	Negative	
82	Negative	Negative	Negative	

DISCUSSION

Culture is accepted as the gold standard in the laboratory diagnosis of brucellosis. However, the sensitivity of blood culture varies according to the clinical condition of the patient, the amount of bacteria circulating in the blood, the method used, the laboratory's practice, the amount of bacteria circulating in the blood and the type of bacteria, and the positivity rate is between 15-70% (**5, 6**). The higher concentration of bacteria in the reticuloendothelial system increases the chance of isolation in bone marrow cultures. However, since relapse develops in 5-40% of acute brucellosis cases, these cases may not always have culture positivity (**6**).

In our study, according to the high titer positivity, the growth in culture was detected as 7/18 and 30.8%. However, it was determined that bacterial incubation was not kept for a long time in patients who were positive for STA / BCGT, but there was no growth in blood culture, and it was reported as no growth after 7 days. Although it is estimated that the chance of production may increase after a longer incubation, the company recommendations are that there will be growth in the blood culture bottle in the first 5 days and that long incubation is unnecessary. It is thought that the bacteria passes from the blood to the reticuloendothelial system, so it is not detected in the blood. Although nucleic acid amplification tests or detection of antigens in the diagnosis of brucellosis are diagnostic for new brucellosis cases, the presence of DNA in the blood of the cases even after successful treatment is not useful in determining the recurrence of brucellosis (**7, 8**). In the first week of the infection, IgM antibodies against lipopolysaccharide (LPS) antigens appear in the serum, and IgG antibodies appear within two weeks. Both types of antibodies reach their highest level in the fourth week. However, the time of serological change during relapse varies from patient to patient. In this case, clinical findings and serological tests are important.

Brucella agglutination tests play an important role in the serological diagnosis of brucellosis. One of the two important criteria in the diagnosis of brucellosis is Brucella spp. isolation; the other is the detection of the presence of high titer specific antibodies in the serum accompanied by clinical findings and the demonstration of seroconversion (**9**). In routine practice, samples found positive by the Rose Bengal test are evaluated by tube agglutination and dilution tests. STA test is a common method used in the diagnosis of brucellosis all over the world and still in our country.

In this test, total antibodies, especially against S-LPS, are detected on the surface of the bacteria. The disadvantage of the STA test is that false positive and false negative results create difficulties in interpretation. False positivity may occur due to cross reactions with other gram-negative bacteria, while false negativity may be due to the very early stage of the infection, the presence of blocking antibodies or the prezone event.

This can be overcome by the complement binding test, the 2-mercaptoethanol test, the CAB test and the ICA test. It is stated that BCGT reacts with B.abortus, B.melitensis and B.suis, is 99% compatible with the Coombs test and is a fast and economical test that can be used for both screening and titration (**10**). Studies have reported that the specificity and sensitivity of CAB and ICA methods are close to each other. Irvem et al. In his study, CAB or ICA was found to be perfectly compatible with BCGT, but since it was the first study, more different studies were required (**11**). In different studies from our country; In the comparison of BCGT with the CAB test and ICA test, it was concluded that BCGT was perfectly compatible with these tests (**12-16**).

CONCLUSION

BCGT method shows excellent compliance with both CAB and ICA tests; the application of the test is practical; It was determined that it gave results in as little as two hours and it was visually easier to evaluate than other methods. In this study, when the serum samples of patients with growth in blood cultures were evaluated, it was found that they were 100% compatible in 7/7. Since BCGT is a new method, there is no other study evaluated together with blood culture. The result of the test in 2 hours shows that it is routinely preferable due to its ease of diagnosis, cheap and safe.

Author contributions: AI; Literature search and study design, labarotary tests, data collection and analyzes **AI;** Writing article and revisions

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. This research did not receive and specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical issues: All authors declare originality of research. Umraniye Training and Research Hospital ethics committee decision no; B.10.1.THK.4.34.H.GP.0.01/29

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MSD

Medical Science and Discovery
ISSN: 2148-6832

A new type of addiction: Emergency service abuse

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ABSTRACT

Objective: Patients repeatedly applying to the emergency department have become a common problem for many hospitals. With this study, the demographic characteristics of the patients who applied to an emergency department in 12 or more times in a year, the patients' hospitalization status, and the rate of using ambulance service were retrospectively examined.

Material and Methods: This study is a retrospective, descriptive, cross-sectional research article. The study was conducted in the 1300-bed Sakarya Training and Research Hospital (SEAH) adult emergency room (ER), the largest hospital in Sakarya Province, the study period was 2019. Adult patients with 12 or more emergency department applications per year were selected for the study.

Results: In the SEAH adult emergency department, 220.296 patients have examined a total of 382.413 times (1.74 per person) during 2019. The applying patients' to the SEAH adult emergency room 12 times or more in a year was 808 in 2019. These patients' emergency examinations' total quantity was 14369, presenting 3.76% of all emergency examinations, 625 (77.4%) were never hospitalized. Of these patients 420 (52%) were male; the median age was 47. The emergency department examinations estimate was 17.78 (±10.98) times averagely, the median amount was 15 times, and was between 12-192. Of them, 305 (37.7%) had also applied to the psychiatry outpatient clinic at least once. A weak but notable correlation was perceived between the number of outpatient clinic admissions and emergency service admissions ($p = 0.001$, $r = 0.245$).

Conclusion: Frequent users visit the ER and other polyclinics regularly. Limitations should be required on these patients using emergency services in non-emergency situations. It is essential to pay specific attention to frequent emergency room users and investigate the motivations for proceeding to the emergency room.

Keywords: Emergencies, patients, emergency service

INTRODUCTION

One of the busiest places of many hospitals is emergency services which all kind of patients are admitted. For instance, the cases who have chest pain, headache, stomachache, trauma, suicidal thoughts, cardiopulmonary arrest, dyspnea, and more can be encountered at emergency service repeatedly. Therefore, emergency physicians have to struggle with various symptoms, diseases, sufferers, also their relatives.

Various deductions explain the intensity endured in emergency services such as; repeated appeals, inadequate emergency room area, insufficient empty beds for hospitalized patients, not having an appointment system, patients who are hospitalized are waiting in the emergency room due to beds reserved for elective cases, and insufficient primary health care services (1). Although the rate of recurrent emergency admissions among patients examined in the emergency department is low, it is an essential factor that enhances the emergency department's exhaustion (2).

Patients repeatedly applying to the emergency department have become a common problem for many hospitals. In recent years, a concept called "frequent users" has emerged due to repeated admissions to the emergency room. Although some describe patients who apply to the emergency department regularly, for example twice, four times, 12 times, 18 times a year, as "frequent users", there is no generally accepted definition (3–5).

Research Article

Received 07-02-2020

Accepted 23-02-2021

Available Online: 24-02-2021

Published 24-02-2021

http://dx.doi.org/10.36472/msd.v8i2.477

Inadequate healthcare services, being uninsured, acute exacerbation of chronic conditions, mental problems (2, 6), fear or uncertainty about their condition (7), substance use (8) can be among the purposes for repetitive emergency service admissions. The higher number of hospitalizations and the exactness of their ailments execute this group more critical for emergency physicians (2).

Considering the emergency services' position in Turkey; more than 20% of patients' examinations in whole state hospitals were performed in emergency departments, and exceeding 90 million cases are inspected annually in state hospitals' adult ER solely (9). For this reason, any thought that will reduce the patient burden of emergency services is of great importance for the health of patients and the sustainability of the quality of health service delivery. Because increasing the emergency room intensity may reduce patient care quality; moreover, expand patient mortality, delay treatments, and a lengthened waiting period in the emergency department (1, 10).

As the emergency services endure more exceeding patients every year, the consequence of "frequent user" enhancing the emergency service's workload, has similarly increased. With this study, the demographic characteristics of the patients who applied to an emergency department in 12 or more times in a year, the patients' hospitalization status, and the rate of using ambulance were examined. Besides, since it is stated in the literature that there is a significant proportion of patients with mental status problems among patients with repeated admissions, the psychiatry outpatient clinic's follow-up status was also investigated (11). It aims to contribute to the current medical literature regarding the problem's solution by discussing the patient group's measures, which need special attention.

MATERIAL AND METHODS

This study is a retrospective, descriptive, cross-sectional research article.

The study was conducted in the 1300-bed Sakarya Training and Research Hospital (SEAH) adult emergency department, the largest hospital in Sakarya Province; the study period was 2019. This hospital's adult emergency service is established on approximately 2000 square meters; moreover, it is a clinic that admits all emergency applications such as; outpatient, ambulance, trauma, and non-trauma cases.

Eighteen-year-old and older cases applying to the SEAH adult emergency department at least 12 times were included in the research regardless of patients' symptoms. According to the Ministry of Health report, the average number of examinations per person in Turkey was 9.5/year, while for Eastern Marmara, where Sakarya province is located, this figure increased to 9.9/year (12). Among the various interpretations proffered for "Frequent Users" in the literature, considering the average number of examinations in Turkey, patients with 12 or more emergency department applications per year were selected for the study.

Patients confirmed to the pediatric or the gynecology emergency; moreover, those who did not proceed to the ER for examination just particularly had injection or dressing were not consolidated in the study.

If the patient file is abstaining, such as insufficiencies in the patient file or the hospital automation system were not endured to the investigation.

Cases that attained to the ER by ambulance were grouped additionally.

Not with standing the patients' length of stay in the ER, solely one application made in a day was covered in the computations.

There was no referral procedure for patients to apply to the emergency room. Patients could apply to the ER without any limitation when they felt the need, and they could be referred to the ER from primary health care institutions or other hospitals.

The patients had to make an appointment with their means to be examined in the polyclinic. The emergency physician could only advise the patients to make an appointment to the outpatient clinic of the relevant branch during discharge but could not refer the patient without an appointment to the polyclinic.

Statistical Analysis: IBM Statistical Product and Service Solutions (SPSS) V21.0 was applied concerning statistical analyses.

Chi-square test was accepted for comparison of categorical data. Results with $p<0.05$ were analyzed statistically meaningful.

The skewness and kurtosis marks were required to be in the ± 2 value range to define whether the decentralized data match the regular distribution (13). An "independent T-test" was adopted to compare independent data with normal distribution within two groups. Results with $p <0.05$ were recognized as statistically notable.

Mann-Whitney U test was utilized to compare independent data that did not submit to the normal distribution, and results with $p <0.05$ were acknowledged significant. Spearman correlation test was applied to correlate data that did not fit the normal distribution; moreover, $p <0.05$ were considered meaningful.

Permission was obtained from Sakarya Training and Research Hospital Chief Physician's Office on 05.02.2021 for this study.

RESULTS

In the SEAH adult emergency department, 220296 patients have examined a total of 382413 times (1.74 per person) during 2019. The applying patients' to the SEAH adult emergency room 12 times or more in a year was 808 in 2019. These patients' emergency examinations' total quantity was 14369, presenting 3.76% of all emergency examinations.

Of these sufferers, 420 (52%) were male, and 388 (48%) were female. Patients' mean age was 47.96 years (±18.82); the median age was 47; furthermore, the age range was 18-108. In comparing the patients' age according to their gender, it was mentioned that the average age of men (mean: 50.65) was significantly higher than women's (mean:45.04) according to the independent samples T-test [$t(806) = 4.284$, $p <0.001$]. The distribution of the patients' genders is presented in Table 1.

 http://dx.doi.org/10.36472/msd.v8i2.477

Table 1. Comparison chart by gender

Category	States	Male	Female	Total	p Value[1]
Other Polyclinics[2]	Yes	369	363	732	0.006
	No	51	25	76	
Psychiatr Policlinic[3]	Yes	175	130	305	0.017
	No	245	258	503	
Addiction Polyclinic[4]	Yes	16	3	19	0.004
	No	404	385	789	
Ambulance[5]	Yes	169	110	279	0.001
	No	251	278	529	
Hospitalization[6]	Yes	94	89	183	0.850
	No	**326**	**299**	**625**	

1Pearson Chi-Square test, 2Application status to other polyclinics in SEAH except for emergency service, 3Application status of SEAH Psychiatr polyclinics, 4Application status to a specialised polyclinic for addicted patients, 5Arriving at the emergency room by ambulance at least once, 6Hospitalization from the emergency department

The emergency department examinations estimate was 17.78 (±10.98) times averagely, the median amount was 15 times, and was between 12-192. The patients' gender had no significant impact on emergency room admittances (p = 0.626). A weak but meaningful positive correlation was remarked between the patients' ages and their ER examinations (r = 0.096, p = 0.006). Thus, it can be stated that as the age of the patients increases, their emergency service admissions raise additionally.

183 (22.6%) patients were hospitalized from the emergency room minimum once, besides 625 (77.4%) were never hospitalised. There was a striking association between hospitalization and the number of ER access, and it was discovered that the admission number was higher in hospitalized patients (p = 0.001). On the other hand, there was no significant discrepancy between the gender of the cases and the hospitalization status (p = 0.850).

732 (90.6%) patients applied to the regular outpatient clinic at least once throughout the research period, and 76 cases (9.4%) did not apply. However, the number of referrals to other polyclinics did not significantly affect the number of admissions to the emergency department (p = 0.150). Considering the patients who also applied to other polyclinics among these patients, it was perceived that the count of polyclinic examinations was between 1-95, an average of 16.83 times (±16.82), the median value was 10. A weak but notable correlation was perceived between the number of outpatient clinic admissions and emergency service admissions (p = 0.001, r = 0.245).

Two hundred seventy-nine patients (34.5%) attained to the ER at by ambulance minimum once, despite that 529 cases (65.5%) never arrived at the ER by ambulance. Nevertheless, it was determined that the number of emergency service admissions of patients who attained by ambulance at least once was statistically higher than those who did not (p = 0.048).

Of the patients, 305 (37.7%) had also applied to the psychiatry outpatient clinic at least once. It was observed that being examined in the psychiatry outpatient clinic had a significant effect on the number of emergency service admissions; moreover, it was ascertained that the ER visits number was higher in these patients (p = 0.001).

DISCUSSION

The demographic data of our study were compared with similar research in the literature. Eduardo and Elaine's reported that patients who frequently used the emergency room were on average, 40; and, younger than 65 years old, while women were more apparent in their study (**2**). Lauren et al. similarly remarked that patients who applied to the emergency department were predominantly female, with an average of 48 (**11**). Roberta et al. and Kristin et al. further observed that frequent users' average age was 40.3 years and 40 years, respectively; most of them were women (**3,7**). In contrast, Ksenija et al. perceived that most of the patients who frequently applied to the emergency department were men, but the patients' average age was 50.3 years, on average, younger than 65, matching with other publications (**6**). The patients' average age in our study was less than 65 years and was 47.96 years; therefore, it was related to other studies. In various other analyses, female cases had a noble rate of re-admissions, whereas, in our investigation, males more applied to the emergency room repeatedly, supporting the study of Ksenija et al.

It is believed that the incapacity to attain a specialist doctor appointment in outpatient clinics can enhance emergency departments' intensity. Sara et al. determined in their study that extended outpatient appointment time could raise emergency administrations (**14**). Garbers et al. also stated that increasing primary health care usage has resulted in a meaningful reduction in unnecessary emergency room applications (**15**)." If people could make an appointment to outpatient clinics, they would not come to emergency departments unnecessarily," the results of this research do not verify this recognition. It was observed that 90.6% of patients applied to other outpatient clinics at least once in the same year, and the fact that they applied to other outpatient clinics did not have a statistically significant impact on the number of emergency demands of patients. On the converse, as increasing outpatient applications number, it was observed that there was a correlated accretion in applying patients' emergency department. The fact that 65.5% of the patients did not apply to the emergency room by ambulance, 77.4% of the patients were discharged from the emergency service may signify unnecessary emergency service utilisations. These people also had intensive applications to other outpatient clinics; furthermore, it could be assumed that they were heavy

health system users. In other studies, it has been stated that the determination that facilitating outpatient appointments leads to a reduction in emergency applications; in contrast, this did not correspond with this research's results about frequent emergency room patients. Publications report a significant proportion of those with mental state ailments repeatedly applying to the emergency department (2,3,6,11,16). Outcomes of this study showed that 37.7% of the patients were observed from the psychiatry polyclinic; moreover, the results were consistent with the medical literature. Consequently, it may be valuable to evaluate patients frequently applying to the emergency department, but do not have a recognized chronic disease, referring them to the psychiatry outpatient clinic, if they do not have any urgent complaints. Roberta et al. asserted in their study of 100 emergency patients who regularly admitted to the emergency room that the patients had negative health system experiences, low socioeconomic status, and chronic mental-physical diseases (3). In this study, no inquiry was made regarding the causes of repeated emergency admittances and the patients' diagnoses. Because of a limited number of studies in the literature to analyze the reasons for excessively using the emergency room, it is seen that more detailed studies and analyses are needed on this subject.

CONCLUSION

One of the factors that increase emergency services density is frequent emergency users, even if their numbers are low. Also, this patient group visits other polyclinics regularly. Limitations should be required on these patients using emergency services in non-emergency situations. Releasing an unlimited number of admissions to the emergency room without restriction may result in remarkable patients' abuse of the emergency health system.

It is essential to pay specific attention to frequent emergency room users and investigate the motivations for proceeding to the emergency room. Since the crucial prevalence of mental disorders in such patients can be interpreted as patients necessitate help, it may be profitable to contact psychiatry.

Author contributions: ED, FG; Literature search and study design, data collection and analyzes **ED;** Writing article and revisions

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. This research did not receive and specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical issues: All authors declare originality of research.

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MSD

Medical Science and Discovery
ISSN: 2148-6832

Case of BRAF mutant Erdheim-Chester disease presenting with multisystem involvement: A case report

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ABSTRACT

Objective: Erdheim-Chester disease is a rare form of non-Langerhans cell histiocytosis of unknown etiology. It is a multisystemic disease that can involve bones, skin, brain, retroperitoneum, cardiovascular system, endocrine system, and lungs. Diagnosis is made by clinical findings, imaging results, and histopathological examination. BRAFV600E (B-rapidly accelerated fibrosarcoma gene) mutation is found in more than half of the cases. When Erdheim-Chester disease is not considered the differential diagnosis, it is not possible to diagnose and treat. In this case report, an Erdheim-Chester case with symptomatic, multisystem involvement, BRAFV600E mutation, and initiated vemurafenib treatment is presented in the light of literature data.

Keywords: Bone pain, non-Langerhans cell histiocytosis, Erdheim-Chester Disease, vemurafenib, case report

INTRODUCTION

Erdheim-Chester disease (ECD) was first described as lipoid granulomatosis in 1930 by Jacob Erdheim and William Chester (**1, 2**). The cases are generally diagnosed between the ages of 40-70. It is slightly more common in men (**2-4**).

Clinical findings are related to the involved organ (**2, 5**). The skeletal system is involved in 95% of the cases. Bilateral cortical osteosclerosis in long bones involving the diaphysis and metaphysis strongly suggests ECD. The axial system is generally preserved. Findings of 18-FDG PET-CT are highly specific. Patients often complain of bone pain (**2, 3, 5**).

Cardiovascular involvement is detected in at least 50% of patients (**2, 5**). Retroperitoneal fibrosis is seen in nearly half of the cases, especially around the kidneys and ureters. Central nervous system involvement is detected in approximately half of the cases (**6**).

Diabetes insipidius as a result of pituitary involvement and exophthalmos as a result of periorbital involvement is seen in 20-30% of cases. Particularly pleural and mediastinal involvement can be seen in the respiratory system. Lesions in the skin, xanthelasma in the eyelids and periorbital region are common. Arterial involvement can be seen in 50-80% of cases (**2, 5**). Additionally, myeloid neoplasia develops in approximately 10% of the cases (**2**). The biopsy is required for the diagnosis of ECD.

The examination shows mixed infiltration rich in histiocytes and rare Touton giant cells and fibrosis. Histiocytes are CD 68 positive and CD 1a negative (**3, 5, 7**). More than half of the cases have the BRAFV600E (B-rapidly accelerated fibrosarcoma gene) mutation (**3, 4, 7, 8**). Corticosteroids, interferon (INF), anti-cytokine therapy, and targeted therapies, BRAF inhibitor, vemurafenib and MEK inhibitors can be used in treatment (**1, 2, 8**).

While Non-Langerhans cell histiocytosis constitutes the rarely seen disease group, we wanted to present a case of ECD, which is one of its subgroups and because of the fact that only a few cases have been reported in the world.

Case Report

Received 16-01-2021

Accepted 01-02-2021

Available Online: 06-02-2021

Published 24-02-2021

http://dx.doi.org/10.36472/msd.v8i2.465

CASE

A 48-year-old male patient has complaints of bone pain and fatigue for 15 years. Thirteen years ago, the patient, who applied to a hospital with these complaints, was diagnosed with histiocytosis X and was given steroid therapy for 10 months. His treatment was discontinued due to the development of iatrogenic Cushing in his follow-up, and central Diabetes Insipidus developed during his follow-up in the hospital. The patient, whose pain in the hip bones was investigated, was diagnosed with steroid-induced avascular necrosis. The patient is first monitored and then operated on 2 years later. During this period, the patient's complaints repeated millitantly. He applied to our hospital with the complaint of extreme fatigue. The laboratory tests in his application are shown in (Table 1).

Table 1: Laboratory results of the patient

	Before treatment	After treatment	Normal range
WBC (10^3/uL)	6,7	8,7	4.6-10.2
Hg (g/dl)	7,6	12,7	12.2-16.2
Hct (%)	25,49	40,76	35.5-48
Plt (10^3/uL)	331	228	142-424
Glucose (mg/dl)	90	89	70-105
AST (U/L)	94	16	5-34
ALT (U/L)	98	13	0-55
Total Bilirubin (mg/dl)	0,41	0,19	0.2-1.2
Creatinine (mg/dl)	0,44	0,71	0.57-1.11
CRP (mg/dl)	164	69	<0.5
ESR (mm/h)	72	36	0-20
Albumin (g/dL)	2,5	4,3	3.5-5
LDH (U/L)	295	209	125-220
Na (mmol/L)	140	141	135-145
K (mmol/L)	4,5	4,3	3.5-5.1
Ca (mg/dl)	8	9,1	8.4-10.2

WBC: white blood cell, **Hg:** hemoglobin, **Hct:** hematocrit, **Plt:** platelet, **AST:** aspartate aminotransferase, **ALT:** alanine aminotransferase, **CRP:** C-reactive protein, **ESR:** erythrocyte sedimentation rate, **LDH:** lactate dehydrogenase, **Na:** sodium, **K:** potassium, **Ca:** calcium

In thoracic computed tomography (CT), low-density nodules with a diameter of 5.5 mm are present in both lung parenchyma, the larger of which is in the lower lobe of the left lung, laterobasal segment. Pleural thickening was observed in the right lung. Spleen size was 155 mm in abdominal CT, and a 10 mm hypodense lesion was observed in the lateral middle part of the spleen. There was an appearance of hypodense soft tissue around both kidneys reaching a thickness of 20 mm on the right and 19 mm on the left. 18-FDG PET/CT imaging showed areas of hypermetabolism in the cranium, sphenoid bone, left frontal bone, bilateral humerus, right iliac bone, adjacent sacroiliac joint, left acetabulum ceiling, and bilateral femur in favor of the involvement of the primary lytic-sclerotic disease (SUV Max:10.1), paravertebral mild hypermetabolic pleural thickenings in the right hemithorax, increased retroperitoneal, hypermetabolic density in the vicinity of the bilateral kidney, increased kidney size, and hepatosplenomegaly (Figure 1). When the patient was evaluated cardiologically, fibrotic aortic and mitral valves, minimal mitral, aortic, and tricuspid regurgitation were detected on echocardiography. The ejection fraction was calculated to be 60%. Erythematous antral gastritis was determined in gastroscopy examination. No pathology was found in the colonoscopic examination. The biopsy was taken from the xanthomatous lesion around the eye. A trucut biopsy was performed from the soft tissue around the kidney with imaging guidance. Pathology was evaluated as compatible with ECD. The BRAF mutation examined was also found to be positive in support of ECD (Figure 2). Vemurafenib was started because studies were showing that it is more effective on the patient's multisystem involvement including the central nervous system, the development of side effects to previous steroid treatment, and the detection of positive BRAF mutation (**9, 10**). Vemurafenib treatment dose was started as 960 mg/ day. An improvement in clinical and laboratory values was detected in the first 3 months of the patient's follow-up (Table 1). The patient, whose general condition is stable, comes for a checkup every 3 months.

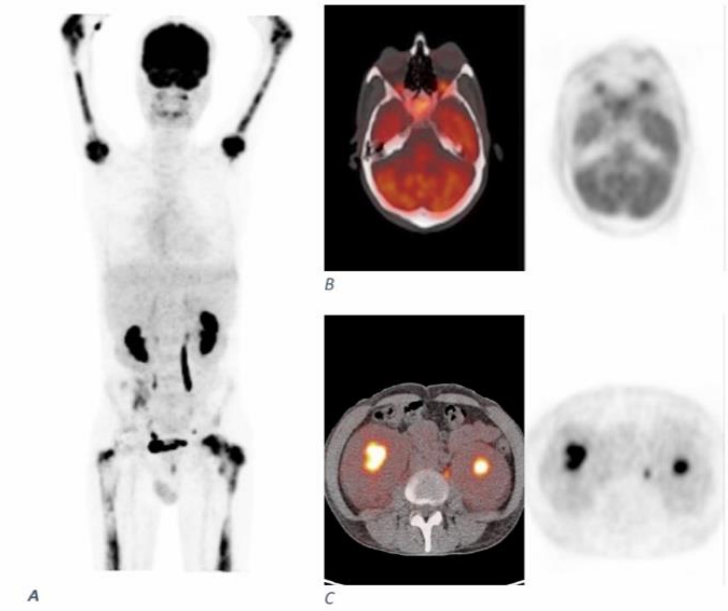

Figure 1. Positron emission tomography(PET) scan showing high pathologic uptake 18F- flurodeoxyglucose in multiple sclerotic bone lesions (**A**), cranium (**B**) and perirenal soft tissue (**C**).

http://dx.doi.org/10.36472/msd.v8i2.465

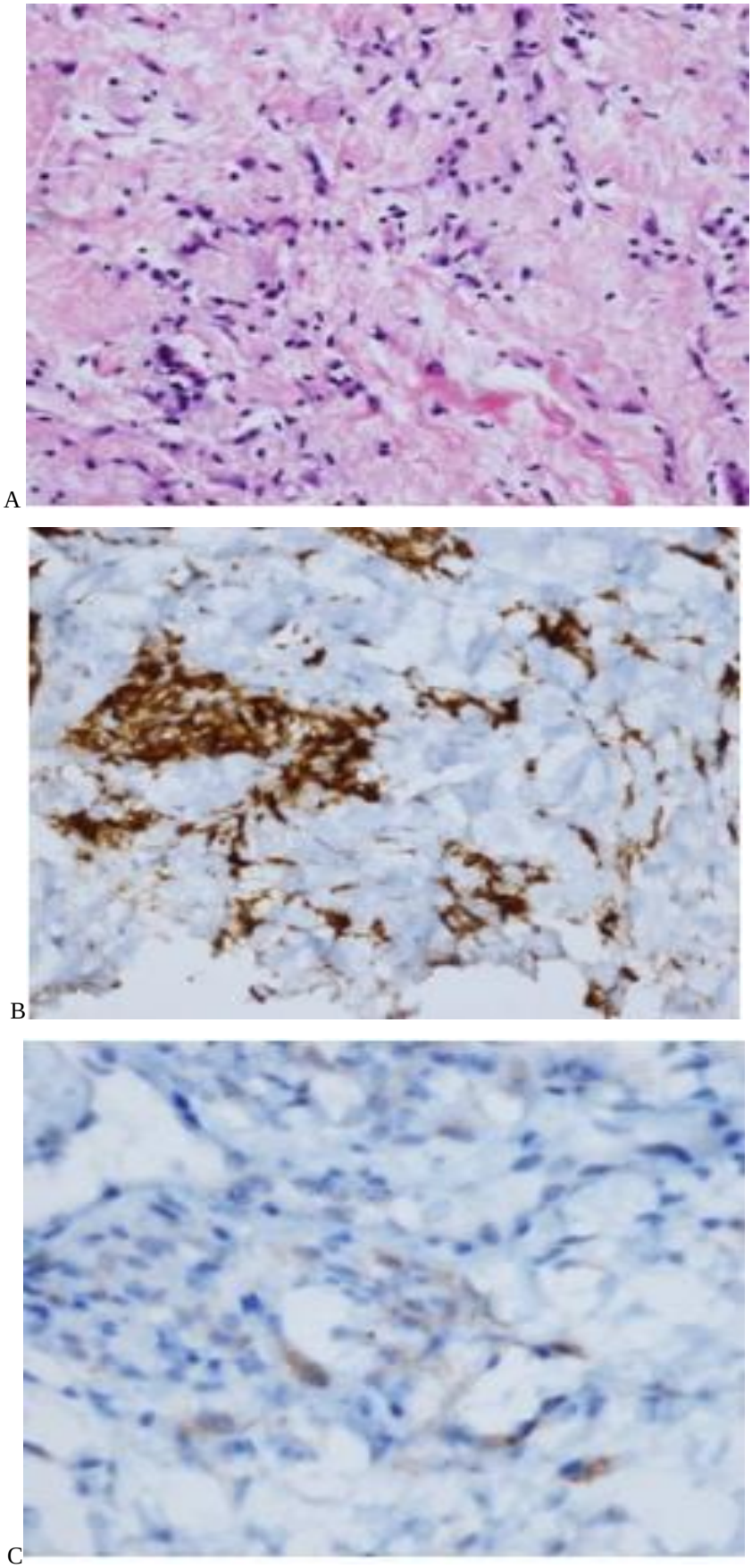

Figure 2. **A**: In samples taken from perirenal soft tissue, histiocytic cells with foamy cytoplasm forming small groups in collagenized connective tissue were noted. **B-C**: The described histiocytic cells showed immunohistochemically positive immunoreactivity with CD163 and BRAF. The result of the V600E mutation in the BRAF gene applied by the real-time PCR method is positive. The mutation is present.

DISCUSSION

Erdheim Chester disease can affect many organs. Since the lesions are not specific to the disease, patients may be diagnosed late (**4, 8**). The main complaints of the patients are bone pain and fatigue. Weight loss, fever, and night sweats may also occur (**1**). In our patient, the first application reason and the complaint during the period were bone pain and fatigue. Laboratory findings are not specific to the disease. However, sedimentation and high C-reactive protein (CRP) are detected in most patients (**1, 4**). Both of the values were high in our patient. Anemia findings observed in our patient are related with chronic inflammation. Liver enzymes are elevated in 10% of patients (**4**). In our patient, liver enzymes that were normal at the beginning were found to be slightly elevated at the final examination. Imaging methods play a crucial role in diagnosis. Bone scintigraphy rather than direct radiography shows long bone lesions. CT is valuable for organ involvement. Particularly, perirenal fat tissue involvement and fibrosis, which is responsible for "hairy kidney" appearance, can be demonstrated by CT. Magnetic resonance imaging (MRI) is more suitable for central nervous system and cardiovascular system lesions (**1, 4, 8**). In our case, pituitary involvement was detected by MRI. 18 FDG-PET/CT is ideal for showing both skeletal system and soft tissue lesions (**1, 4, 8**). In addition, response to treatment can be followed by this method. Extensive bone involvement was determined with 18 FDG-PET / CT in our patient. Organ involvement and retroperitoneal involvement around the retroperitoneal kidney were also shown. Lymph node involvement is very rare in ECD (**4**). In our case, there was no finding of lymph node involvement.

Biopsy of pathological tissues is required to confirm the diagnosis of Erdheim-Chester disease. In order for the biopsies to be evaluated accurately in pathology, considerations about clinical and pre-diagnosis should be consulted with pathology. Biopsy can be performed on different areas of involvement such as bone, skin, retro-orbital and retroperitoneal region. Mixed infiltration rich in histiocytes and sparse Touton giant cells and fibrosis are seen. Histiocytes are CD68 positive and CD1a negative (**1, 4, 7, 8**). In our case, biopsies were performed from the xanthomatous lesion around the eye and perirenal tissue. In the biopsy sample taken, staining in histiocytes was CD68 (+) and CD1a (-), and staining was selected with BRAF in the lesions. The biopsies taken had features that could be compatible with Erdheim Chester. The prognosis of Erdheim Chester disease depends on the degree of the organ involved and the treatment given (9). Early diagnosis and timely initiation of correct treatment are important for patient's morbidity and mortality. With the increasing knowledge of the pathogenesis, BRAF and MEK inhibitors are used. Cases with a favorable response with vemurafenib therapy have been reported in patients with BRAF mutants (**10, 11**). In our case, in the third-month follow-up of vermurafenib treatment, clinical remission, increase in haemoglobin, and regression in inflammatory markers such as CRP and ESR were found (Table 1). In our case, diabetes insipidus, pituitary involvement, testicular insufficiency, diffuse bone involvement, xanthetasis in the skin, pleural thickening in the lungs, perirenal/retroperitoneal involvement, splenomegaly, hepatomegaly, and chronic anemia were detected. As in our case where many organs are involved, defined ECD is limited in number. Its diagnosis is difficult due to its low awareness, extreme rarity and involvement of many different organs. When ECH disease is not known, it will not be possible to make a diagnosis and to give the correct treatment, even if there are typical kidney appearance and bone lesions. We wanted to present a very rarely found case to the literature and raise the awareness of the disease.

Author contributions: NHK, CK; Patient examination and project design, **TK;** Pathological examinations, **AE, GC;** Hemalotological tests,. **NHK;** review of the literature, analyzes and writing of the manuscript.

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. This research did not receive and specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical issues: All authors declare originality of research.

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MSD

Medical Science and Discovery
ISSN: 2148-6832

Second Branchial Cleft Cyst: A Case Report

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ABSTRACT

Objective: Branchial anomalies are congenital pathologies that are seen in the lateral region of the neck and are generally benign. The branchial clefts develop in the 2nd-7th weeks of fetal life as embryonal development. The branchial anomalies are caused by non-disappearance, abnormal development and, incomplete emerger of the branchial clefts and pockets during embryonal development. The branchial anomalies are generally seen as the cyst. The most common cyst was the second branchial cleft cyst with 95%. Their diameter is usually a few centimeters.

Case: A 37-year old male patient was admitted to the hospital because of a swelling on the left side of his neck for four years. Physical examination revealed a mobile cystic mass in level 2 at the upper left jugular region of the neck. The cystic mass and the surrounding lymph nodes were excised and sent to the pathology laboratory. A cystic mass, approximately 5x4x3.5 cm in size, containing cystic areas was observed macroscopically. Microscopically, the cyst was lined with squamous epithelium and contained large lymphocyte groups in the subepithelial area. The case was reported as the branchial cleft cyst.

Conclusion: Branchial cleft cysts should be excised before reaching large sizes, as they may cause pain and pressure on the surrounding tissue. In addition, it should be kept in mind that malignancy may develop from branchial cleft cysts, although rare.

Keywords: Branchial, cleft, cyst

INTRODUCTION

Branchial anomalies are congenital pathologies that are usually benign and seen in the lateral neck region (1,2). Branchial anomalies are generally seen as cysts, sinuses, and fistulas (1,2). These anomalies originate from the branchial cleft (3). Of these cysts, malignancy may rarely develop, the most common being squamous cell carcinoma (2,4). Branchial clefts begin to form in the second week of embryonic life and close between the 6th and 7th weeks (5). Branchial anomalies are caused by the destruction, abnormal development, and incomplete fusion of branchial clefts and pockets during embryonic development (1,3). A human has five branchial clefts, five branchial sacs, and six branchial arches on either side of the neck (6). The most common is the 2nd branchial cleft cyst with 95% (1). First branchial cleft cysts are usually located in the preauricular region (7). Second branchial cleft anomalies are localized anterior to the sternocleidomastoid muscle (7). Third and fourth branchial cleft cysts develop from the piriform fossa and end in the paratracheal region of the neck (8). Treatment of branchial cleft cysts involves total excision of the lesion (9).

CASE REPORT

A 37-year-old male patient was admitted to the hospital with the complaint of swelling on the left side of the neck for four years. On physical examination, a mobile cystic mass of approximately 5 cm was found in the upper jugular area of the neck at level 2. There were mild redness and pain in the area of the mass. Surrounding the mass, there were three lymph node-like lesions. No pathological finding was detected in the examination of the patient's mouth, oropharynx, and larynx.

Case Report

Received 03-02-2020

Accepted 23-02-2021

Available Online: 24-02-2021

Published 24-02-2021

http://dx.doi.org/10.36472/msd.v8i2.483

A minimal increase in C-reactive protein and sedimentation was observed in laboratory examinations. No pathology was found in the chest x-ray. The cystic mass and surrounding lymph nodes were excised and sent to the pathology laboratory.

Macroscopically, the lesion was 5x4x3.5 cm in size and contained cystic areas (Figure 1).

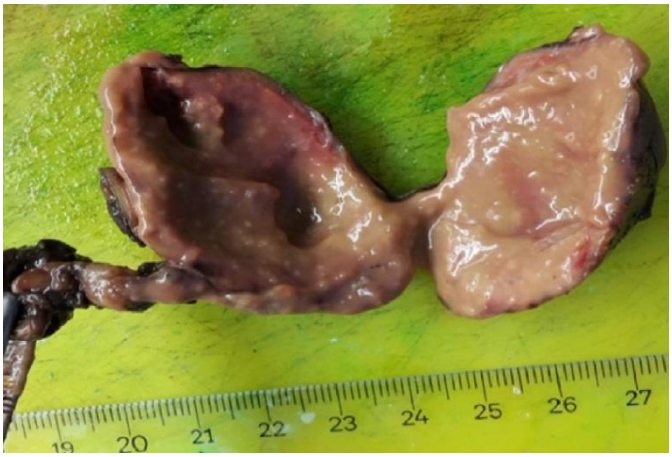

Figure 1: Approximately 5 cm cystic lesion, macroscopic view.

The thickness of the cyst was 0.1-0.7 cm. There was light brown necrotic material in the cyst. 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, the cyst was lined with squamous epithelium and there were lymphocyte groups of different sizes in the subepithelial area (Figure 2).

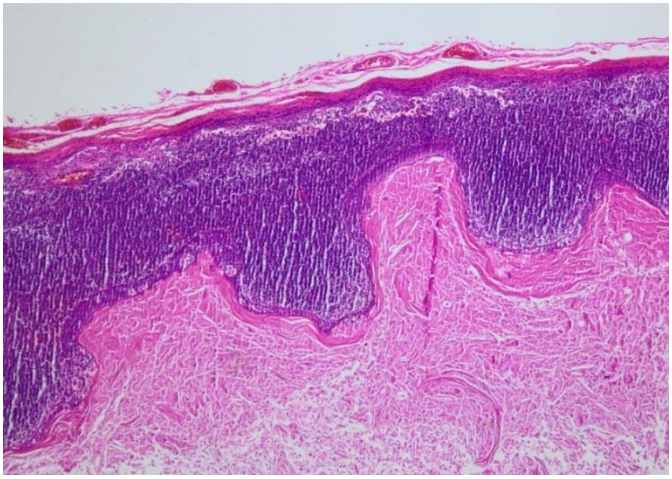

Figure 2: Cystic lesion lined by squamous epithelium, containing lymphocyte groups of different sizes in the subepithelial area (HEx40).

The contents of the cyst consisted of eosinophilic necrotic material. The case was reported as the branchial cleft cyst. Three lymph node-like lesions were reported as active chronic lymphadenitis.

DISCUSSION

Branchial anomalies are the most common congenital neck pathologies in the lateral neck (4). Branchial cleft cysts are usually seen as cystic masses on the left lateral side of the neck (1,2). These cysts can be found at any age, but findings generally occur in young adults between the ages of 20-40 (2,4). It usually occurs unilaterally with no significant sex predilection (10). These cysts are often seen as neck masses following respiratory tract infection attacks (2,4). Infection may develop in cases of branchial cysts. Of these cysts, malignancy may rarely develop, the most common being squamous cell carcinoma (2,4).

In addition to physical examination, ultrasonography, computed tomography, and magnetic resonance imaging methods are used for diagnosis (11). Fine needle aspiration biopsy and observation of cholesterol crystals, epithelial cysts and squamous cells in the material taken may be helpful in diagnosis (12). The definitive diagnosis of branchial cysts and carcinomas is made by the removal of the lesion and then the histopathological examination of the mass (13,14).

Second branchial cleft cysts range in size from 1 to 10 cm (15). Alimoglu Y et al. Found the mean diameter of second branchial cleft cysts to be 3.29 in their study (16). In our case, the large diameter of the lesion was 5 cm.

CONCLUSION

Removal of these lesions at an earlier stage will increase the quality of life of the patient. In addition, since they are rarely malignant, more successful results will be obtained with early diagnosis and treatment.

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

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. This research did not receive and specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical issues: All authors declare originality of research.

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Medical Science & Discovery

International Journal of
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Open Access Scientific Journal
ISSN: 2148-6832
Lycia Press LONDON U.K.
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