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REVIEW ARTICLE OPEN ACCESS

Curtailing the Risk of COVID-19 Infection Among Medical Students Through Safer Handling of Cadavers During Anatomical Dissection

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The novel COVID-19 pandemic has produced broad consequences on medical training globally. The resulting disturbances led to the shift from physical to virtual learning. A serious feature of medical training demanding pressing consideration is the avoidance of COVID-19 spread among medical students during cadaveric dissection. This can be attained by guaranteeing the protection of cadavers from COVID-19 afore been used for anatomical dissection. This article highlights the safety procedures necessary for handling cadavers and its' significances on the SARS-CoV-2 virus spread among medical students amidst the pandemic.

Keywords: COVID-19, prevention, transmission, cadaveric dissection

Introduction

The novel COVID-19 pandemic has produced broad effects and drastic impacts on medical education all over the globe. Its influence and consequent disruptions led to the shift from face-to-face to virtual learning, thus affecting millions of learners to suspend learning or learn remotely (1, 2). A critical aspect of medical training demanding pressing attention is the prevention of COVID-19 transmission among medical students during cadaveric dissection (3-5). This is necessary as cadaver dissections are widely practiced in most medical schools, and arguably remain the gold standard in gross anatomy teaching globally (4, 6-8).

Although the advent of COVID-19 has brought a major setback in teaching anatomy via cadaveric dissections resulting from cessation or sharp decline in body and organ donations, the use of cadavers for anatomical dissection can hardly be completely erased or canceled (9). The use of cadaver dissection for anatomy training during and post- COVID-19 pandemic is possible and necessary (10). However, for medical schools that rely heavily on donor or

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unclaimed body to resume cadaveric dissection, there is a need to guarantee the protection of cadavers from potential COVID-19 infection afore been used for anatomical dissection (3-5). The article explores the safety measure needed for cadaver handling and its consequences on the SARS-CoV-2 virus spread among medical students amidst the COVID-19 pandemic.

Global Rising Cases of COVID-19 Infections, Public Health and Safety Concerns

According to John Hopkins University and Medicine (JHUM), about 164,284,766 confirmed cases and 3,406,261 deaths arising from COVID-19 have been reported globally as of May 19, 2021 (11). This novel COVID-19 pandemic produced by SARS-CoV-2 has brought about huge effects on populations (12), socio-economic, and health care systems universally (13-16). These disruptions including; prohibition of several human activities such as restraint on movements (17), ban of mass gatherings (18), use of face masks, hand hygiene (washing and sanitizers), physical distancing among other strict actions are the result of the spike in COVID-19 cases (19,20). These interventions are necessary to be enforced by several countries and governments to curtail its further spread and the tendencies of succeeding wave of infections following the WHO recommendations and standards.

Public Health Concerns About Cadaveric Dissection During COVID-19 Pandemic

While the pandemic unfolds, many health regulatory authorities and public health organizations have raised critical questions and issues concerning the health, safety, and ethics of body and organ donations to medical schools (21). Although there is currently no evidence of contagion resulting from exposure to COVID-19 associated bodies, the alleged danger of COVID-19 infection from individuals who died from the disease to medical students dissecting such bodies cannot be ignored (3-5).

The International Federation of Associations of Anatomists (IFAA) stated in a published report that direct contact with body fluids and intrusive procedures on the bodies, like dissection, autopsy, or related techniques that produce droplets or aerosols could increase the likelihood of disease spread (22). This stemming situation has placed a grave problem on medical education largely, and precisely cadaveric dissection. Thus, there is a dire need to explore the safety measures needed for cadaver handling during anatomical dissection, and its consequences on the SARS-CoV-2 virus spread among medical students amidst the COVID-19 pandemic.

Adoption and Curricular Integration of Effective Alternative Models for Teaching Gross Anatomy

Though the advent of the recent pandemic has rejuvenated the need and call for adoption and use of other alternatives models for studying gross anatomy such as virtual dissection amongst others (4), cadaveric dissection remains a chief, renowned, and widely accepted standard for teaching gross anatomy in anatomy education (6-8). The importance and gains that cadaveric dissection gives to students cannot be over-emphasized. More significantly is that students do not derive anatomical understanding alone, but likewise life teachings in humanity and ethics (23).

In this light, notwithstanding that the pandemic had and is still causing difficulties with medical education delivery; medical schools must constantly strive to pursue means to guarantee pedagogies like dissection are incorporated into the revised curriculum (9).

Pressing on, medical schools will certainly trust profoundly on technologies including anatomical lectures and online dissection videos. Nevertheless, cadaveric dissection possesses immense and valuable educational opportunities, experiences, and advantages for medical schools to forever curb its usage as an anatomical pedagogy (10, 23). Thus, it is obligatory for medical schools and educators to continually provide a well-balanced dissection-centered anatomy education (10).

Curtailing the Risk of COVID-19 Infection Through Safer Handling of Cadavers

A high level of safety concerns rest on nations and medical schools who depends mostly on free bodies and/or donor program for anatomical dissection, as medical histories to establish the reason of death may be absent, hence posing a risk for students observing cadaveric dissection during and after the coronavirus pandemic (3-5). Thus, providing a COVID-19 infected body for dissection will be disastrous if not well handled.

It is, therefore, necessary for medical students to dissect cadavers following the standard operating procedures (SOPs) and all safety procedures and guidelines (3-5), while fully protected with necessary personal protective equipment and maintaining proper personal hygiene (PPE) (22,24). In addition, before students are permitted to dissect cadavers, it is expected that all the essential COVID-19 assessments should be carried out. They must also strictly follow all laboratory and COVID-19 safety precautions and guidelines, to avert unintended infection spread as the battle against COVID-19 remains (3-5).

Conclusively, if the spike in COVID-19 deaths raises the availability of cadavers for anatomy teaching, there is a need to enforce strict safety measures for cadaver handling by medical educators and students during cadaveric dissections to prevent COVID-19 transmission. Some of the recommended safety measures include the following: learners need to be fully protected with necessary personal protective equipment (PPE) during dissection, aerosols-producing techniques, and smearing of polluted fluids must be highly discouraged as the cadavers may still be infectious.

More so, it is vital to conduct screening for COVID-19 and/or obtain comprehensive medical histories of cadavers and bodies donated to medical schools to ensure educators and students have complete information before handling such bodies. It is also imperative that medical educators and students are well skilled in the spread and avoidance of COVID-19 infection, as negative laboratory investigation results might not completely exclude the chance of COVID-19 infection.

Conflicts of Interest

The authors have declared no conflict of interest for the present article.

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ORIGINAL ARTICLE OPEN ACCESS

Dosimetric Comparison of Scalp Protection in Whole Brain Radiotherapy Due to Brain Metastasis

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Introduction: The objective is to demonstrate dosimetrically the preservation of scalp in whole-brain irradiation in the treatment of brain metastases and to make dosimetry at 20 points determined on the rando phantom while comparing between Linac and Tomotherapy devices.
Materials and Methods: 10 randomized patients, who had previously undergone radiotherapy for whole-brain metastasis cancer, were determined prospectively. In Helical Tomotherapy (HT) and Linac devices, Intensity-modulated radiation therapy (IMRT) and volumetric modulated arc therapy (VMAT) were planned to be 30 Gray(Gy) 10 Fractions for the whole brain region.
Results: The average target volume (PTV), Homogeneity index (HI), conformity index (CI), and integral dose (ID) for IMRT and VMAT were 0.075, 0.77, 0.94, 0.97 and 29.67, 23.57, respectively. Mean median doses for scalp IMRT and VMAT were 19.71Gy and 18.01 Gy ($p<0.005$). Lenses and body doses were significant for IMRT and VMAT. The mean median plan doses for Rando phantom scalp were 19.43Gy and 19.55Gy in IMRT and VMAT, respectively. The mean median film doses for Rando phantom scalp were 17.03Gy and 20.64Gy in IMRT and VMAT, respectively ($p<0.005$).
Conclusion: By using both VMAT and Helical Tomotherapy techniques, it is possible to dry the lens and scalp without low PTV doses in whole-brain irradiation.
Keywords: Brain metastasis, scalp, intensity-modulated radiotherapy, volumetric-modulated arc therapy

Introduction

Brain metastasis is known as the spread of a tumor occurring in tissues and organs other than the brain through the blood circulation or lymphatic system and forming a tumor on the brain. These types of tumors are the most common cases in the brain. With the treatment of visible and hidden lesions, reduced symptoms, and rapid application, whole-brain radiotherapy is a common treatment feature (1).

Although the most common clinical findings in brain metastase include headache, vomiting, convulsion, and different neurological findings, diagnosis can be made without any clinical symptoms, and approximately 20% of lung, breast and colorectal cancers are cancer types that cause brain metastasis (2-4).

Upon diagnosis, radiotherapy, chemotherapy, surgery, and supportive treatments are often

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applied to brain metastases. Radiotherapy is an important and effective treatment method in the treatment of brain metastases(3,5). Since brain metastases spread through the blood and lymph system, one of the precautions to be taken against this situation is to consider the whole brain as a target and apply whole-brain radiotherapy.

There are many significant parameters in determining the treatment option for brain metastases. The most significant of these are listed the age, general condition, quality of life and performance of the patients, and the toxicities that may occur (4, 8). Temporary or permanent hair loss is observed in whole-brain radiotherapy (WBRT). Together with hair loss (alopecia) is an important psychological problem in terms of the quality of life of patients, it has a deeply negative impact on their social life (6, 7).

When the dose and fraction values prescribed in whole-brain radiotherapy treatments were compared; the best dosage regimen in terms of mean survival, local control, and neurological function is the administration of a 3 Gy dose in 10 fractions with a total of 30 Gy. This dose fractionation regimen is used as a standard in WBRT (9, 10).

In this study, we aimed to minimize such negativities, 10 patients randomly selected with Intensity-modulated radiation therapy (IMRT) and volumetric modulated arc therapy (VMAT) techniques in scalp-protected Helical Tomo therapy and Linac devices in whole-brain radiotherapy, and dosimetric comparison of scalp doses in different techniques, and the same techniques on the rando phantom by planning the parietal and occipital bones at 20 points.

Material and Method:

Patient Selection

Ten randomized patients, who had undergone radiotherapy for whole-brain metastasis cancer previously were determined prospectively. Our study is approved by the ethics committee following the Helsinki Declaration.

Simulation and Contouring of Targets and OARs

Computed tomography (CT) images of 3 mm thickness were obtained by thermoplastic head mask to provide head & neck immobilization in the supine position of all patients.

From the computerized tomography (CT) images recorded in the planning computer of 10 randomly selected patients, the whole brain was contoured prospectively and the planning target volume (PTV) was determined by giving the whole brain a 3-millimeter margin. The organs at risk (OARS) (lens, eye, optic nerve) were contoured (11).

Between skull and skin tissue was determined as scalp by giving a margin of 3 millimeters to the planning target volume and drawing 2 millimeters from the external (skin tissue) contour. Based on these drawn contours, IMRT and VMAT planning was made in Helical Tomotherapy (HT) and Linac devices with 30 Gray 10 Fractions to the whole brain region. Similarly in the Rando phantom, scalp contouring with PTV was made with a 30Gy 10 fraction planning, and 20 points were determined on the rando phantom, 10 points were determined on the frontal-upper parietal and occipital bones, 5 points were determined in the right parietal and 5 were determined with 2 cm intervals in the left parietal, and a CT image was taken. In addition, planning was made in both devices, and irradiation was performed in tomotherapy and linac devices by

placing gafchromic EBT3-1417 film on these points. Homogeneity and conformity indices and integral dose calculations were made for plan evaluation.

Treatment Planning

The planning target volume (PTV) was obtained by giving a 3-millimeter margin to the whole brain. By giving a margin of 3 millimeters to the planning target volume and drawing 2 millimeters from the external contour, between the skull and skin tissue was determined as scalp. Prescription dose 30 Gy was determined as 10 fractions. The dose was prescribed to cover 95% of PTV.

A 0.03cc volume in any PTV was planned not to receive>110% of the prescribed dose. Compliance with critical organ doses determined by RTOG protocol was demanded. In the Helical Tomotherapy device, planning was made with Intensity-modulated radiation therapy (IMRT) to be 30Gray 10 Fractions to the whole brain region. During the treatment, the gantry rotates 360 degrees continuously and at a constant speed and applies RT. During helical treatment, the MLC layout changes in each projection by dividing 360 degrees into 51 projection angles while Linac rotates continuously.

Tomotherapy planning system (Hi-Art Tomo therapy, version 5.1.2, Accuray, Madison, WI, USA) was used in planning HT. MRT and VMAT plans were contoured at the Velocity contouring station. For Helical Tomotherapy device, field size shaping field width (jaw) field width 2.5cm, opening and closing time of MLCs modulation factor (MF) 2.8, table movement speed pitch factor 0.215, and fine dose calculation grid were used.

In the Linac device, planning was made with volumetric modulated arc therapy (VMAT) to be 30Gray 10 Fractions for the whole brain region. Varian Eclipse planning system (version 13.7-Varian Medical Systems, Palo Alto, USA) was used in VMAT planning. The structures contoured in the Velocity contouring station were transferred to the Eclipse planning system in DICOM (Digital Imaging and Communication in Medicine) format.

Two full arcs were used in the VMAT plans at 181°-179° clockwise and 179° 181° counter clockwise. A total of 177 firing points were used at 2 degrees for each full arc. PO 13.6 algorithm was used for fiber positions, dose rate, gantry speed. The maximum dose rate was determined as 600 MU/min. Dose calculation matrix resolution 2.5mm and final dose calculation Anisotropic Analytical Algorithm (AAA) photon dose calculation algorithm was used for all plans. To reduce the tongue and groove effect of the fibers, 30˚ and 330˚ collimator angles were used in arc treatment.

120-fiber (central 20-cm of field uses, 0.5-cm-wide leaves, the outer field uses 1-cm-wide leaves) dynamic multileaf collimator (MLC) was used in VMAT plans. The maximum MLC speed was determined to be 2.5 cm/s.

According to RTOG protocol, 50% of the doses (D50)-V5(volume of received 5 grays), V10(volume of received 10 grays), V15 (volume of received 5 grays), V20(volume of received 20 grays) doses of critical organs and scalp dose were measured with maximum, minimum and average doses (24). 6MV energy was used in all VMAT and HT plans.

Homogeneity and conformity indices and integral dose calculations were made for plan evaluation.

Evaluation Tools

Plan evaluation was made by examining all slides one by one and looking at dose-volume histograms (DVHs). Homogeneity index (HI) was calculated as HI=D2-D98/Dp. D2 is the minimum dose for 2% of the target volume, D98 is the minimum dose for 98% of the target volume, and Dp is the anticipated dose. This is the commonly used formula in the literature. The ideal value of this formula, which evaluates the degree of dose distribution homogeneity of in the target volume, is equal to 0 (12).

Conformity index (CI) was calculated as RTOG CI=VRI/TV. VRI is the volume of the reference dose (cm3), TV is the target volume. CI is defined as the ratio based on the exact volume of the prescribed dose to match the target volume. Its ideal value is equal to 1 (13). Integral dose (ID) calculated= Mean dose (Gy) * volume (L); Calculated as dose-volume histogram received by normal tissue (14).

Statistics

Analyzes were performed using the Statistical Package for Social Science version 22.0, software (SPSS, Chicago, IL, USA). Data were summarized as mean±standard deviation (SD). To evaluate the normality of distribution, Unpaired T-test, data with normal distribution were analyzed with repeated measures analysis of variance, and Bonferroni post-hoc method was used. Non-normally distributed data were analyzed by the Independent Samples test and a Bonferroni adjusted pairwise comparison was used. The significance level was admitted as 0.05 in all analyzes.

Results

Significant differences were observed in tomotherapy and linac device in the maximum, median, and minimum values for the planned target volume ($p<0.005$). In the plans made with Tomotherapy IMRT, the maximum (max) dose value of PTV is 108%, the median (med)

Table 1. PTV Parameters comparative dosimetric comparison

Variables	Groups	N	Mean (Gy)	Std. Deviation	P value
PTV max	*Tomotherapy*	10	32,52	0,32	0,001
	Linac	10	33,19	0,34	0,001
PTV med	*Tomotherapy*	10	30,89	0,19	0,001
	Linac	10	31,21	0,03	0,001
PTV min	*Tomotherapy*	10	28,52	0,27	0,001
	Linac	10	29,57	0,43	0,001
HI	*Tomotherapy*	10	0,075	0,14	0,744
	Linac	10	0,077	0,12	0,744
CI	*Tomotherapy*	10	0,947	0,003	0,002
	Linac	10	0,971	0,02	0,005
ID	*Tomotherapy*	10	29,67	15,84	0,29
	Linac	10	23,15	10,34	0,293
MU	*Tomohterapy*	10	6389,0	521,25	0,001
	Linac	10	569,5	53,05	0,001
BOT	*Tomotherapy*	10	446,79	35,62	0,001
	Linac	10	56,95	5,30	0,001

value is 102%, the minimum (min) values are 95%, while this value is 110% maximum, 103% median and 98% minimum in Linac VMAT.

For the target volumes with calculated dosimetric data, for HI, CI, ID, monitor unit (MU), and beam-on time (BOT) values; Although there is no significant difference in HI, ID dose calculations (p>0.05), CI, MU, and BOT in Linac VMAT plans were found to be higher than HT IMRT (p<0.005). Comparison values are shown in table 1.

Although the maximum values in scalp doses according to both plan criteria are not close to each other (p>0.05), the median and minimum values and the VMAT plan technique are significantly superior with the V5(volume of received 5 grays), V10, V15 and V20 and 50% of the doses (D50) (p=0.005). The comparison values are shown in table 2.

Table 2. Scalp Parameters' dosimetric comparison

Variables	Groups	Mean *(Gy)*	SD	P
Scalp Max	*Tomotherapy*	29,73	0,86	0,938
	Linac	29,75	0,32	0,938
Scalp Med	*Tomotherapy*	19,71	0,52	0,001
	Linac	18,01	0,17	0,001
Scalp Dmin	*Tomotherapy*	4,33	1,30	0,001
	Linac	1,85	0,51	0,001
D50	*Tomotherapy*	19,68	0,87	0,018
	Linac	18,90	0,34	0,024
V5	*Tomotherapy*	99,36	0,89	0,001
	Linac	95,39	1,93	0,001
V10	*Tomotherapy*	95,5	2,14	0,001
	Linac	89,95	2,13	0,001
V15	*Tomohterapy*	81,76	5,44	0,003
	Linac	75,29	2,13	0,005
V20	*Tomotherapy*	48,16	6,65	0,004
	Linac	39,55	4,79	0,004

Although there is no significant change in IMRT and VMAT plans in the dose values taken at 20 points in the plan made on Rando phantom, the values are close to each other and the dose values are shown in Table 3.

Table 3. Dosimetric comparison with Randofantom plan

Groups	N	Mean *(Gy)*	SD	P
Tomotherapy Fantom Plan	20	19,43	4,95	0,652
Linac Fantom Plan	20	19,95	1,22	0,654

Table 4. Dosimetric comparison with Randofantom film

Groups	N	Mean *(Gy)*	SD	P
Tomotherapy Film	20	17,03	4,68	0,003
Linac Film	20	20,64	2,01	0,004

There was a significant difference (p<0.005) in the irradiation made with gapchromic film to 20 points in the plan made on the Rando phantom, and the HT IMRT plan was superior to the VMAT plan. Comparison values are shown in table 4. While lens doses and body doses are lower in HT IMRT, these values are higher in the Linac VMAT plans (p<0.05). Comparison values are shown in table 5.

Table 5. Dosimetric comparison in terms of OAR

Variables	Groups	N	Mean *(Gy)*	SD	P
Right Lens Max	*Tomotherapy*	10	4,78	0,50	0,001
	Linac	10	6,69	0,42	0,001
Left Lens Max	*Tomotherapy*	10	4,77	0,44	0,001
	Linac	10	6,58	0,51	0,001
Body	*Tomotherapy*	10	32,58	0,35	0,001
	Linac	10	33,19	0,34	0,001

Discussion

In this study, we searched for an answer to the question of whether scalp protection is possible in patients undergoing WBRT with two different modern techniques and is there any difference between these techniques. In previous studies, it has been shown that scalp doses with the IMRT technique are considerably lower than conventional WBRT treatments (15,16). In the study conducted by Kao et al., conventional WBRT and IMRT-WBRT were compared. While the mean scalp dose was 26.2 Gy in conventional treatment, it was 16.4 Gy with IMRT (26.2Gy vs. 16.4Gy, p<0.001). PTV 30 was lower in the IMRT arm (38.4 Gy vs. 32 Gy, p<001). Hair protection was provided 50% in 4 of 15 patients, and 25-50% in 6 patients (17). In the study of Witek et al, it was shown that IMRT and WBRT can protect not only the hippocampus but also the scalp. Mean scalp V10 and V20 were found to be 46% and 35%, respectively (18). In our study, scalp Dmean is found 19.7 Gy with HT and 18.01 for VMAT; V10 is 95.5% for HT and 89.95% for VMAT; the V20 value is 48.16% for HT and 39.55% for VMAT.

Volumetric modulated arc therapy (VMAT) is a new radiation technique that can provide highly compatible dose distributions with better target volume range and preservation of normal tissues compared to conventional radiotherapy techniques (19). In a prospective study conducted in Australia, 9 patients who underwent VMAT-WBRT were given scalp protection with hippocampus protection. PTV was prescripted at 30Gy. The scalp was divided into 2 as superior and inferior 4 cm above the most superior pinna. Superior scalp Dmax was found as 20.5Gy, Dmean: 10.4Gy, and for inferior scalp Dmax: 23.1 Gy and Dmean: 12.2 Gy (20).

Helical Tomotherapy is a novel method and arc-based application of IMRT. During the treatment, the gantry rotates 360° at a constant and fixed speed to apply RT. In a study conducted by Hu et al., scalp doses could be reduced up to 52% of the PTV prescribe dose in the application of radiotherapy to the brain region (21). There are many studies comparing Tomotherapy and VMAT techniques for various tumor types. Similar studies have been conducted in whole-brain radiotherapy, as well. In the study of Doğan et al. in which they applied whole-brain radiotherapy for prophylactic cranial radiotherapy (PCI) with hippocampus protection; the minimum, maximum and mean values of PTV brain doses were higher in the VMAT arm(p=0,0001). When HI and CI values were compared, PTV was significantly superior in VMAT CI (p=0.033), however, there was no significant difference in HI values. When the lens doses were analyzed, it was observed that the mean and maximum dose values of the right and left lenses were much lower in helical tomotherapy (22).

In the study of Rong et al., HI was also found to be 0.15 and better than other techniques, IMRT and VMAT (23). In our study, on the other hand, PTV max, PTV median, and PTV min were found lower in HT (p<0.001). While there was no difference in terms of HI; CI was superior in VMAT. MU and Beam on time values were found to be much lower with the VMAT technique. In our study, the measurements made on TPS were measured and verified with Gafcromic films at 20 different points on the phantom. The measurement results were found to be similar.

The limitations of the present study are that Gafrocrmic film measurements are made on the phantom and not on the real patient.

Conclusion

By using both VMAT and Helical Tomotherapy techniques, it is possible to dry the lens and scalp without low PTV doses in whole-brain irradiation.

Ethical Statement

The present study was approved by the Inonu University Clinical Research Ethics Committee (Approval number: 2019/218).

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ORIGINAL ARTICLE OPEN ACCESS

Evaluation of Biomarkers in Patients with Sepsis Diagnosis in Pediatric Intensive Care Unit

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Introduction: Sepsis is one of the leading causes of mortality and morbidity in intensive care units. In this study, we aimed to investigate the etiological cause, focus of infection, culture sample results, and inflammatory markers among patients treated for sepsis at pediatric intensive care units (PICUs).
Materials and Methods: We retrospectively reviewed the medical records of 70 patients aged 1 month to 18 years who were treated for sepsis at PICU between January 2014 and May 2019.
Results: The median age of the patients was 37 months. The most common underlying etiology was respiratory failure (70%). The most common site of infection causing sepsis was the respiratory system (n:40, 57%). The most commonly isolated agents were Proteus mirabilis and Acinetobacter baumannii. Whereas C reactive protein (CRP) was normal at the time of the diagnosis of sepsis in 28.5% (n=20) of the patients, procalcitonin (PCT) was elevated in all of them. A comparison of the laboratory parameters in the first 24 hours after the diagnosis of sepsis and at the end of the treatment revealed a significant difference between White blood cell (WBC) count, neutrophil-lymphocyte ratio (NLR), the levels of C reactive protein (CRP) and Procalcitonin ($p<0.05$). In addition, positive correlations were detected between NLR, CRP and PCT ($p=0.036$, $p=0.012$/ $r=0.251$, r:0.299, respectively).
Conclusion: We believe that PCT, CRP, and NLR can be used as biomarkers for monitoring patients with sepsis.
Keywords: CRP, NLR, PICU, procalcitonin, sepsis

Introduction

Sepsis is one of the leading causes of mortality and morbidity in intensive care unit. Multiorgan failure and death may occur unless the infection causing sepsis is effectively treated. Thus, it is of vital importance to have a thorough knowledge of sepsis and start appropriate treatment as quickly as possible (1, 2).

Sepsis is a clinical syndrome characterized by systemic inflammatory response syndrome, which is associated with infection-mediated immune system abnormalities, microcirculatory disorders, and end-organ failure. It has complex pathophysiology mediated by cytokine release, and it results from the effects of the circulating products of bacteria originating from constant

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bacteremia. There are some clues that the pathophysiology of pediatric sepsis is multi factorial, thus no single pathogen, mediator, or pathway is the sole culprit (3-6).

The introduction of advanced pediatric intensive care procedures, guidelines for early diagnosis and treatment, and support programs have all contributed to lower sepsis-related pediatric mortality, particularly in developed countries (7). Many laboratory parameters are used to diagnose inflammatory diseases and to monitor immune system response. Several specific laboratory tests determine the type and severity of an ongoing infection; however, only a few parameters exist for monitoring critically ill patients and assessing their treatment response (8-13).

In the present study, we aimed to investigate the effects of the etiological cause, focus of infection, culture sample results, and inflammatory markers on monitoring patients treated for sepsis at Dicle University Faculty of Medicine, Department of Pediatrics, Pediatric Intensive Care Units between 2014 and 2019.

Materials and Methods

This study enrolled 70 patients of any sex and age between 1 month and 18 years who were treated for sepsis at Dicle University Faculty of Medicine, Pediatric Intensive Care Unit (PICU) between January 2014 and May 2019. The patients' clinical and laboratory data were retrospectively obtained from the medical records and available digital media. We recorded full blood count parameters, C-reactive protein (CRP), procalcitonin, culture samples, neutrophil-lymphocyte ratio (NLR), thrombocyte lymphocyte ratio (PLR), and other laboratory parameters, which were studied in the first 24 hours after the diagnosis of sepsis and immediately after the treatment for sepsis was completed at the Pediatric Intensive Care Unit. NLR and PLR were calculated from the full blood count data.

Full blood count samples were collected in tubes containing 1-2 ml EDTA and studied with CELL DYN 3700 Hematology Analyzer device. Procalcitonin was studied with a RADIOMETER AQT90 device using the immune assay method; a level above 0.1 mg/dl according to the reference value was considered to be elevated. CRP was studied with nephelometer (SIEMENS) NnII model device using the nephelometry technique, with the normal reference range being 0-0.5 mg/dl and levels above >0.5 mg/dl being considered high. Blood cultures were obtained at a volume of at least 3 ml under sterile conditions. The samples were studied with the BACTEC FX fully automatic blood culture system at the microbiology laboratory. Aerobic blood culture samples were monitored in the device for 5 days. A sample was prepared from a culture sample signaling proliferation, and a preliminary microbiological identification was performed with Gram staining of that sample. The colonies that grew in the growth medium were identified by genus and/or species with Maldi Biotyper 3.1 (Bruker, Germany) system using the MALFU TOF mass spectrometry technique. Among hematological parameters, leucocyte count was evaluated by age. The values below the lower limit of normal by age were defined as leukopenia, and those above the lower limit of normal by age were defined as leukocytosis. Body temperature above 38.5 °C was defined as hyperthermia and below 36.0 °C as hypothermia.

Patients with the following features were excluded: contaminated blood culture receiving chemotherapy/glucocorticoids, HIV infection, underlying immune deficiency syndrome.

Sepsis was diagnosed based on the criteria established by the International Pediatric Sepsis Consensus Conference dated 2005 (7). These criteria are as follows;

SIRS: The presence of at least two of the following four criteria, one of which must be abnormal temperature or leukocyte count:

1. A core temperature of 38.5°C or 36°C.
2. Tachycardia, defined as a mean heart rate 2 SD above normal for age in the absence of external stimulus, chronic drugs, or painful stimuli; or otherwise unexplained persistent elevation over a 0.5- to a 4-hr time
3. Mean respiratory rate 2 SD above normal for age or mechanical ventilation for an acute process not related to underlying neuromuscular disease or the receipt of general anesthesia.
4. Leukocyte count elevated or depressed for age (not secondary to chemotherapy-induced leukopenia) or 10% immature neutrophils

Sepsis: SIRS in the presence of or as a result of suspected or proven infection.

Ethical statement

The study was conducted in compliance with the criteria of the Helsinki Declaration. All the participants (family members) accepted the informed/written consent. It was approved by the Dicle University Faculty of Medicine Institutional Ethics Committee (Date: 23.05.2019 No: 200).

Statistical Method

Statistical analysis of the data was done using the IBM Statistics Version 22 package program with 95% confidence. Chi-Square test, Fisher's exact Chi-Square test, and Chi-Square Trend were used for comparison of categorical data between groups, and Mann-Whitney U statistical analyzes were used for comparison of continuous variables between groups since the data were not suitable for normal distribution. The power of NLR values to predict bacteremia with ROC analysis; The survival times according to bacteremia, medical/surgical status, and NLR groups were evaluated by Kaplan Meier survival analysis, $p<0.05$ was considered significant.

Results

Out of 70 patients enrolled in the study, 36 (51.4%) were male, and 34 (48.6%) were female ($p>0.05$). The median age of the patients was 37 (min 3-max 188) months. The age range of the study population was 0-5 years for 49 patients (70%); 5-10 years for 18 (25.7%); and 10-18 years for 3 (4.3%). The most common indication for PICU admission was respiratory failure(70%). The indications for PICU admission were shown in Table 1, with some patients having more than one indication.

Table 1. Indications for intensive care unit admission

Parameters	n	%
Respiratory Failure	49	70
Spinal Muscular Atrophy	9	12.6
Convulsion	6	8.5
Cerebral Palsy	6	8.4
Hypoxic-İschemic Encephalopathy	5	7.1
Laringomalacia / Tracheomalacia / Bronchomalacia	4	5.6
Down Syndrome	4	5.6
Neurometabolic Disease	3	4.2
Neuropathy & Myopathy	3	4.2
Trauma	2	2.8
Intracranial Bleeding	2	2.8
Bronchiolitis Obliterans	2	2.8
Shunt Infections	2	2.8

* More than one etiology was found in the same patient

The most common foci of infection-causing sepsis in decreasing frequency were respiratory infections (n: 40, 57%), urinary system infections (n:14, 20%), circulatory system infections (n:9, 12.8%), and gastrointestinal system infections (n:9, 12.4%) (Table 2).

Table 2. Focus of infection-causing sepsis

Focus of Infection	N	%
Respiratory System	40	57
Urinary System	14	20
Gastrointestinal System	9	12.8
The Circulatory System	9	12.8
Others	2	2.8

* More than one focus of infection existed in 4 (4.2%) patients

A microorganism proliferated in the culture samples of 52 (74.2%) of 70 patients while no proliferation occurred in the samples of 18 (25.8%) patients. Culture positivity was detected in the tracheal aspirate fluid in 44.2% of the patients, urine samples in 27%, blood samples in 17.3%, stool samples in 17.3%, and other samples in 2%. An analysis of microorganism proliferation in culture samples showed that Proteus mirabilis proliferated in 9 (17.3%) patients, Acinetobacter baumannii in 8 (15.3%), Carbapenem-resistant enterococci (CRE) in 8 (15.3%), Pseudomonas aeruginosa in 7 (13.4%), Methicillin-resistant staphylococcus aureus (MRSA) in 6 (11.5%), Klebsiella pneumoniae in 4 (7.7%), E.coli in 4 (7.7.%), Candida albicans in 4 (7.7%), Vancomycin-resistant enterococci (VRE) in 2 (3.8%), and other microorganisms in 2 (3.8%) (Figure 1).

Two patients were found to have multiple simultaneous proliferation in different culture samples. An analysis of the acute phase reactants in septic patients in the first 24 hours after the diagnosis of sepsis showed that 20 (28.5%) patients had CRP levels within the normal reference range and 50 (71.5%) patients had CRP levels above the normal reference range. Procalcitonin level was high in all patients (100%).

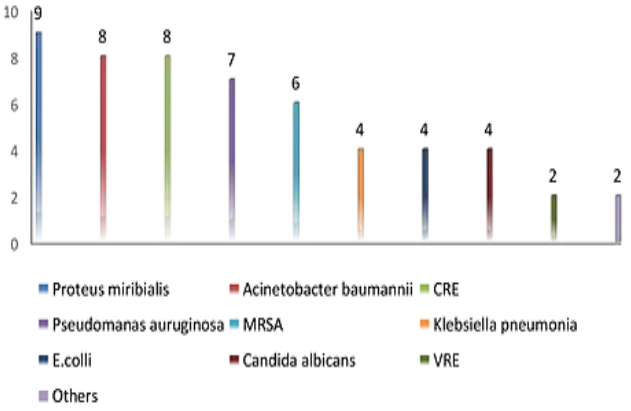

Figure 1. Distribution of the microorganisms detected in the culture samples

A comparison of the laboratory parameters measured in the first 24 hours after the diagnosis of sepsis and at the end of the treatment showed significant differences between WBC, NLR, CRP, and Procalcitonin levels ($p<0.05$, Table 3, Figure 2). There was no significant difference regarding the other parameters. A correlation analysis between NLR and the other parameters showed a positive correlation between NLR, CRP and PCT ($p=0.036$, $p=0.012$/ $r=0.251$, $r:0.299$, respectively).

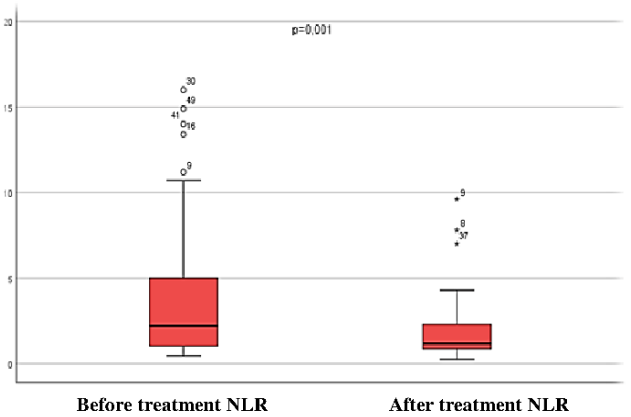

Figure 2. Comparison of pre and post-treatment NLR levels using Box plot analysis

Table 3. Comparison of the laboratory results at the time of the diagnosis of sepsis, before and after the treatment

Parameters	Before treatment *(Mean±Sd)*	After treatment *(Mean±Sd)*	P value
WBC *(10^3/uL)*	14,51±5,52	12,03±4,43	<0.001
Neutrophil count *(10^3/uL)*	8,9±5,06	33,02±227,2	0,377
Lymphocyte count *(10^3/uL)*	4,04±2,78	4,57±2,64	0,066
Hemoglobin *(g/dl)*	10,97±1,6	11,17±1,49	0,31
MPV *(fl)*	8,28±1,9	8,04±1,61	0,247
NLR	3,71±3,76	1,79±1,69	<0,001
PLR	118,3±85,4	106,49±75,33	0,335
Prokalcitonin *(ng/ mL)*	2,58±9,68	0,17±0,25	0,04
CRP *(mg/dL)*	4,55±6,51	2,1±10,11	0,029

WBC: White blood cell, **CRP**: C-reactive protein, **MPV**: Mean platelet volume, **NLR:** Neutrophil-Lymphocyte Ratio, **PLR:** Platelet-Lymphocyte Ratio. *****Pre-treatment laboratory parameters were measured in the first 24 hours after the diagnosis of sepsis was considered.

Discussion

Pediatric intensive care units are services that serve diverse groups of patients requiring a multidisciplinary approach. Mortality risk can be high in patients managed in intensive care units. Sepsis is one of the leading risk factors for increased mortality (14,15).

Pediatric intensive care units admit patients aged 1 month to 18 years for treatment. According to our literature review, Xiao et al.(16) reported that 80.8% of patients admitted to PICU for treatment of sepsis were children aged 1 month to 5 years; Navin et al. reported that the same age group comprised 68% of patients. Our study findings showed similarity with literature data, with 70% of our patients being 1 month to 5 years old. Sepsis can be observed in any age group, and care should be exercised to detect sepsis during intensive care unit follow-up of children under the age of 5 and to remember that early diagnosis may be life-saving in those patients. A review of the previous studies investigating the cause of intensive care unit admission showed that respiratory infections were the most common cause, affecting 76% of patients in the study of Xiao et al. (16), 34.6% of patients in the study of Workman et al. (17), 21% of patients in the study of Rey et al. (10), and 36.8% of patients in the study of Aygün (13). Similarly, our study found that respiratory infections were the most common etiological cause, being responsible for 70% of PICU admissions. A separate analysis of the former studies regarding the focus of the infection-causing sepsis showed that respiratory and urinary infections were the most common foci (10,16,17). In our study, the most common foci of infection-causing sepsis, in decreasing order of frequency, were respiratory and urinary infections. It is of vital importance to determine the foci causing sepsis and to start empirical antibiotic therapy as fast as possible at an early stage. It should be kept in mind that the respiratory and urinary systems are typically responsible for sepsis, and empirical therapy against these foci should be initiated until culture results become available.

Variable results have been reported in the literature regarding the detection of micro organisms in culture samples of children with sepsis. Katu et al. (18) found culture positivity in 66.7% of patients, Demirdağ et al. (19) in 77%,

and Workman et al. (17) in 67%. We detected culture positivity in 74.2% of our patients. The most commonly isolated bacteria in our patients, in decreasing order of frequency, were Proteus mirabilis, Acinetobacter baumanii, Pseudomonas aeruginosa, Carbapenem-resistant enterococci and Methicillin-resistant staphylococcus aureus (MRSA). Using blood samples to diagnose sepsis is of great importance for preventing mortality in critically ill patients with a life-threatening infection. However, it is not always a simple task to obtain an adequate blood volume for cultures in children and infants. Furthermore, possible contamination of blood culture samples especially by skin saprophytes despite efforts to reduce contamination continues to be an important clinical problem. Despite several challenges posed by blood cultures, they continue to guide clinicians for the treatment of sepsis for the sake of efficacy and, if necessary, modification of the treatment.

Sepsis is a pathophysiological process rather than a specific syndrome and is too complex to be defined by a single parameter. A perfect marker is yet to be found to diagnose sepsis. Since the clinical signs and symptoms of sepsis are not specific and may frequently vary, there is a potential for some biomarkers to be used for determining the severity of sepsis, clinical monitoring, and assessing treatment response. There are a plethora of studies in this field (8-12, 19).

Among such biomarkers, PCT and CRP are the most widely used ones. Procalcitonin is a protein containing 116 amino acids, which is a precursor protein of the calcitonin hormone released by the medullary neuroendocrine C cells of the thyroid gland, typically in response to a high calcium level in blood. Procalcitonin level is thought to increase 2 hours after the onset of an infection, to become detectable by 4 hours, to peak at 6 hours, and to maintain its level for 8-24 hours. Thanks to such favorable kinetics, PCT is an ideal candidate for use as a biomarker (20). C-reactive protein (CRP) is a pentameric acute-phase protein of hepatic origin circulating in the blood. Serum CRP level rises dramatically in the case of inflammation, tissue injury, and cytokine-mediated reaction against an infection. Quantification of acute-phase reaction may provide clinical information about the presence of tissue injury or inflammation as well as the treatment response (21). Serum CRP level is elevated by all inflammatory processes including infections and sepsis. Despite being a classical and sensitive biomarker of inflammation, CRP cannot distinguish bacterial and other types of inflammatory infections (20). Using PCT for the diagnosis of sepsis may offer an advantage since it may have better specificity for bacterial infections, unlike many other biomarkers that may be elevated in conditions other than bacterial infection (22).

Bustos et al. (8) reported that, unlike CRP and lactate, PCT was a good predictor of mortality and septic shock, being capable of categorizing patients by the severity of sepsis at the time of pediatric intensive care unit admission. In a study in adults, Luzzani et al. (22) found that PCT was a better marker of sepsis and showed a better correlation to the severity of infection and organ dysfunction than CRP. Rey et al. (10) found that PCT was a better diagnostic marker than CRP among critically ill children with sepsis. Nargis et al. (23). studied the specificities of PCT and CRP in patients treated for sepsis and reported a specificity of 72.2% for PCT and 33.3% for CRP.

Our comparison of the laboratory parameters measured in the first 24 hours after the diagnosis of sepsis and at the end of the treatment detected significant differences in WBC, CRP, PCT, and NLR levels. While 20 (28.5%) of our patients had a normal CRP level and 50 (71.5%) of them had an elevated CRP level in the first 24 hours of sepsis, all of our patients had an elevated PCT level. These results support the literature data indicating that PCT rapidly rises and reaches a detectable level in blood at an earlier stage than CRP; they also suggest that PCT may be a better parameter than CRP for assessing bacterial sepsis. In addition, we believe that CRP and PCT should be effectively used to monitor patients with sepsis and to assess their treatment response.

Neutrophils are the first-line cellular defense cells of innate immunity against infectious agents. Lymphocytes play an important role in adaptive immunity. The immune response against various triggers causes an increase in neutrophil count and a decrease in lymphocyte count. Neutrophil lymphocyte ratio (NLR) is a readily available, rapidly obtained, and inexpensive parameter. Several researchers have studied NLR with CRP and PCT as an important marker for an early diagnosis of bacteremia and assessment of the outcomes of sepsis (24, 25, 26). Several studies have shown that, in comparison to standard diagnostic biomarkers, NLR made meaningful contribution to the early prediction of sepsis (25-27). Furthermore, several studies in infants and newborns have indicated that NLR was an accurate predictor of sepsis along with CRP (28,29). We detected a significant difference between the NLR levels at the onset of sepsis and at the end of the treatment; we also demonstrated that NLR had a positive correlation with CRP and PCT. We believe that the neutrophil-lymphocyte ratio can be used for disease monitoring similarly to CRP and PCT.

Conclusion

Respiratory and urinary infections are the most common foci of infection among patients treated for sepsis at pediatric intensive care units. The proliferation of microorganisms in culture samples is time-consuming and sometimes even not possible; we, therefore, believe that empirical antibiotic treatment should be commenced as soon as possible after determining the most probable focus and microorganism.

We believe that PCT, CRP, and NLR, the effectiveness of which has been recently shown, may be used as biomarkers to support the diagnosis of sepsis and to assess treatment efficacy. In the light of our results, we may suggest that PCT is a more effective marker than CRP in the first 24 hours among patients suspected to have sepsis. In conclusion, markers such as PCT, CRP, and NLR should not be used alone as the definitive tests but should be used in conjunction with physical examination, clinical features, and microbiological culture results to diagnose sepsis and to assess treatment response.

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Conflicts of Interest

The authors declared no conflict of interest for the present article.

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ORIGINAL ARTICLE OPEN ACCESS

Evaluation of Plasma Selenium, Zinc and Malondialdehyde Levels in Newly Diagnosed Preeclamptic Women at A Teaching Hospital

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Introduction: Preeclampsia is a clinical condition unique to humans which forms a major part of hypertensive disorders in pregnancy. Although its exact etiology remains unknown, an imbalance between lipid peroxides and antioxidants is implicated. The study aimed to evaluate plasma selenium, zinc, and malondialdehyde (MDA) levels in newly diagnosed preeclamptic women attending antenatal visits at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH), Awka in Anambra State.
Materials and Methods: A total of 81 female participants were recruited and grouped into three (A, B, and C) comprising 21 newly diagnosed preeclamptic women, 30 healthy pregnant control, and 30 non-pregnant control participants respectively.
Results: The newly diagnosed preeclamptics had significantly lower plasma selenium levels when compared with the healthy pregnant and non-pregnant controls ($p<0.05$). The plasma zinc level in the preeclamptics did not differ significantly from the level in healthy pregnant women ($p>0.05$), it was however significantly lower in the preeclamptics and healthy pregnant women when compared with the non-pregnant women ($p<0.05$). Plasma MDA level was significantly elevated in the preeclamptics compared to the healthy pregnant and non-pregnant control participants ($p<0.05$).
Conclusion: Newly diagnosed preeclamptics have lower plasma selenium and zinc levels with higher plasma MDA levels, and additional sources of these micronutrients may be required.
Keywords: Preeclampsia, pregnancy, selenium, zinc, malondialdehyde

Introduction

Preeclampsia is a human pregnancy specific disorder which forms a major part of hyper tensive disorders (1). It presents with elevation of blood pressure to ≥140/90 mmHg and significant proteinuria of ≥0.3g/day or ≥30mg /mmol of urinary creatinine in random sample after 20 weeks of gestational age (2). It may as well occur as late as 4 - 6 weeks postpartum (3). It is a major cause of long term maternal and perinatal mortality and morbidity worldwide (4, 5). In as much as preeclampsia remains a threat to pregnancy, its exact aetiology and pathophysiology remains unknown.

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Malondialdehyde (MDA), which is a product of lipid peroxidation has been reported to be increased in certain disease conditions including preeclampsia (6, 7) which could lead to oxidative stress. Antioxidant trace elements such as selenium and zinc are essential elements incorporated into antioxidants enzymes like glutathione peroxidase and superoxide dismutase respectively to prevent oxidative stress. Meanwhile, studies have shown conflicting reports on levels of blood trace elements in preeclampsia (8-10). Preeclampsia affects women in developing countries more than women in developed nations (11), and Nigeria is known to be among the six countries in the world with very high maternal mortality rate due to preeclampsia (12). Worst still, preeclampsia and eclampsia are shown to be the leading cause of maternal mortality in Nigeria (13).

This study was therefore aimed at evaluating plasma levels of MDA and some antioxidant trace elements: selenium and zinc in preeclamptics attending antenatal visits at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital in Awka, Anambra State, South East Nigeria.

Materials and Methods

Participants

This case control study evaluated the plasma levels of Selenium, Zinc and MDA in newly diagnosed preeclamptic women who have not commenced antihypertensive medications attending antenatal visits at Chukwuemeka Odumegwu Ojukwu University - Teaching Hospital (COOUTH), Awka in Anambra State from June to December 2019.

A total of 81 female participants were recruited for this study and divided into three groups (A, B and C) which comprised of 21 newly diagnosed preeclamptic women (group A), 30 pregnant control participants (group B) and 30 non-pregnant control participants (group C) .

Participants aged between 20 and 35 years, preeclamptic women with minimum blood pressure of 150/100 mmHg, pregnant and non-pregnant women with normal blood pressures (<140/90 mmHg) were recruited for the study.

Pregnant women with the following conditions were excluded: chronic hypertension, diabetes mellitus, thyroid diseases, liver diseases, kidney diseases and other metabolic disorders, mild hypertension, patients on mineral supplements, herbal medications and anti-hypertensives. Pregnant women with previous history of preeclampsia or eclampsia, pregnant women who did not book before 20 weeks of gestational age. Women who smoked or consumed alcohol at any gestational age of pregnancy or women with BMI>30kg/m^2.

Ethical Statement

The ethical approval for this study was issued by the Ethics Committee COOUTH, Awka, Anambra state, Nigeria. Written informed consents were sought after brief explanation of the purpose of this study, and they freely signed up to participate.

Sample Collection

Five milliliters (5 ml) of random blood samples were collected from ante-cubital vein of each participant under aseptic conditions into lithium-heparin bottle. Each blood sample was centrifuged, at 3000 revolutions per minute for 5 minutes, immediately after collection at the Chemical Pathology Laboratory COOUTH. The plasma were separated into plain tubes and stored at –20^{o}C until required for analysis. All analyses were done within two weeks of sample collection. Pregnant participants were tested for

significant proteinuria using Combi 9 strips dipped in their early morning midstream urine collected in a clean universal container. Pregnancy was ruled out in non-pregnant participants by choronic gonadotrophin strips.

Laboratory Procedures

Selenium and zinc levels were determined by Atomic Absorption Spectrophotometer (AAS) according to the method of American Public Health Association (14) while MDA level was determined according to the method of Gutteridge and Wilkins (15).

Statistical Analysis

Statistical Package for Social Sciences (SSPS) version 23.0 (SPSS Inc, Chicago, IL, USA) was used in analysis of data. The results were expressed in mean±standard deviation. One way analysis of variance (ANOVA) was used for comparison of means among the three groups, post-hoc analysis was used to determine the intergroup variability, and p value<0.05 was considered to be statistically significant.

Results

The result of this study (Tables 1&2) showed that the newly diagnosed preeclamptic women had significantly lower mean plasma level of selenium when compared to the pregnant and non-pregnant control participants (p=0.024 and 0.0001 respectively). The pregnant control also had significantly lower plasma selenium level when compared to the non-pregnant controls (p=0.0001).

There was no significant difference in the mean plasma zinc level in the newly diagnosed preeclamptic women compared to the pregnant controls (p=0.053), however, this was significantly reduced in the newly diagnosed preeclamptics when compared to the non-pregnant control participants (p=0.0001) as well as in the pregnants when compared to the non-pregnant control participants (p=0.003). The mean plasma MDA level was significantly elevated in the newly diagnosed preeclamptics in comparison to the pregnant and non-pregnants (p=0.003 and p=0.0001 respectively), however, there was no significant difference in the level of this parameter in pregnant controls when compared to the non-pregnant control participants (p=0.61).

Discussion

Preeclampsia is a life threatening complication of pregnancy which may threaten the lives of mother and/or fetus. The lower plasma selenium levels observed in preeclamptics as well as in healthy pregnant control participants

Table 1. Plasma Levels of Selenium, Zinc and MDA among study participants

Parameters	Groups				
	A (n=21)	B (n=30)	C (n=30)	F	*p-value*
Selenium *(µg/l)*	44.29±13.07	53.67±11.68	86.63±11.79	91.27	0.0001*
Zinc *(µg/dl)*	74±15.59	88.97±17.74	107.9±28.03	15.55	0.0001*
MDA *(nmol/ml)*	2.83±0.9	1.96±0.63	1.66±0.51	11.05	0.0001*

Table 2. Post-hoc test showing mean differences and p-values of study groups

Parameters	Mean Difference			p-value		
	A vs B	A vs C	B vs C	A vs B	A vs C	B vs C
Selenium *(µg/l)*	-9.38	-42.35	-32.97	0.024*	0.0001*	0.0001*
Zinc *(µg/dl)*	-14.97	-33.9	-18.93	0.053	0.0001*	0.003*
MDA *(nmol/ml)*	0.87	1.16	0.29	0.003*	0.0001*	0.61

may be associated with placental transfer of selenium from the mother to the developing fetus (16) as well as hemodilution resulting from increased plasma volume in pregnancy. This is in line with previous studies which also reported lower plasma selenium levels in preeclamptics (17, 18) and healthy pregnant women (19). Karita et al. (20) however reported otherwise. Venderlelie and Perkin (18) had earlier reported that an average selenium level of ≥95ug/l is required for optimal activity of the antioxidant enzyme, glutathione peroxidase (GPx), and from this study, the average plasma selenium level of preeclamptics and healthy pregnant women are suboptimal for glutathione peroxidase function. This may result to increased production of reactive oxygen species (ROS) and oxidative stress.

In this study, plasma level of zinc was also significantly lower in preeclamptics compared to the non-pregnant control participants as well as in pregnant participants compared to non-pregnant participants, and this may as well be attributed to placental transfer to the growing fetus (21, 22). This is supported by studies which reported higher plasma zinc levels in fetuses compared to their mothers (23). This decrease may also be as a result of increase in plasma volume which in turn reduces plasma zinc concentration (24). Additionally, the low plasma zinc levels may be partly due to increase in urinary excretion of zinc in preeclamptics (25), and serum cortisol which is known to increase during normal pregnancy, with a much higher level in preeclampsia may as well stimulate increased zinc excretion (26). Similarly, supplemental iron of ≥65mg per day of elemental iron may decrease intestinal zinc absorption (27) or limit its bioavailability because both zinc and iron have same plasma carrier protein, transferrin. Hambidge et al. (28) showed that iron therapy in doses typically prescribed by obstetricians has an acute measurable effect on maternal zinc status.

Our findings agree with the respectively reported lower levels of zinc in preeclamptics compared with non-pregnant women in Bangladesh, Saudi Arabia and India (29, 30, 10). Our finding however differs from those of Golmohammad et al. (31) and Ugwuja (32) who did not observe any significant difference in the plasma zinc levels between the preeclamptics and controls. Zinc is an essential component of the antioxidant enzyme, superoxide dismutase (SOD) involved in the removal of free radicals or ROS (33), and as such, reduction in the level of this essential element may cause insufficiency of SOD (34) thereby exposing pregnant women to oxidative stress, which may lead to the development of preeclampsia.

The elevated plasma level of MDA in preeclamptics may be due to increased generation of reactive oxygen species (ROS) which could lead to lipid peroxidation. This is consistent with reports beyond Nigeria (35)(7) and within Nigeria (36). Guptha et al. (37) however, did not demonstrate any significant difference between preeclamptics and non-pregnant controls.

Conclusion

This study suggests that preeclamptic women have lower plasma concentrations of zinc and selenium and higher MDA concentration compared to healthy pregnant and non-pregnant women. Therefore pregnant women in this environment (Nigerian Africans), especially those at high risk of developing preeclampsia, should take zinc and selenium rich diets.

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ORIGINAL ARTICLE OPEN ACCESS

The Epidemiology of Hyperprolactinemia in A Single Tertiary Care Center: The Importance of Drug History and Role of An Endocrinologist

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Introduction: The underlying causes of hyperprolactinemia differ between studies. The study aimed to determine the causes, initial signs, and treatment methods of hyperprolactinemia.
Materials and Methods: Prolactin (PRL) measurement was requested from 16241 patients between January 2016 and December 2019. A total of 176 patients whose serum prolactin levels above 29.9 ng/mL in two consecutive measurements were included in this study. Electronic Health Records (EHR) of these patients were reviewed.
Results: Forty-Seven (26.8%) of 176 patients had a prolactinoma. Among the prolactinoma group, 63.8% of the patients had microadenoma. Polycystic Ovary Syndrome (PCOS)(29.5%), drugs (20.9%), and pituitary disorders other than prolactinoma (13.2%) were the most common causes of hyperprolactinemia in the non-prolactinoma group. Galactorrhea (38.3%) was the most common initial sign. Cabergoline's starting dose in the Endocrinology clinic was 1 mg/week, and 87.2% of the cases started with a 1 mg/week dose. All of the other cases (12.8%) who were diagnosed by other departments received inappropriate doses of cabergoline.
Conclusions: Drug-induced hyperprolactinemia may be much more common than previously thought. Referring these patients to the Endocrinology clinic will be much more beneficial to determine both the correct dosage of cabergoline and the cause of hyperprolactinemia.

Keywords: Hyperprolactinemia, prolactinoma, cabergoline

Introduction

Hyperprolactinemia is defined as an elevation of serum prolactin levels (1). The definite diagnosis of hyperprolactinemia is based on two consecutive measurements of serum prolactin. The prevalence of hyperprolactinemia in the adult age group was around 0.4% in the previous studies (2). There are numerous etiologies of hyperprolactinemia (3, 4). Most of the studies point out prolactinoma as the most common cause of hyperprolactinemia. However, recent studies indicate that drug-induced hyperprolactinemia is probably the actual leading cause of hyperprolactinemia (5). If there is a suspicion of drug-induced hyperprolactinemia, guidelines recommend

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stopping the suspicious medication and remeasure prolactin levels after 72 hours (6). Pituitary Magnetic Resonance Imaging (MRI) is the preferred method of imaging in these patients to exclude a potential hypophyseal or hypothalamic lesion. Prolactinoma is the most common pituitary adenoma and can be treated successfully by medical treatment in most cases. This study aimed to review both clinical features and etiology of hyperprolactinemia and treatment approaches in our center.

Materials and Methods

A total of 176 patients who were admitted to our center between January 2016 and December 2019 (4-year period) older than 18 years, and, whose serum prolactin levels more than 29.9 ng/ml in two consecutive measurements were included in the study (The upper limit of the average prolactin level, according to our biochemistry laboratory references was 29.9 ng/ml). Electronic health records (EHR) of the patients were reviewed. In this period, prolactin measurement was ordered from 16241 patients. Six hundred forty-one of these patients had at least one value of serum prolactin level more than 29.9 ng/ml. Among these cases, 321 patients had hyperprolactinemia in at least two consecutive measurements. However, there was not enough data in the EHR system other than 176 patients.

Serum prolactin, clinical features, presenting complaints, cause of hyperprolactinemia, initial admissions, and treatment modalities of these patients were reviewed. Prolactinoma was the cause of hyperprolactinemia in 47 patients, and all of them started cabergoline (CAB) treatment. Treatment responses in terms of prolactin levels and maximum tumor diameter on MRI were evaluated additionally in these 47 patients. Tumor diameter measurements were done through MRI. The maximum tumor diameter was identified in the initial imaging study, and the evolution of the same width was assessed in consequent imaging tests. Maximal diameter change of adenoma after treatment was calculated and recorded as a percentage relative to initial maximal diameter.

Ethical Statement

This study was conducted in compliance with the principles of the Declaration of Helsinki. It was approved by the local Ethics Committee (Ethical committee decision date: 12.06.2016 no:2016/164).

Statistical Method

The compliance controls of the data in each group were checked with the Shapiro Wilk test. For the data that conform to the normal distribution, the mean and percentile values were given as the descriptive statistics and for the data that did not meet the median (minimum-maximum) were given. Number and percentage values were given for categorical data. In the test of whether there is a difference between the two groups' averages, the student t-test was used for the parameters that fit the normal distribution, and the Mann Whitney U test was used for the parameters that did not fit the normal distribution. While the chi-square test was used to analyze the relationship of categorical data, the correlation coefficient was calculated for continuous data. Statistical significance was taken as $p<0.05$.

Results

A total of 176 patients were included in our study 47 (26.8%) of the patients had a prolactinoma and 63.8% of these patients had microadenoma. Among all cases, 152 (86%) of them were female. While the most common

symptoms in female patients with hyper prolactinemia were menstrual irregularity (37.5%), galactorrhea (14.5%) and infertility (11.2%); loss of sexual desire (41.7%), impotence (8.3%), and headache (8.3%) were the most common symptoms in male patients. In the non-prolactinoma, 46.5% of the patients applied to the Obstetrics & Gynecology Clinic.

Polycystic Ovarian Syndrome (PCOS) (29.5%), drugs (20.9), and pituitary disorders other than prolactinoma (13.2), respectively, were the most common causes of hyperprolactinemia in the non-prolactinoma group. In 15 (11.6%) of the patients, it was observed that detailed investigations were not made; they were evaluated as idiopathic hyperprolactinemia (Table-1). None of these 15 patients were enrolled in the Endocrinology department.

Table-1. Causes of hyperprolactinemia in the group of non-prolactinoma (n:129)

Variables	N	%
Pituitary disorder	17	13.2
Hypothalamic lesion	1	0.8
Head trauma/surgery	2	1.6
Pregnancy	5	3.9
Hypothyroidism	12	9.3
Chronic Kidney Disease	9	7
Chronic liver disease	3	2.3
PCOS	38	29.5
Drug	27	20.9
Idiopathic	15	11.6
Total	129	100

Abbreviations. PCOS: Polycystic Ovary Syndrome

Twenty-seven (20.9%) patients had drug-induced hyperprolactinemia. Escitalopram and Risperidone were the most common causative drugs (29.6% to 25.9%, respectively) in the drug-induced hyperprolactinemia (Table-2).

Table-2. Causative drugs in patients with drug-induced hyperprolactinemia

Variables	N	%
Escitalopram	8	29.6
Risperidone	7	25.9
Olanzapine	2	7.4
Sertraline	2	7.4
Venlafaxine	2	7.4
Amisulpiride	1	3.7
Amitriptyline	1	3.7
Lamotrigine	1	3.7
Methylphenidate	1	3.7
Paliperidone	1	3.7
Valproic Acid	1	3.7
Total	27	100

When the most common initial signs and symptoms were evaluated in patients with prolactinoma, galactorrhea (38.3%) was the most common sign. When the most common initial signs and symptoms were assessed according to gender: while galactorrhea (47.4%) was the most common one in female patients, loss of sexual desire (55.5%) was the most common in male patients.

Table-3. Starting and maintenance CAB doses in patients with prolactinoma

Starting CAB dose *(mg/week)*	N	%	Maintenance CAB dose *(mg/week)*	N	%
0.25	0	0	0.25	16	34
0.5	1	2.1	0.5	9	19.1
1	41	87.2	1	4	8.5
1.5	4	8.5	1.5	9	19.1
2	0	0	2.	2	4.3
2.5	0	0	2.5	1	2.1
3	1	2.1	3	5	10.6
4	0	0	4	1	2.1

Abbreviations. CAB: Cabergoline

All of the patients with prolactinoma were started on CAB treatment. The CAB starting dose was 1 mg/week in 41 (87.2%) of the patients. It was noticed that patients with starting doses other than 1 mg/week were diagnosed and started on treatment in clinics other than Endocrinology. The maintenance CAB dose was 0.25 mg/week in 34% of the patients (Table-3).

At the admission, mean PRL levels were 55.55±36.15 ng/mL (29.98-208) in patients with hyperprolactinemia without prolactinoma and 411.72±940.46 ng/mL(100-4700) in patients with prolactinoma. PRL returned to normal in 25 (83.3%) of the patients with microadenoma after CAB treatment. PRL levels of the patients with microadenoma were 148.6±50.8 ng/mL before treatment and 12.8±16.79 ng/mL on CAB treatment (p<0.001). While the mean basal maximal diameter of hypophyseal adenoma was 5.7±2.26 mm before treatment, it was 3.8±3.15 mm after CAB treatment. Percent diameter reduction after treatment was found to be 39.88±44.44% in the patients. PRL levels were normalized in 10 (58.8%) of the patients with macroadenoma after starting CAB. Mean PRL levels were 876±358 ng/mL and 101±78.3 ng/mL before and after treatment, respectively (p<0.001). The mean basal maximal diameters of adenoma were 19±11.7 mm before treatment and 13±10.3 mm after the treatment. Percent diameter reduction after treatment was found to be 26±6.7% in these patients. Reduction in maximal diameter and percent diameter reduction was not statistically significant.

After treatment, basal PRL levels and maximal diameter were significantly different between microadenoma and macroadenoma groups (p=0.009 and p<0.001, respectively). There was a statistically relevant relationship between basal maximal diameter and PRL normalization (p=0.032). PRL normalization was not related to the percent reduction diameter and gender. Table-4 reveals some characteristics of CAB treatment responses, differences between microadenoma and macroadenoma groups.

Table-4. Characteristics and implications of treatment responses in the patients with micro and macroadenoma

Variables	Micro adenoma	Macro adenoma	P value
Number of cases (n)	30	17	
PRL normalization (n, %)	25 (83.3%)	10 (58.8%)	0.068
Basal PRL (ng/ml)	148.6±50.8	876±358	0.009
PRL after treatment (ng/ml)	12.8±16.79	101±78.3	0.135
Basal maximal diameter (mm)	5.7±2.26	19±11.7	<0.001
Maximal diameter after treatment (mm)	3.8±3.15	13±10.3	<0.001
Reduction in diameter (%)	39.88±44.4	26±6.7	0.283
Starting CAB dose (mg/week)	1.07±0.21	1.11±0.17	0.344
Maintenance CAB dose (mg/week)	0.84±0.64	0.68±0.55	0.001

Abbreviations: CAB: Cabergoline, PRL: Prolactin

Discussion

We tried to review the etiological causes of patients with hyperprolactinemia in our center. With this study, we found that there are many drug-related hyperprolactinemia cases in our center. In addition, we think that this study is important because we found that both the elucidation of the etiological cause and the treatment according to the guidelines are applied much better in the Endocrinology clinic in cases of drug-induced hyperprolactinemia.

The cause of the hyperprolactinemia was prolactinoma in 26.8% of the patients. This rate

was similar to the other previous studies (6, 7). Although microadenoma incidence among patients with hyperprolactinemia was about 90% in other studies, the microadenoma ratio was 63.8% in our study (8).

The three most common symptoms in female patients were menstrual irregularity (37.5%), galactorrhea (14.5%), and infertility (11.2%) in our study. The frequency of symptoms differs significantly from study to study. The most common symptoms were infertility (48%), headache (39%), and oligomenorrhea (29%) in a study, which included a total of 104 female patients whose ages were between 30 and 44 years(9). Being conducted in an infertility clinic may explain this high frequency of infertility in their study. Decreased sexual desire (41.7%), infertility (8.3%), and headache (8.3%) were the three most common symptoms among male patients in this study. Symptom distribution in male patients was parallel to other studies in the literature (5, 10, 11).

We found PCOS as the most common cause of hyperprolactinemia in patients without prolactinoma (29.5% of all patients, 33.3% of the female patients). There are diverse data about the association between PCOS and hyperprolactinemia in the medical literature. A study indicated that there was mild hyper prolactinemia in 30% of the patients with PCOS (12). Other than this study, multiple studies are revealing similar incidences of hyperprolactinemia in PCOS (13, 14). However, these studies were mostly conducted before the definition of Rotterdam criteria. A recent review presented that the mean prevalence of hyperprolactinemia in newer studies designed per the new criteria in PCOS patients was 11.8% (15). Thus, the authors of this review believe that the link between PCOS and hyperprolactinemia is more of a myth than a well-established medical reality, and there is a need for a more comprehensive standard etiological investigation of hyperprolactinemia.

Pregnancy-related hyperprolactinemia was present in 3.9% of our patients. As one of the physiological causes of hyperprolactinemia is pregnancy, many guidelines recommend that all female patients with hyperprolactinemia be screened with a pregnancy test before further evaluation (6). 82.2% of our patients firstly applied to the Endocrinology and Gynecology outpatient clinic. However, due to the Psychiatry Department's registration privacy, we were able to include only 3 of these patients who were consulted directly to the Endocrinology clinic. When we screened a total of 649 patients with hyperprolactinemia at the beginning of the study, we realized that 102 (15.7%) of the patients were psychiatry patients. None of these 102 patients could be included in our study. 15.3% of the whole patients and 20.9% of the patients without prolactinoma had drug-induced hyperprolactinemia. Especially considering that psychiatric drugs in the anti psychotic group may cause hyperprolactinemia frequently, it can be estimated how many patients applied to Psychiatry Outpatient Clinic with hyperprolactinemia. Therefore, it can be said that the ratio of drug-induced hyper prolactinemia may be much higher in our patients. This is one of the main limitations of this study.

Since drugs can cause hyperprolactinemia, drug-related hyperprolactinemia should be considered in the differential diagnosis of patients with hyperprolactinemia. Therefore, the drugs used by patients should be recorded. One of the most relevant results in our study was the high incidence of hyperprolactinemia

in patients using Escitalopram. Although the hyperprolactinemia rate associated with Escitalopram was not that elevated in other studies, Escitalopram was the number one drug in our patients with drug-related hyper prolactinemia. On the other hand, the causative drug was sertraline in two patients and venlafaxine in the other two patients in our study. It was also an important finding in this study because sertraline and venlafaxine-induced hyperprolactinemia were relatively rare in the literature (16, 17). A multicenter study with 1234 patients revealed that drugs were the cause in 14.5% of the patients (18). PROLEARS study suggested that drug-induced hyperprolactinemia would be the leading cause of hyperprolactinemia (5). In our study, drug-induced cases were 20.9% of the non-prolactinoma group. In the light of all this information, it can be said that drug history is very important in these patients.

The recommended starting dose of CAB is 1-2 mg/week (6). After diagnosing prolactinoma in our center, regardless of adenoma size, serum PRL level, and symptoms, it was observed that cabergoline treatment was initiated at a dose of 1 mg/week in the Endocrinology Department for each standard patient. Six of 47 patients with a prolactinoma in our study were diagnosed by the Neurosurgery Department and referred to Endocrinology after CAB treatment was initiated. Starting the CAB dose was 1.5 mg/week for four patients, 3 mg/week in one patient, and 0.5 mg/day in one patient. While the literature on CAB dose was reviewed, it was observed that the initial dose was generally 0.5-1 mg/week. However, studies conducted in different centers also attracted attention to patients receiving various doses of CAB up to 11 mg/week (19-21). Although high doses of cabergoline have many side effects, the most important dose-related adverse effect is cardiac valvulopathy (22).

PRL normalization by CAB treatment was in 74.4% of the patients with a prolactinoma in this study. This success rate was parallel with other published studies (19, 23-26). PRL normalization rates in microadenoma and macroadenoma were correspondingly 80% and 58.8%. However, this difference was statistically insignificant (p=0.068).

Conclusion

This study is important as it demonstrates the high frequency of drug-related hyper prolactinemia and reminds once again the importance of the Endocrinology clinic in elucidating the etiology of hyperprolactinemia. Although the number of patients is relatively low, we find this study important in terms of emphasizing the importance of anamnesis and showing that referring patients with hyper prolactinemia to the Endocrinology department will be more beneficial.

Conflict of Interest

None of the authors has no conflict of interest

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ORIGINAL ARTICLE OPEN ACCESS

Is Fasting Necessary for the Assessment of Clinical Biochemical Parameters?

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Introduction: Blood tests are usually requested for patients visiting polyclinics in the morning following fasting. However, as hospitals provide services for 24 hours, blood is collected from patients at every hour of the day, and fasting status cannot be completely ensured. Therefore, our study aimed to compare the fasting/non-fasting biochemical parameters of individuals.
Materials and Methods: Our retrospective study examined 18 different most frequently requested blood tests of patients who visited certain polyclinics at our hospital only once as fasting and non-fasting values based on their hours of being requested.
Results: In our study which analyzed a total of 70,352 individuals, statistically significant differences were observed in the fasting/non-fasting values. However, when the effect size values were measured for the tests, they were not clinically significant (0.006-0.104).
Conclusion: There was no noticeable difference between the fasting and non-fasting parameters. It was concluded that, as long as there is no doubt on routine results, individuals could give blood samples throughout the day with or without fasting.
Keywords: Fasting, non-fasting, biochemistry parameters, effect size

Introduction

The in vitro reflection of nutrition in the clinic is the changes in the biochemical parameters in blood samples as a result of metabolic changes in the body. As a result of digesting food, serum glucose, amino acid, and triacylglycerol levels increase.1As a response to ingestion of food, the pancreas reduces glucagon secretion by increasing insulin secretion and tries to keep the blood glucose level in the reference range. This change in the insulin/glucagon levels triggers the anabolic stage in tissues (especially the liver, muscle, and adipose tissues). The liver forms glycogen and lipids from the substrates that are supplied. While the glycogen that forms is stored in the liver, lipids are transferred to the

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blood via very-low-density lipoproteins (VLDL). Adipose tissue removes free fatty acids from lipoproteins, synthesizes triacylglycerols, and stores them in the form of insoluble droplets. When the metabolism is in the state of non-fasting, the brain and the heart use glucose as their energy source. Only after long-term fasting, they can use only ketone bodies (1, 2).

While transitioning from non-fasting to fasting, the body secretes the glucagon hormone from the α cells of the pancreas. The main purpose of glucagon is to try to keep the blood glucose level around normoglycemic levels in fasting (3). By regulating the metabolism, the insulin/ glucagon hormones regulate lipolysis/ lipogenesis and glycogenolysis or glycogen synthesis in a balanced way. Therefore, all bodily enzymes/hormones function in a way to keep the metabolism in balance despite fasting or non-fasting (4).

While the metabolism is conducting all these processes, a difference in blood biochemistry occurs between the states of fasting and non-fasting. However, this difference is on a negligible level for many tests (5-7). Importance is paid to the states of fasting and non-fasting in Turkey in terms of biochemical tests. For this reason, in our study, by taking the biochemical parameters of samples arriving at our laboratory, it was aimed to determine whether or not there were differences between the fasting and non-fasting blood samples in the statistical and clinical sense, and if any, how this was reflected on the clinic.

Materials and Methods

Ethical Statement & Subjects

The data that were retrospectively used in this study were obtained from the automation system (ENLIL) of the Hatay Mustafa Kemal University (HMKU) Health Application and Research Hospital. Ethics committee approval was obtained (19.YL.024). The groups of the study were formed by using the hospital database information of patients who visited the polyclinic between 01.01.2019-31.10.2019.

Materials

Our study covered a total of 70,352 individuals including 42,696 (60%) women and 27,656 (40%) men. Test results were obtained from outpatients who received treatment from the polyclinics of otorhinolaryngology, orthopedics, ophthalmology, dermatology, neurology, physiotherapy & rehabilitation, general surgery, endocrinology, infectious disease, hematology, internal medicine, and gastroenterology. Inpatient data and the data of the oncology service and polyclinic, pediatrics, emergency services, nephrology polyclinic, and intensive care unit were not included in the analysis.

Methods

The study included 18 different biochemical tests used most frequently by clinics. As we considered that the probability of blood samples taken at early hours of the morning to be fasting blood samples would be higher than the same probability for those taken towards the afternoon and in the afternoon, the patients' blood tests were categorized as fasting for 07:00-10:00 and non-fasting for 10.01-17:00. The blood samples that were transferred to the laboratory under appropriate conditions were kept for 20 min, centrifuged for 10 minutes at 4°C and 4000 rpm, separated into their serums, and analyzed with an ADVIA 1800 Clinical Chemistry System autoanalyzer.

Statistical Analysis

In studies in general, the significance of the difference between groups is statistically analyzed and expressed in terms of units as $p<0.05$. In the literature, it was stated that

the "p" value is affected by the size of the sample that is analyzed, and in studies with large samples, the p-value could turn out to be smaller than 0.05 even if there are very small differences between the means-medians belonging to the groups that are not clinically significant (8).

A measurement parameter showing whether or not the difference between the results of the groups in a study is clinically significant is the effect size. The Effect Size is defined as the minimum change at which we want to be able to accurately determine the test result or the minimum degree of difference that will be clinically significant. As a general proposition of Cohen, it is stated that d values of smaller than 0.2 indicate a weak effect size, a value of 0.5 indicates a medium effect size, and a value of higher than 0.8 indicates a strong effect size (9, 10). In our study, SPSS 21 was used to analyze. The outlier values in all parameters found in the dataset were examined by a Box Plot and excluded from the study. After summarizing the data with descriptive statistics, Student's t-test and Mann Whitney U test were used for the analyses. The level of significance for all tests was accepted as $p<0.05$.

Results

Our study covered a total of 70,352 individuals including 42,696 (60%) women and 27,656 (40%) men. Among these individuals, 46,731 (66%) were assessed as fasting, and 23,621 (34%) were assessed as non-fasting. As seen in Table 1, while there was no significant difference in Amylase (AMY), Iron, Lipase (LIP), Phosphor, Total cholesterol, High-density lipoprotein, Triglycerides, and Alkaline Phosphatase (ALP), some differences were observed in Albumin (ALB), Alanin Aminotransferase (ALT), Aspartate Aminotransferase (AST), Creatinine (CRE), Calcium (Ca), Gamma-Glutamyl Transferase (GGT), Glucose (GLU), Blood Urea Nitrogen (BUN), Total Protein (TP), and Creatinine kinase (CK) at $p<0.05$. As shown in Table 2, between the fasting and non-fasting values of the women, there was no significant difference in AMY, AST, Phosphor, Total cholesterol, CRE, iron, ALP, TP, Triglycerides, High-density lipoprotein and CK, while there were significant differences in ALB, ALT, Ca, GGT, GLU, BUN, and LIP at $p<0.05$.

Table 3 has demonstrated that, between the fasting and non-fasting values of the men, while there was no significant difference in amylase, Total cholesterol, CRE, Iron, GGT, BUN, LIP, Triglycerides, ALP, and High-densitylipoprotein, there were significant differences in ALB, ALT, AST, Phosphor, Ca, GLU, TP, and CK at $p<0.05$. However, according to the effect size values that were calculated, even the parameters that were found to be statistically significant were not significant (effect size min-max=0.006-0.104) (Table 2-3).

Additionally, while there was no significant difference among the women in AST, IRON, TP, ALP, High-density lipoprotein, and CK (Table 2), there was no significant difference among the men in IRON, GGT, BUN, ALP, and High-density lipoprotein (Table 3). As it may be seen here, although some parameters such as CK and AST were not statistically significantly different among the women between the fasting and non-fasting values ($p>0.05$), the differences in the men were significant ($p<0.05$) (Tables 2-3). Again, while parameters such as GGT and BUN were not significantly different among men, they were significantly different among the women. According to the effect size values that we measured to determine whether or not the parameters that turned out to be statistically

Table 1. Values of Parameters in Fasting/Non-Fasting States

	Fasting				Non-Fasting				p	Effect Size
	N	Mean±SS	Median	Min - Max	N	Mean±SS	Median	Min - Max		
Age	45929	49,49±16,96	50	18-98	23074	46,66±18,08	46	18-106	0,001	-
ALB	16356	4,31±0,35	4,35	3-5,66	7159	4,34±0,38	4,38	3-5,5	0,001	0,082
AMY	3670	70,05±27,5	66	7-185	2126	69,6±27,7	65	13-183	0,248	0,031
AST	26525	21,7±6,9	21	0-49	13221	22,04±7	21	1-49	0,001	0,037
ALT	38063	21,3±9,73	19	0-59	18936	21,62±10,08	19	0-59	0,007	0,023
Pi	6781	3,5±0,68	3,6	1,1-6,3	3233	3,61±0,66	3,6	1,1-6,2	0,164	0,03
Ca	20110	9,41±0,48	9,43	7,37-11,54	8379	9,46±0,49	9,48	7,3-11,54	0,001	0,103
CHOL	4880	188,2±43,06	184	35-379	2579	187,3±43,05	185	41-362	0,366	0,021
CRE	40471	0,743±0,22	0,7	0-1,64	19835	0,75±0,22	0,72	0,07-1,6	0,001	0,043
IRON	8807	63,69±37,54	58	1-225	3762	65,06±38,86	60	1-226	0,067	0,035
GGT	8114	21,82±13,42	18	1-72	5597	21,2±13,24	17	1-72	0,008	0,046
GLU	31376	93,89±16,09	90	36-150	15550	93,09±15,72	90	36-150	0,001	0,05
BUN	23778	14,21±5,79	13	1-36,9	14087	14,09±5,65	13	0,6-36,9	0,044	0,02
LIP	3387	37,41±12,37	35	12-84	1915	36,79±12,13	34	2-84	0,078	0,05
TP	9264	7,24±0,056	7,26	4,82-9,53	4187	7,27±0,057	7,29	4,89-9,66	0,002	0,053
TRIG	10082	148,56±78,46	129	0-469	4263	150,08±82,3	129	26-468	0,307	0,018
ALP	10070	74,05±25,83	70	8-179	4723	73,88±25,88	70	19-179	0,717	0,006
HDL	4089	47,08±12,69	45,5	7-103,9	2330	47,29±13,26	46	14-103,9	0,527	0,016
CK	7327	87,9±45,9	77	1,44-269,7	3115	92,78±47,8	81,35	9-269,7	0,001	0,104

Units: GLU, BUN, CRE, HDL, LDL, T-Cholesterol, TRIG, Ca, Pi: mg/dL; ALP, AMY, LIP, CK, ALT, AST: U/L; TP, ALB: g/dL; IRON: ug/dL

Table 2. Fasting / Non FastingValues of Female Individuals

Variables	Fasting				Non-Fasting				p	Effect Size
	N	Mean±SS	Median	Min - Max	N	Mean±SS	Median	Min - Max		
Age	28697	48,05±16,45	48	18-98	13305	45,49±17,66	45,49	18-106	0	-
ALB	10003	4,29±0,32	4,33	3-5,66	4079	4,31±0,35	4,31	3,02-5,37	0,03	0,059
AMY	2090	67,19±25,48	64	7-182	1155	66,78±26,16	66,78	13-183	0,659	0,015
AST	16611	20,93±6,53	20	0-49	7474	20,92±6,48	20,92	1-49	0,86	0,001
ALT	24685	19,82±8,68	18	0-59	11166	19,47±8,78	19,47	0-59	0	0,04
Pi	4074	3,68±0,66	3,6	1,1-6,3	1830	3,68±0,64	3,68	1,1-6,2	0,908	0
Ca	12952	9,41±0,48	9,42	7,38-11,54	4898	9,43±0,49	9,43	7,38-11,54	0,01	0,042
CHOL	2701	193,61±43,19	190	35-376	1296	192,42±43,07	192,42	41-362	0,413	0,027
CRE	25463	0,66±0,18	0,63	0-1,64	11369	0,66±0,18	0,66	0,07-1,63	0,138	0
IRON	6208	59,2±35,29	54	2-225	2599	59,99±36,38	59,99	2-226	0,341	0,022
GGT	4943	18,83±12,19	15	1-72	3232	17,56±11,15	17,56	1-72	0	0,108
GLU	20905	92,43±15,27	89	38-150	9267	91,97±15,21	91,97	46-150	0,016	0,03
BUN	13327	13,20±5,68	12	1-36,9	7548	12,97±5,48	12,97	1-36,9	0,005	0,041
LIP	1878	37,30±12,23	35	12-84	1047	36,31±11,35	36,31	2-82	0,031	0,083
TP	5248	7,23±0,54	7,25	4,9-9,53	2212	7,23±0,55	7,23	5,2-9,66	0,917	0
TRIG	6205	139,98±73,42	122	15-469	2379	138,96±76,95	138,96	33-468	0,577	0,013
ALP	6306	72,11±25,75	68	15-179	2661	71,06±25,73	71,06	19-177	0,076	0,04
HDL	2150	51,91±12,71	50	7-103,9	1135	52,48±13,31	52,48	14-103,9	0,226	0,043
CK	5402	81,71±41,45	72,85	1,44-269	2013	82,97±40,55	82,97	9-269,28	0,239	3

Units: GLU, BUN, CRE, HDL, LDL, T-Cholesterol, TRIG, Ca, Pi: mg/dL; ALP, AMY, LIP, CK, ALT, AST: U/L; TP, ALB: g/dL; IRON: ug/dL

Table 3. Fasting/Non Fasting Values of Male Individuals

Variables	Fasting				Non-Fasting				p	Effect Size
	N	Mean±SS	Median	Min - Max	N	Mean±SS	Median	Min - Max		
Age	17232	51,9±17,52	54	18-94	9769	48,25±18,54	48,25	18 - 101	0	-
ALB	6353	4,34±0,39	4,39	3-5,49	3080	4,38±0,42	4,38	3 - 5,50	0	0,099
AMY	1580	74,86±29,52	69	16-185	971	73,02±29,07	73,02	14 - 183	0,123	0,062
AST	9914	23,21±7,49	22	0-49	5747	23,51±7,37	23,51	3-49	0,016	0,04
ALT	13378	24,27±10,85	22	2-59	7770	24,71±11	24,71	0 - 59	0,004	0,04
Pi	2707	3,46±0,7	3,4	1,3-6,3	1403	3,53±0,68	3,53	1,6 - 6,2	0,004	0,101
Ca	7158	9,42±0,49	9,44	7,37-11,41	3481	9,49±0,51	9,49	7,45 - 11,4	0	0,141
CHOL	2179	181,66±41,9	179	69-379	1283	182,18±42,4	182,18	71 - 332	0,722	0,012
CRE	15008	0,87±0,2	0,85	0,2-1,64	8466	0,88±0,19	0,88	0,15 - 1,64	0,378	0,051
IRON	2599	74,42±40,45	70	1-225	1163	76,39±41,72	76,39	1- 226	0,17	0,047
GGT	3171	26,49±13,9	23	26-72	2365	26,18±14,21	26,18	2-72	0,42	0,022
GLU	10471	96,82±17,25	93	36-150	6283	94,73±16,29	94,73	36 - 150	0	0,124
BUN	10451	15,49±5,68	14,2	1-36,9	6539	15,37±5,57	15,37	0,6 - 36,9	0,166	0,021
LIP	1509	37,55±12,55	35	13-84	868	37,37±12,98	37,37	12-84	0,749	0,014
TP	4016	7,25±0,58	7,27	4,82-9,42	1975	7,32±0,58	7,32	4,89 - 9,63	0	0,12
TRIG	3877	162,3±84,11	141	0-468	1884	164,1±86,71	164,12	26 - 467	0,445	0,021
ALP	3764	77,29±25,64	73	8-179	2062	77,53±25,64	77,53	19 - 179	0,735	0,009
HDL	1939	41,72±10,28	40,5	8-88,7	1195	42,37±11,17	42,37	16,6 - 98	0,108	0,06
CK	1925	105,29±52,82	94,83	6,82-269,75	1102	110,70±54,42	110,7	15,07-269,7	0,007	0,1

Units: GLU, BUN, CRE, HDL, LDL, T-Cholesterol, TRIG, Ca, Pi: mg/dL; ALP, AMY, LIP, CK, ALT, AST: U/L; TP, ALB: g/dL; IRON: ug/dL

significantly different at $p<0.05$ between the fasting and non-fasting groups were clinically significant, no clinically significant difference was observed in any of the parameters. While the smallest effect size value was 0.016 for High-density lipoprotein, the largest one was 0.104 for CK, and there was no clinical significance according to Cohen's d (Table 1).

Discussion

In the literature, while it had been accepted until the 2010s that the appropriate metabolic state is the fasting state since the length of stay is desired to be shortened and since the laboratory is asked for serving all applicant patients 24 hours a day, blood is taken from patients at any time of the day, and it is not possible to be sure of the fasting state of these patients (11,12). Due to the crowdedness of blood collection units in the early morning, patients who arrive at the hospital to provide blood samples at 8 am may have to wait until noon. The fact that this waiting process is difficult for diabetic, pregnant, elderly, or pediatric patients, in particular, or the fact that these people cannot wait on an empty stomach, may cause distress for phlebotomists and patients.

In fact, outside the hours of the day, one is required to fast, the body metabolism is in a state of non-fasting most of the day. It might not be the right thing to still assess individuals with fasting metabolism tests (6,13). While research on the difference between fasting and non-fasting values in humans is mostly on lipids, a limited number of studies covered other biochemistry parameters including albumin, bilirubin, and uric acid (13-15).

Langsted et al. assessed lipids, lipoproteins, apolipoproteins, and albumin in diabetic and non-diabetic individuals at different periods following their latest meal. As a result, they reported that the plasma Triglycerides amounts in a non-fasting state increased by only 0.2 mmol/L in comparison to fasting in diabetic and non-diabetic individuals, non-High-density lipoprotein and Apolipoprotein-B stayed constant before and after meals, LDL-CHOL and albumin amounts decreased in diabetic and non-diabetic individuals, and this was probably caused by hemodilution as a result of fluid intake. They also emphasized that the use of non-fasting blood in lipid profile measurements is more useful than fasting blood (16). Mora et al. assessed lipid profiles and reported changes in Triglycerides by 0.2 mmol/L, Total cholesterol by 0.1 mmol/L, and LDL-CHOL by 0.2 mmol/L, while there was no significant change in High-density lipoprotein (17). Similarly, in our study, no significant difference was seen between the fasting and non-fasting values for Total cholesterol, Triglycerides, and High-density lipoprotein ($p=0.366$, $p=0.307$, $p=0.527$, respectively) (Table-1). In the review by Nordestgaard et al. which was supported by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM), according to the results of studies assessing fasting and non-fasting lipid profiles, it was proposed that non-fasting should be the standard, several countries (the United Kingdom, Canada, Scandinavian countries, etc.) have changed their clinical guidelines, and fasting is not required in lipid profile measurements in either diabetic or non-diabetic individuals, those with or without cardiovascular diseases and children or adults (5,18). They argued that fasting samples could be repeated for only patients with a doubt in routine results, and fasting samples could be collected based on their own guidelines in

patients with non-fasting triglyceride levels of >5 mmol/L (440 mg/dL) or chronic hyper triglyceridemia (5).

Plumelle et al. assessed the fasting and non-fasting results of 77 tests (37 biochemistry, 16 hematology, 3 coagulation, 21 endocrine parameters). They stated that, among the 37 biochemistry parameters, 29 were not affected by fasting or non-fasting (ALT, ALB, ALP, AMY, Apo A1, AST, B_2M, BIC, BUN, Ca, Total cholesterol, CK, GGT, HbA1c, High-density lipoprotein, Iron, K, LDH), the remaining were affected by food ingestion at $p<0.05$ (UA, TBIL, BNP, CREA, GLU), but as they did not affect the total change limit (TCL), they did not have clinical significance ($TCL=\sqrt{(2.77CVa)2+(0.5CVb)2}$).14 In our study, similar to the findings of the aforementioned studies, there were no differences in the ALT, ALP, AST, ALB, BUN, Ca, GGT, IRON, CK, TP, and Total cholesterol parameters.

The Bispebjerg study conducted by Sennels et al. prospectively examined the effects of fasting and non-fasting states on the circulating concentrations of 14 frequently used clinical biochemical parameters. Consequently, among the parameters common with ours in the 14 examined parameters, while there was no difference between fasting and non-fasting values in creatinine, there were differences in creatine kinase, ALT, and AST (19). Additionally, Plumelle et al. stated that there was no significant difference between fasting and non-fasting in ALT and AST values, and therefore, regardless of the hour of the day, measurements on the metabolism did not differ based on non-fasting or fasting states (14).

In our study, the p-value between the fasting and non-fasting values was 0.001 for creatinine, 0.007 for ALT, and 0.001 for AST (Table 1).

While studies on the effects of fasting and non-fasting on only the lipid profile among the 18 parameters that were examined in our study could be encountered in the literature, there is a limited number of studies regarding the other parameters we included. For this reason, we were not able to compare our results on some parameters to values reported in the literature.

Conclusion

Considering the mean values of the ALB, ALT, AST, Ca, CRE, GGT, Glu, TP, CK, and BUN parameters, it was found that they were close to each other between the statuses of fasting and non-fasting, but there were differences. However, when effect size analysis that is used in studies with large samples was applied, no clinical significance was found. In addition to this, no significant difference was found between the fasting and non-fasting values in the parameters of High-density lipoprotein, Triglycerides, Phosphor, Total cholesterol, AMY, Lipase, and ALP. Moreover, while there were differences in some parameters when this situation was assessed based on sex, again, clinically insignificant results were obtained.

In conclusion, biochemical values obtained by collecting blood at any time of the day may be used to make diagnoses of diseases without considering fasting/non-fasting status.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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ORIGINAL ARTICLE OPEN ACCESS

The Transmission Panorama and Epidemic Characteristics of SARS-CoV-2 of Jining City of China

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Introduction: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread in hundreds of countries and made millions of people infected and more than two hundred thousands of death.
Methods: This study retrospectively analyzed the data for the SARS-CoV-2 epidemic which ranged from Jan 24 to Feb 16, 2020. The source of the data was the reports about SARS-CoV-2 issued by the authority of Jining City. The materials, the traveling history, the transmission, the gender, and the age of the infected persons were deeply analyzed.
Results: There were 52 cases with SARS-CoV-2 infection in Jining City, including 20 females (38.5%). The average age for the infected persons was 45.3 years, and the cases numbers of the age stage of 31-40 and 41-50 years were all 14 cases with a total proportion of 53.8%. 23 cases were the primary infectors and 14 persons had obvious traveling experience in other provinces. There was one event of four generations of transmission; most of the primary infectious persons did not transmit the virus to others.
Conclusion: Jining City has the relative specific epidemic characteristic of SARS-CoV-2. This character of restricted transmission indicates that the prevention strategy is effective and worthy to learn.

Keywords: SARS-CoV-2, transmission panorama, epidemic, Jining City

Introduction

Currently, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread in hundreds of countries and made millions of people infected and more than two hundred thousands of death (1). In this pandemic, lots of cities in the world have been invaded by the virus; and among them, there are some cities of China at the primary stage of the epidemic. As we have known, Wuhan is the epidemic epicentral in China, and many other cities of and out of Hubei Province are involved (2), such as Huanggang, Shanghai, Hangzhou, and so on. The epidemic and clinical characteristics of SARS-CoV-2 infection in first-tier cities have

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been described in some reports (3, 4). However, relatively few reports about virus transmission in the little cities.

As one of sixteen prefecture-level cities of Shandong province, Jining City belongs to a third-tier city in China. However, it is famous in the world for Confucius' hometown – Qufu which is a county that belongs to Jining. In this pandemic of SARS-CoV-2, Jining can not escape by a piece of sheer luck. In this article, we retrospectively collected and analyzed the epidemic data related to SARS-CoV-2, further presented the transmission panorama and demographic characteristics of the city, and hope to pose some information or ideas for future relative researches on the aspects of prevention, control, and epidemic analysis.

Materials and Methods

According to the reports of Jining Municipal Health Committee, Rencheng District Health Committee of Jining City, and other local authority's media, the detailed information relates to the cases infected with SARS-CoV-2 was collected, and analyzed. The date range was from Jan 24 to Feb 16, 2020. The informed consent form was signed by all the objects.

Ethical Statement

This study is supported by the Research Fund for Academician Lin He New Medicine (JYHL2018FMS08), and the Project of scientific research support fund for teachers of Jining Medical University (JYFC2018FKJ023). The study has been approved by the Ethic Committee.

Results

The Distribution of the Cases with SARS-CoV-2

Twenty-two cases with SARS-CoV-2 infection were reported in Jining City to date. In the total 11 counties or districts, except Weishan, Liangshan, and Yutai County, there were different numbers of infectious cases in the remainder 8 administrative regions. The urban district of Jining City, Qufu City, and Yanzhou District were the top three regions with a higher number of infected cases, and the cases number was 22, 14, and 8, respectively (Figure 1).

Figure-1. The urban district of Jining City, Qufu City, and Yanzhou District

The Basic Materials

The confirmed dates for 52 cases with SARS-CoV-2 infection were ranged from Jan 24 to Feb 16, 2020. Among the cases, 14 infected persons had traveling experiences in the cities out of Shandong Province, and the involved cities included Wuhan, Chongqing, Zhengzhou, Hankou, Beijing, Chengdu, and so on (Table 1).

The Membership Analysis

There was 20 female in 52 cases, and the percentage was 38.5%. 37 persons infected with SARS-CoV-2 came from the city including Jining City and its affiliated counties and districts; the ratio was high to 71.2%. 5 persons came from town (9.6%) and 10 persons (19.2%) came from the countryside. Most of the infected cases had no traveling history to other provinces (38, 73.1%). The detailed analysis was shown in Figure 2.

The Age Distribution

In the infected population, the eldest age was 91, and the youngest age was only 4. The cases numbers of the age stage of 31-40 and 41-50 years were all 14 cases, and the total proportion was 53.8%. 16 persons were older than 50 years and the percent was 30.7%. The sum for the persons aged older than 71 years and less than 10 years was 6 (11.5%) and 3 (5.8%), respectively. There was no case at the age stage of 11-20 years (Figure-3).

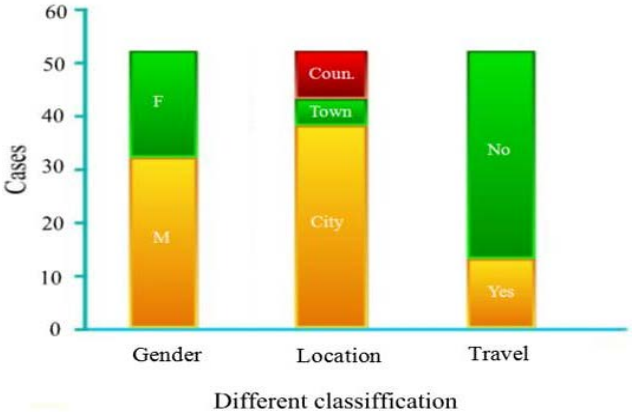

Figure-2. Patients' classifications according to gender, location and travel.

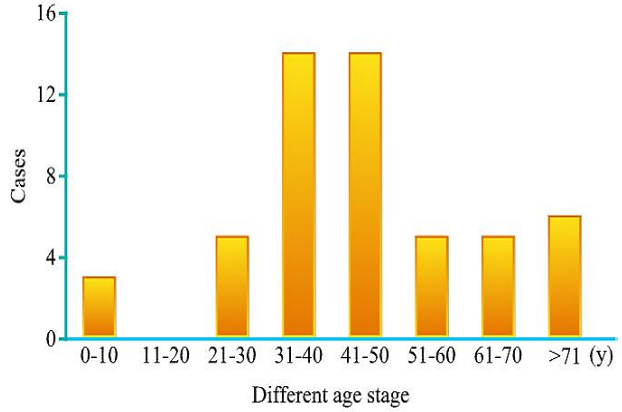

Figure-3. Different age stages of the cases

The Graphics for the Infection Route

According to the infectious source, confirmed date, and the relationship between the infected persons, graphics were drawn out to reflect the transmission of SARS-CoV-2 in Jining City (Figure 4). There were 23 cases in the primary generation. The number for the second, third and fourth generation was 21, 2, and 4 cases, respectively.

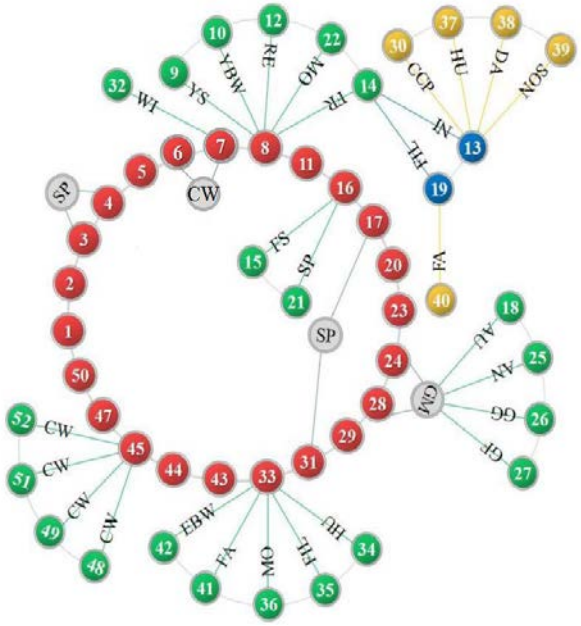

Figure-4. The Graphics for the Infection Route

Discussion

The pandemic of SARS-CoV-2 has been a severe concern threatening the human health of the whole world (5). In China, the novel coronavirus spread in many cities at the beginning of 2020. Except for Dongying City, the cases of SARS-CoV-2 emerged in the other fifteen cities of Shandong Province. This study puts the eyes on one of the prefecture-level cities — Jining City which is famous for Qufu city where is the hometown of Confucius, a well-known thinker, and educator (6).

As the results have shown, there were 52 cases with SARS-CoV-2 infection in nine counties or districts of Jining City. The urban district of Jining City, Qufu City, and Yanzhou District were the top three regions with a higher number of infected cases. The reason for this status is that Jining, Qufu, and Yanzhou are the

Table 1. The information for 52 patients with SARS-Cov-2 infection

Case	Sex	Age	Location	Confirm date	Trav. His.	Case	Sex	Age	Location	Confirm date	Trav. His.
1	F	57	Town	1.24	WH	27	M	58	City	2.5	—
2	M	43	Town	1.24	WH	28	F	61	City	2.5	WH
3	M	36	Coun.	1.25	WH	29	M	35	Coun.	2.5	ZZ
4	F	34	Coun.	1.25	WH	30	F	32	City	2.5	—
5	M	48	City	1.26	ZZ	31	F	30	City	2.5	—
6	M	33	City	1.29	CQ	32	F	33	Coun.	2.5	—
7	M	35	Coun.	1.29	CS	33	F	40	City	2.6	HB
8	M	48	City	1.29	BJ	34	M	44	City	2.6	—
9	F	44	City	1.30	—	35	M	77	City	2.6	—
10	F	38	City	1.30	—	36	F	65	City	2.7	—
11	M	25	City	2.1	HK	37	M	48	City	2.7	—
12	M	21	Coun.	2.1	—	38	F	6	City	2.7	—
13	F	40	City	2.1	—	39	M	4	City	2.7	—
14	F	65	City	2.1	—	40	M	61	City	2.10	—
15	M	31	City	2.2	—	41	M	71	City	2.10	—
16	M	58	City	2.2	—	42	F	42	City	2.11	—
17	M	33	City	2.2.	—	43	M	67	Coun.	2.12	—
18	M	30	City	2.2	—	44	M	73	Coun.	2.13	—
19	M	91	City	2.3	—	45	M	47	City	2.13	—
20	M	53	City	2.3	—	46	M	49	City	2.13	—
21	F	55	City	2.3	—	47	F	46	Coun.	2.14	—
22	F	72	Town	2.3	—	48	M	43	Town	2.14	—
23	M	40	City	2.4	JX	49	M	47	Town	2.14	—
24	F	5	City	2.5	WH	50	M	34	Coun.	2.15	—
25	F	29	City	2.5	—	51	M	48	City	2.15	—
26	F	82	City	2.5	—	52	M	50	City	2.16	—

Abbreviations: BJ, Beijing; Coun., Countryside; CQ, Chongqing; CS, Changsha; F, Female; HB, Hubei; HK, Haikou; JX, Jiangxi; M, Male; Trav. His., Travel History; WH, Wuhan; ZZ, Zhengzhou

prefecture-level, the traveling, and the railway transport city, respectively, and the number of infection cases is associated with the degree of population mobility (7). In the gender analysis of 52 cases, the percent of the female and male was 38.5% and 61.5%, respectively. This was similar to Chan's report in which the female and male ratios were 32% and 68%(8). In Ryu's investigation (9), more than two-third (10 in 15) of persons infected with SARS-CoV-2 were male. In Huang's study (10), the percent of the male was high to 73.2%, while the rate in Wang's paper was only 54.3% (11). Although there were differences in the ratios of the male in different reports, it was obvious that the case number of the male was generally more than that of the female. In the infected population, the age almost covered every phase: from elder men to little children, and the average age was 45.3 years. This was very near to Fang's report, in which the average age of the patients with SARS-CoV-2 infection was 45.1 years (12). It is also consistent with the mean age of 47.0 years in Guan's study (13). Certainly, there were inconsistent references about the average age of the infected person: 37 years for Yang's

report (4) about Chongqing and 32.5 years for a study on Nanjing (14). As the epicenter of this epidemic, the mean age was reported as 55.5 and 56 years in two reports focused on Wuhan (15, 8), and it was obvious that the data were higher than in others cities. Whether the average age of the people infected with SARS-CoV-2 in non-epicenter usually is younger than that of the epicenter area needs further observation.

To analyze the cases with different age stages, we found that the percent of 31-50 years stage was 53.8%, which was significantly higher than another report (47.3% & 36.3%)(4,16). However, all the percents of this age stage were more than one-third and higher than those of other age stages in these studies. The ratio for the age more than 71 years was 11.5%, which was similar to another report in which the corresponding ratio of 12% (16). In the range of fewer than 10 years, there were only 3 cases with SARS-CoV-2 infection in this study, and the percentage was 5.8%. It was obviously higher than 1.2%, 0.1% and 3.85% reported in other references (16-18). Interestingly, there was no case aged 11-20 years in all the objects of Jining City. Although the rate of this age stage was more than zero in other studies, the value was rather low that it always was less than 2% (17,19). These findings probably indicated that the youngster was not easy to be infected with SARS-CoV-2, and the reason possibly was that this population has consisted of the students of primary and middle school. They usually lived in a relatively small space and had very little social activity, and hence, they had very low chance to be infected with SARS-CoV-2.

Of 52 cases, 71.2% came from the city which including Jining City and the affiliated counties and district of the city; while less than 30% of the persons with SARS-CoV-2 infection came from town and countryside. This finding was consistent with Chen's and Lin's reports (15, 17). This point was determined by the relatively greater popular mobility of city dwellers. According to such a character, it is suggested that the city is of the primary importance for controlling the transmission of SARS-CoV-2.

According to an investigation reported by the local health authority (20), graphics were drawn out to describe the transmission of SARS-CoV-2 in this study. 23 index persons were taken as the initial transmission source. These index cases were responsible for the local epidemic of SARS-CoV-2 to some extent. Among them, 14 cases had clear travel histories out of Shandong Provinces. These indicated that the inputting cases were the main infectious source for a city of non-epicenter (4) such as Jining City. In all of the spread brought by 23 index infectors, there was only one time for the four generations of transmission, two generations of transmission happened five times, and most of the primary infectious person did not transmit the virus to others. The reality strongly proved that the preventing strategies against SARS-CoV-2 infection were very effective, such as home isolation, collective centralization quarantine, keeping social distance, wearing face masks (21, 22). Besides, from Figure 4 we could found that the transmission usually happened between the relatives of a family, so the family cluster was the main element for virus spreading in Jining City. Therefore, the prevention and control of SARS-CoV-2 transmission between the family members or relatives are rather important during the SARS-CoV-2 epidemic (23).

Conclusion

According to the panorama and epidemic characteristics of SARS-CoV-2 in Jining City, it is

obvious that the prevention and control are effective. Although these data are extracted from the primary period of the epidemic, this study again indicates that the strategy for the fight against SARS-CoV-2 is effective to some extent and worthy to learn by the members of the global village.

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ORIGINAL ARTICLE OPEN ACCESS

Dermatophytosis in Bhairahawa, Nepal: Prevalence and Resistance Pattern of Dermatophyte Species

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Introduction: Dermatophytosis is colonization by dermatophytic fungus of the keratinized tissues like hair, nails and skin. They are considered important as a public health problem. The study was aimed to isolate, identify, and detect the in-vitro antifungal sensitivity pattern of various dermatophytes isolates from clinically diagnosed cases of dermatophytosis.
Materials and Methods: One hundred and sixty patients of all age group and both sexes and clinically diagnosed with dermatophytosis were recruited in this study. The specimens included skin scales, hair pluckings and nail clippings. Identification and isolation were done by microscopic examination, culture and biochemical analysis.
Results: Dermatophytosis was more common in males (60.62%) than females (39.37%). Tinea corporis (31.25%) was the most common clinical presentation followed by Tinea faciei (25%). *Trichophyton rubrum* (36.19%) was the most common isolate followed by *Trichophyton mentagrophytes* (15.23%). Out of four antifungal drugs used, fluconazole was found most resistant while Itraconazole was most effective drug.
Conclusion: The epidemiology of dermatophyte infections may change with time. Antifungal susceptibility testing will aid the clinician in initiating prompt and appropriate antifungal therapy and prevent emergence of resistance.
Keywords: Antifungal sensitivity, dermatophytosis, tinea infection, trichophyton

Introduction

Dermatophytes are keratinophilic hyaline molds that can cause disease in keratinized tissues like hair, skin, and nail (1). The members of this dermatophytic group include *Trichophyton*, *Microsporum* and *Epidermophyton* (2). Based on the reservoir and route of transmission, dermatophytes may be of anthropophilic (human), zoophilic (animals), or geophilic (soil) origin. These organisms are named according to the affected body site: Tinea capitis (head), T. corporis (trunk), T. cruris (perianal area), T. pedis (foot and interdigital area), and T. unguium (nail) (3). The most common etiological agents are *Trichophyton rubrum*, *T. mentagraphytes*, *T. interdigitale*, *T. tonsurans*,

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and *Microsporum canis*. *T. rubrum* is the most frequently isolated agent in clinics (1). Nepal is such a country where a wide variation in climate, socio - economic status, religion and customs is quite prevalent in different parts of the country. In developing countries, other than hot and humid climatic conditions, low hygiene, poor access to water, overcrowding contact also plays significant etiological role for dermatophytosis (4-9).

Treatment options for dermatophytosis are topical as well as systemic antifungal drugs. But during course of time dermatophytes have also evolved drug resistance for single as well as multiple drug simultaneously. Studies conducted worldwide show that resistance among dermatophytes is not uncommon (10, 11). Due to high temperature and increased humidity, there are increased cases of dermato phytosis and other fungal infections especially in terrain and hilly region of Western Nepal. Since there was increased incidence of drug resistance observed over a period of time to the antimycotic drugs commonly used for the treatment i.e., fluconazole, terbinafine and clotrimazole, the need for testing of antifungal susceptibilities of dermatophytes has become apparent. Recently CLSI (Clinical and Laboratory Standards Institute) has approved a micro broth dilution method for antifungal susceptibility testing of molds, but these tests are cumbersome and difficult to be performed in routine laboratory setup. The agar-based disc diffusion (ABDD) is an easy method to determine the antifungal susceptibility of dermatophytes, but data regarding these methods are scarce and not standardized (2, 12, 13). The application of in vitro antifungal susceptibility testing for guidance of antifungal drug therapy has been limited due to uncertain correlation between in vitro and in vivo action of drugs (2, 14).

This study was planned to determine the prevalence of dermatophytes infection in Bhairahawa, Nepal as well as the resistance of the recovered dermatophyte species to antifungal drugs. So far, skin fungal infection is empirically treated and fungal culture and sensitivity is not routine recommended in our region; therefore, only handful of data is available regarding incidence of skin infection and drug resistance. Therefore, this study was planned to find out the same.

Materials and Methods

A hospital-based prospective observational study was conducted at Universal College of Medical Sciences Teaching Hospital (UCMS-TH) Bhairahawa from March 2019 to October 2019.

Ethical Statement

Ethical approval was taken from the institutional review Committee (IRC) of UCMS-TH prior to the sample collection (I.R.C. Reg. No. UCMS/IRC /036/019). A total of 160 patients of all age group and both sexes attending Dermatology outpatient and clinically diagnosed with dermatophytosis were recruited in this study after informed consent. Patients with surface infections, accidental and surgical cases and also patients who were already on antifungal treatment were excluded from the study. A detailed history of selected cases was recorded that included name, age, sex, address, duration of illness and other complaints. All the clinically diagnosed 160 cases were subjected to mycological work. The specimens included skin scales, hair and nails. The site of the lesions was cleaned with 70% alcohol, samples were collected in a sterile paper folds and labelled with details of patients. All the samples were

subjected to direct microscopy and culture. One part of the specimen was directly observed under microscope by potassium hydroxide (KOH) mount using 10% for skin and 40% for hair and nail samples. Another part of the sample was inoculated on slants of Sabouraud's dextrose agar (SDA) with chloramphenicol (0.05 mg/ml) and cycloheximide (0.5mg/ml). Culture tubes were examined thrice weekly for appearance of growth, cultures were incubated for 1 month before discarding them as negative. Cultures yielding growth were evaluated to species level-based colony morphology, microscopic properties in Lacto phenol cotton blue (LPCB) mount and urease test. The LPCB was obtained from Hi-Media Laboratories Pvt. Ltd., Mumbai, India. The isolates were subjected to the agar-based disc diffusion method to the study of sensitivity pattern of antifungals using antifungal drugs as described by Nweze et al, (12) and Prabhat Kiran Khatri et al.(15). All the dermatophytes were sub cultured on potato dextrose agar and incubated at 28^0C to enhance sporulation for 1 week. Following growth, conidia were harvested in sterile saline and conidial suspension was adjusted to between 1.0×10^6 and 5×10^6 spores/ml by microscopic enumeration with cell counting hemocytometer (Neubauer chamber) (16). Four antifungal drugs were tested against dermato phytes isolates. The following commercially available antifungal drugs were obtained from HiMedia Laboratory Pvt. Ltd., Mumbai, India; fluconazole (25 µg), itraconazole (10 µg) and ketoconazole (10µg). Plates of non-supplemented Muller Hinton Agar (MHA) were streaked evenly in three directions with a sterile cotton swab dipped into the standardized inoculums suspension. Plates were allowed to dry then antifungal disc were applied over MHA plates, after which the plates were incubated at 28^0 C for 5-7 days.

Trichophyton mentagrophytes ATCC 9533 and *Trichophyton rubrum* ATCC 28188 strains served as control. After the colonies grew, the zones of inhibition around the disc were measured in millimeters and recorded as sensitive, intermediate or resistant (9, 12, 13). Control plates with fungus inoculum and without antifungal disc were also tested.

All the data from cases was fed in MS Excel (Microsoft office 2018) and then analyzed by Statistical Package for Social Service (SPSS) for window version; SPSS 22, Inc., Chicago, IL). All data were expressed in terms of percentage.

Results

Out of 160 clinically diagnosed cases of dermatophytosis, males (60.62%) were more affected than females (39.37%) with male: female ratio 1.54:1. Most of the affected patients belonged to the age group of 15-29 years (33.75%) followed by 30-44 years (26.87%) which is shown in Table-1. Majority of the affected patients belonged to low socio economic status and were involved in active physical works like manual laborer, farmers, carpenter, tailor, domestic help etc.

Table 1. Distribution of patients according to age and sex

Age group *(in years)*	Males	Females	Total
0-14	11 (6.87%)	7 (4.37%)	18 (11.25%)
15-29	31 (19.37%)	23 (14.37%)	54 (33.75%)
30-44	24 (15%)	19 (11.87%)	43 (26.87%)
45-59	18 (11.25%)	9 (5.62%)	27 (16.87%)
>60	13 (8.12%)	5 (3.12%)	18 (11.25%)
Total	N_1 = 97 (60.62%)	N_2 = 63 (39.37%)	N = 160 (100%)

Tinea corporis (31.25%) was the most common clinical presentation followed by Tinea faciei

(25%) and Tinea capitis (14.37%). There was higher incidence of Tinea corporis and Tinea faciei in males compared to females i.e. 27 (16.87%), 22(13.75%) respectively which is shown in Table-2.

Table 2. Distribution of clinical types of dermatophytosis

Clinical Types	Males	Females	Total
Tinea Corporis	27 (16.87%)	23 (14.37%)	50 (31.25%)
Tinea Faciei	22 (13.75%)	18 (11.25%)	40 (25%)
Tinea Barbae	13 (8.15%)	0	13 (8.12%)
Tinea Capitis	12 (7.5%)	11 (6.87%)	23 (14.37%)
Tinea Pedis	10 (6.25%)	4 (2.5%)	14 (8.75%)
Tinea Unguium	7 (4.37%)	3 (1.87%)	10 (6.25%)
Tinea Cruris	6 (3.75%)	4 (2.5%)	10 (6.25%)
Total	97 (60.62%)	63 (39.37%)	160 (100%)

Out of 160 samples processed, 130 (81.3%) were positive for KOH mount while 105 (65.6%) were culture positive. Out of 130 KOH positive samples, 102 (63.8%) were both KOH positive and culture positive, rest were culture negative which is elucidated in Table-3.

Table-3. Correlation between results obtained by direct microscopy (KOH mount) and culture

KOH Results	Number of cases		
	Culture (+ve)	Culture (-ve)	Total
KOH (+ve)	102 (63.75%)	28 (17.5%)	130 (81.25%)
KOH (-ve)	3 (1.88%)	27 (16.88%)	30 (18.75%)
Total	105 (65.62%)	55 (34.37%)	160 (100%)

Samples from patient with Tinea cruris resulted 100% KOH positivity followed by those from cases of Tinea capitis which showed 78.26% KOH positivity. Highest cultural positivity was observed in cases of Tinea corporis (74%) followed by Tinea faciei (70%) and Tinea barbae (61.5%). *Trichophyton rubrum* (36.2%) was the most common isolate followed by *Trichophyton mentagrophytes*(15.2%),*Trichophyton tonsurans* (13.3%) and *Trichophyton violaceum* (12.4%). *Trichophyton rubrum* was the most common dermatophyte isolated from 38 clinical types of dermatophytosis. All four isolated dermatophyte species were recovered from Tinea corporis, the most common clinical presentation which is shown in Table-4.

Antifungal susceptibility testing showed itraconazole as the most sensitive antifungal agent, while ketoconazole was the least sensitive. Among the dermatophyte isolates, *M. audouinii* showed 100% sensitivity against Itraconazole followed by *T. rubrum* (84.21%) whereas the least sensitivity was shown by *M. canis* (55.56%). *T. mentagrophytes* showed 68.75% sensitivity against fluconazole. Similarly, *T. violaceum* showed highest sensitivity i.e., 76.92 % against ketoconazole followed by *T. tonsurans* (71.43%) and *T. mentagrophytes* (62.5%) which is shown in Table-5.

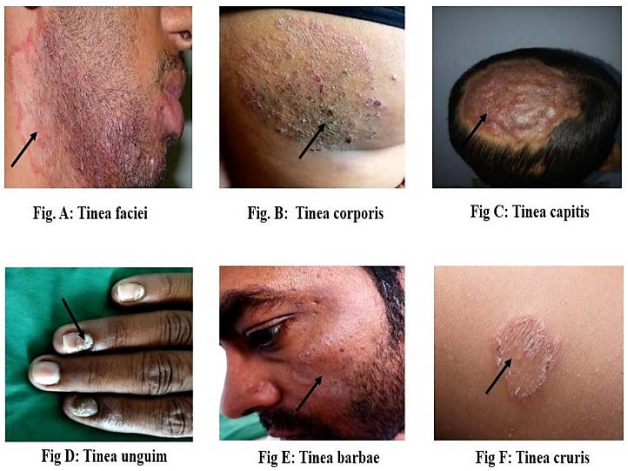

Figure 1. Clinical Pictures of Dermatophytosis Infection. A (Tinea faciei showing erythematous annular lesions with central clearing), B (Annular erythematous scaly plaques with advancing margin of tinea corporis), C (Tinea capitis showing patch of alopecia and ring formation at the periphery), D (Destruction of nail plates due to Tinea unguium). E (Tinea barbae showing erythematous annular lesions over bearded skin); F(Tinea cruris with erythematous lesions at groin region).

Table 4. Correlation between clinical presentations and isolated dermatophytes

Clinical Diagnosis	KOH Positive	Culture Positive	Dermatophyte isolated						
			E. floccosum	*M. audouinii*	*M. canis*	*T. mentagrophytes*	*T. rubrum*	*T. tonsurans*	*T. violaceum*
Tinea Barbae	10	8	0	0	2	0	6	0	0
Tinea Capitis	18	13	0	1	0	6	4	2	0
Tinea Corporis	41	37	5	2	4	9	5	9	3
Tinea Cruris	10	5	0	2	0	1	1	0	1
Tinea Faciei	32	28	1	1	2	0	18	2	4
Tinea Pedis	10	8	1	0	1	0	2	1	3
Tinea Unguium	9	6	2	0	0	0	2	0	2
Total	130 (81.3%)	105 (65.6%)	9 (8.6%)	6 (5.7%)	9 (8.6%)	16 (15.2%)	38 (36.2%)	14 (13.3%)	13 (12.4%)

Table 5. Antifungal susceptibility pattern of isolated dermatophytes

Antifungal Discs	S/R	*T. rubrum* (n=38)	*T. mentagrophytes* (n=16)	*T.tonsurans* (n=14)	*T. violaceum* (n=13)	*M. audouinii* (n=6)	*M. canis* (n=9)	*E. floccosum* (n=9)
Itraconazole	S	32 (84.21%)	11 (68.75%)	12 (85.71%)	8 (61.54%)	6 (100%)	5 (55.56%)	6 (66.67%)
	R	6 (15.78%)	5 (31.25%)	2 (14.28%)	5 (38.46%)	0	4 (44.44%)	3 (33.33%)
Fluconazole	S	7 (18.42.3%)	5 (68.75%)	2(14.28%)	3 (23.07%)	2 (33.33%)	4 (44.44%)	6 (66.67%)
	R	31 (81.57%)	11 (31`.25%)	12 (85.71%)	10 (76.92%)	4 (66.67%)	5 (55.56%)	3 (33.33%)
Ketoconazole	S	19 (50%)	10 (62.5%)	10 (71.43%)	10 (76.92%)	1 (16.67%)	5 (55.56%)	2 (22.22%)
	R	19 (50%)	6 (37.5%)	4 (28.57%)	3 (23.08%)	5 (83.33%)	4 (44.44%)	7 (77.78%)

Discussion

Identification of species responsible for the dermatophytoses and their sensitivity pattern is of great importance not for epidemiology but also for therapeutic point of view. Our study site bears tropical climate where high level of humidity and high temperature favor the growth of fungi causing dermatophytoses.

In our present study about 33.75% of dermato phytes were isolated from patient belonging to the age groups 15-29 years age. Our results are similar to other studies (17-20) who also reported higher infections in young adults. The higher prevalence is mainly due to the physical activity, hot humidity and high temperature in the region. This leads suitable wet condition for dermatophytes to grow. In this study, out of 160 patients, 97 (60.62%) were males and 63 (39.37%) were females, with male to female ratio being 1.54:1. Male dominance is reported in many places of South Asia (21-23). High prevalence of dermatophytes in males is due to frequent interaction with the society.

The predominant of clinical manifestations of dermatophytoses vary considerably to different studies in literature. In this study tinea corporis was the most dominant clinical manifestation involving 31.25%. Our findings are in accordance with the study by Balakumar S and et al, (24) who also reported Tinea corporis as the dominant clinical diagnosis. High rates of Tinea corporis could be attributed to its symptomatic nature (pruritus) which leads the patient to seek medical advice

(5).Whereas study by Hemendra Kumar Sharma et al, (25) showed *Tinea unguium* as the dominant clinical diagnosis. This variation observed in the clinical type of dermato phytoses is due to varied climate conditions, livelihood, type of occupations, type of occupation, pathogen and host relationship.

In the study, out of 160 clinical samples, 130 (81.25%) samples were positive by direct microscopy by KOH mount and 105 (65.62%) samples were culture positive. Out of 130 KOH positive samples, 102 (63.75%) samples were both KOH positive and culture positive, while the rest 28 (17.5%) were culture negative. The direct microscopy and culture findings of present study are relatively in agreement with study done by Basak P et al, (26) (71.1% KOH positive and 59.8% culture positive), Dhyaneswari GP et al, (27) (72.6% KOH positive) and Mahale RP et al, (28) (61.01% culture positive). There is a difference between KOH positivity rate and culture positivity rate in our present study this is because fungal elements were seen under direct microscopy but samples failed to grow on culture which might be due to various factors like unsatisfactory collection of samples containing dead fungal hyphae (29, 30). In this study, some specimens did not show any fungal elements when seen under direct microscopy but showed growth on culture. This might be due to presence of scanty fungal elements which were missed during direct microscopic examination or due to fungal elements in inactive sporulating form, which could not be visualized under microscopy (30).

In this study genus *Trichophyton* represented 77.14% of the isolates of dermatophytes, followed by *Epidermophyton* (8.57%) and *Microsporum* (14.28%). The most isolated was *Trichophyton rubrum* (36.19%) followed by *Trichophyton mentagrophytes* (15.23%) *Trichophyton tonsurans* (13.33%), *Trichophyton violaceum* (12.38%), *Microsporum canis* (8.57%). The other species isolated was *Microsporum audouinii*, and *Epidermophyton floccosum*. Our findings are in accordance with study by Dhyaneswari GP et al, (27) (*Trichophyton rubrum* 59.6%, *Trichophyton mentagrophytes* 26%), Walke HR et al, (31) (*Trichophyton rubrum* 56.37%, *Trichophyton mentagrophytes* 19.39%), R.K Agarwal et al, (32) (*Trichophyton rubrum* 42.63%, *Trichophyton mentagrophytes* 41.81%), and Basak P et al, (26) who have reported dermatophyte *Trichophyton rubrum* as the dominant species. However, there are studies such as by Hemendra Kumar Sharma et al, (25) who has reported *Trichophyton mentagro phytes* as most common species isolated.

The determination of in-vitro antifungal susceptibility was reported to be important for the ability to eradicate pathogenic dermato phytes. Most clinical types of dermatophytoses respond well to topical antifungal therapy, while Tinea unguium, Tinea capitis and extensive type of dermatophytoses require systemic therapy. Recently, there has been a rise in antifungal resistant strains of fungi. Therefore, early initiation of correct antifungal therapy is essential for proper treatment and prevention of spread of disease. In the present study, antifungal susceptibility testing by agar-based disc diffusion method (12, 32) was performed for five antifungal drugs: ketoconazole, fluco nazole, itraconazole and nystatin. Itraconazole (76.19 %) was the most sensitive followed by nystatin (63.8%) and ketoconazole (54.28%) and fluconazole (27.61%) was the least sensitive. Present study findings are almost similar with the findings of Basak P et al, (26) itraconazole (97.9%) was the most sensitive antifungal drug

while fluconazole (2.7%) was least sensitive). Our findings about poor susceptibility of dermatophytes to fluconazole is compatible with the studies by Hemendra Kumar Sharma et al (25), Basak P et al, (26) and EI Damaty et al (33). The higher resistance to fluconazole may be due to its availability at pharmacies, self-medication by patients due to its over the counter (OTC) availability and rampant practice of irrational prescription by compounder.

In this study, out of 105 isolates, 80(76.19%) were sensitive to Itraconazole, while 20.83% were resistant. Itraconazole is a much more affordable antifungal drug. Our study was in according to the Basak P et al, (31) and EI Damaty et al, who also showed Itraconazole as the effective drug. It has effectiveness against dermatophytes; hence, it must be a preferred treatment option for better outcome in patients suffering from dermatophytoses. In this study out of 105 dermatophyte isolates, 54.28% were sensitive to Ketoconazole while 45.71% were resistant. We have observed average sensitivity to Ketoconazole which is in agree ment with study by Hemendra Kumar Sharma et al,(25) which has suggested Ketoconazole as an average choice for the treatment of dermato phytosis. Our work suggests that disk diffusion antifungal susceptibility testing is simple, inexpensive, and does not require high cost equipment. It allows for a comparison between different antifungal agents and may help optimize the therapy for treating patients with dermatophytosis.

Conclusion

This report documents the emergence and occurrence of dermatophytoses and its agent in Western part of Nepal. Males are more affected than female with dermatophytoses infection. KOH examination is shown to be more sensitive than culture. Majority of the cases were Tinea corporis followed by Tinea pedis and the Tinea faciei and the commonest mycological isolate with *Trichophyton* taking the lead, among them the commonest species was *Trichophyton rubrum*. The fungal infections can be treated by a proper dose of itraconazole than other antifungal drug therapy. MIC values should be determined by broth microdilution test to determine the proper dose.

Recommendation

Present study has highlighted the frequency of dermatophytosis in tertiary care hospital which also reflects the overall similar picture in other part of our country. On the basis of the study, it has made following recommendation: Any clinical diagnosis needs to be supported by laboratory diagnosis. Since microscopy and culture are easy to perform, cost effective and this should be done in all suspected cases of dermatophytosis. As antifungal susceptibility testing facilities are now available for dermato phytes, every isolate should be tested against antifungal drugs so that increasing resistance among dermatophytes can be reduced. This may help in surveillance and epidemiological study of resistant strains.

Author Contribution

Subhash Lal Karn: Conceptualization, draft editing, reviewing, writing, Ajay Gurung: draft preparation, Writing, Amit Kumar Shrivastava: Preparation, reviewing, writing, original draft, Sulochana Khatiwada Poudel: writing, Shristi Raut: Writing, Chandra Mohan Sah: Writing.

Conflict of Interest

The authors declared no conflict of Interest in the present study.

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ORIGINAL ARTICLE OPEN ACCESS

Is Vitamin D Deficiency the Invisible Part of the Iceberg in Preschool Children?

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Introduction: Vitamin D deficiency continues to be a serious public health problem in all age groups in Turkey. The aim of this prospective study was to evaluate the vitamin D status in preschool children after the initiation of support programs and to determine risk factors for vitamin D deficiency.
Materials and Methods: The study included 135 preschool children, >24 months to <84 months of age, who presented to the pediatric clinic between January and September 2018. The demographics, risk factors, diet, and daily vitamin D intake were recorded. Serum levels of 25(OH)D, calcium, phosphorus, and alkaline phosphatase were measured. Serum 25(OH)D levels were evaluated in relation to several risk factors including age, gender, body mass index, number of siblings, socioeconomic status, education level of the family, vitamin D intake during pregnancy and infancy, daily sunlight exposure, and season of presentation.
Results: The mean 25(OH)D level was 19.6±8.5 ng/mL. According to the vitamin D levels, more than half of the children had vitamin D deficiency (n=31, 23%) or insufficiency (n=42, 31.1%). Significantly lower 25(OH)D levels were found in children with low socioeconomic status (p=0.01), a low maternal education level (p=0.02), low regular vitamin D intake during infancy (p=0.04), less daily sunlight exposure (p=0.03), and in those who presented in winter (p=0.01). Laboratory parameters did not differ across the vitamin D deficient, insufficient, and sufficient groups.
Conclusion: Despite the current preventive strategies and supplementation programs, vitamin D deficiency continues to be an important problem not only for risk groups but also for preschool children.
Keywords: Vitamin D, deficiency, children, preschool

Introduction

Vitamin D (Vit-D), a prohormone, is important for calcium-phosphorus balance and bone mineralization and is produced by the kidneys (1-3). Vit-D deficiency is not only associated with rickets and osteomalacia but may also predispose individuals to many diseases such as cardiovascular diseases, rheumatic diseases, neuropsychiatric dysfunction, and immune system disorders (4-7).

In both adults and children, vit-D deficiency is a public health problem in Turkey. Therefore, vit-D supplementation programs have been initiated for newborns and infants since 2005 and pregnant women since 2011. It has also

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been shown that the support program for infants reduces the incidence of rickets, especially under 3 years of age (8-9). However, recent research also shows that vit-D deficiency and insufficiency are still major problems (9). Most of the studies evaluating vit-D status following supplementation programs generally involve the risk groups; thus, data are limited in the case of vit-D preschool children. Given its predisposing role to many metabolic and systemic diseases, preventive strategies against vit-D deficiency can be promoted in this pediatric population.

This study aims to determine the vit-D status in preschool children and to determine the important risk factors for vit-D deficiency in this population.

Materials and Methods

This study was carried out prospectively in the Pediatrics Department of the Medical Hospital following the principles of the Declaration of Helsinki.

Ethical Statement

All applications were approved by Biruni University Medical Ethics Committee (2018/15-18). The informed consent was also obtained from the parents for the children.

135 preschool children aged 24-84 months, who presented to the pediatric outpatient clinic between January and September 2018, were included in the study. All parents completed a questionnaire at enrollment to collect medical history and socio-economic demographics. Exclusion criteria for children included age <2 or >7 years, the attendance of chronic disease, congenital disease or malformation, use of any medication that may assume the 25(OH)D level, and refusal of parental consent. A detailed data collection was made involving, gender, age, body mass index (BMI), height, weight number of siblings, socioeconomic position, parental education level, daily exposure to the sun, vitamin D intake during the first year of life, maternal vitamin D intake through pregnancy, diet and the season in which the child visited the clinic. Daily exposure to sunlight for more than 30 minutes was evaluated as adequate sun exposure. Mothers and their children were divided into groups according to their vit-D intake during infancy and pregnancy; none, irregular intake, and regular intake.

Dietary intakes of calcium (Ca) and vit-D were also recorded, with adequate consumption considered to involve two fish meals and three eggs per week and daily intake of milk and dairy products. BMI was calculated by dividing the weight in kilograms by the square height in meters (10). To determine 25(OH)D, alkaline phosphatase (ALP), phosphorus (P), Ca levels of the children, blood samples were collected and analyzed on the same day. Serum ALP, P, and Ca levels were analyzed by photometry on the Cobas Integra 400-Plus automatic analyzer (Roche Diagnostics, Germany). 25(OH)D levels were analyzed by enzyme-linked fluorescence assay on a Mini Vidas automated system (Biomerieux, France). There are also different classifications for evaluating vitamin D status in the literature. In our study, serum 25(OH)D levels were classified according to the 'Global Consensus Recommendations on the Prevention and Management of Nutritional Rickets' (deficiency: <12 ng/mL, insufficiency: 12-20 ng/mL, sufficient: >20 ng/mL) (11).

Statistical Analysis

Data were analyzed using the SPSS Statistics 20 program. G * Power Version 3.1 was used for the sufficient sample size. The minimum value for the total sample size was 134 and the effect

size was 0.35, the power was 0.8 and the type 1 error was 0.05. Descriptive data were expressed as a percentage, minimum, maximum, mean, median, and standard deviation. Student t-test and one-way analysis of variance (ANOVA) were used for normally distributed data, and Kruskal-Wallis test was used for non-parametric data to test the differences between means. The Chi-square test was used to determine the significant differences between categorical variables. A p-value of less than 0.05 was considered to be significant for type 1 error.

Results

Of 135 children, 55 (40.7%) were females and 80 (59.3%) were males. The mean age was 45.6±13.7 months. The mean 25(OH)D level was 19.6±8.5 (range 5.7-42.9) ng/mL and the mean Ca, P, ALP levels were 9.5±0.5 mg/dL, 4.5±0.7 mg/dL, and 179.5±41 U/L, respectively. Some of the mothers could not give an accurate account as to whether they had received vit-D during pregnancy (58; 43%) or for their babies (41; 30%).

Serum 25(OH)D levels showed no noteworthy differences among children by age, gender, BMI, number of siblings, father education level, and vit-D intake during pregnancy (Table 1). A higher socioeconomic status (p=0.01), a higher maternal education level (p=0.02), regular vit-D intake during infancy (p=0.04), the presence of daily sunlight exposure (p=0.03), and summer visits (p=0.01) had significant effects on higher serum 25(OH)D levels (Table 1). Serum 25(OH)D levels were meaningfully lower in children who presented in winter and spring seasons than in those who presented in fall and summer seasons (p=0.01).

Children were divided into groups according to their 25 (OH) D levels as deficient, inadequate, and sufficient(11) (Table 2). More than half of the children fell into deficient (n=31, 23%) or insufficient (n=42, 31.1%) categories. As expected, the mean 25(OH)D level in the deficient group (9.3±1.8 ng/mL) was significantly lower than those of the insufficient (15.9±2.3 ng/mL) and sufficient groups (27.2±5.6 ng/mL) (p=0.01). Ca, P, ALP levels were similar across the three groups.

Serum 25(OH)D levels of children were also evaluated concerning the consumption of nutrients rich in Ca and vit-D. Children with higher consumption of nutrients rich in Ca and vit-D had higher serum 25(OH)D levels than those with an inadequate ingesting of nutrients wealthy in Ca and vit-D, but with no significant difference (for Ca intake, 19.3±9 vs. 17.1±9.2 ng/mL, p=0.16; for vit-D intake, 19.1±8.4 vs. 18.3±9.4 ng/mL, p=0.48).

Discussion

This prospective study provides more evidence to the literature that vit-D deficiency is an ongoing major health problem in the preschool age group. The incidences of deficiency (23%) and insufficiency (31.1%) were high among preschool children, with serum 25(OH)D levels being significantly affected by socio-economic status, mother education level, vit-D intake during the first year of life, sunlight exposure, seasons and vit-D status.

The reported prevalence of vit-D deficiency in children shows a wide range from 1 to as high as 80%, depending on geographical distribution and countries. Two studies in the U.S.A reported the prevalence of serum vit-D levels at <20 ng/ml were 18% and 12.1% (12,13). In a meta-analysis of the European pediatric population, the prevalence ranged from 1-40% (14). In two studies conducted in China and India, the prevalence was reported as 38.8% and 40-80%, respectively (15,16).

Table 1. Serum 25(OH)D levels of children about their characteristics

Variables	n *(%)*	25(OH)D levels (ng/mL)	p value
All Children	135 (100%)	19.6±8.5	
Age (years)			
>2-≤4	79 (58.5%)	19.6±8.4	[1]0.9
>4-<7	56 (41.5%)	19.6±8.7	
Gender			
Male	80 (59.3%)	19.8±8.5	[1]0.7
Female	55 (40.7%)	19.3±8.6	
BMI *(kg/m²)*			
5-<85 p.	107 (79.2%)	19±7.8	[1]0.1
85-<95 p.	28 (20.8%)	21.8±10.6	
Number of Siblings			
0	41 (30%)	21.3±9.6	[2]0.07*
1	44 (33%)	19.4±9.7	
≥1	50 (37%)	16.3±7.3	
Socio-Economic Status			
Low	104 (77%)	17.5±8.6	[1]0.01**
Moderate	31 (23%)	23.3±9	
Mother Education Level			
Primary	34 (25%)	17.8±8.1	[3]0.02
Secondary	70 (52%)	17.6±9.7	
High	31 (23%)	22.8±7.4	
Father Education Level			
Primary	28 (21%)	16.9±7	[3]0.05*
Secondary	73 (54%)	17.2±9.2	
High	34 (25%)	23.9±8.5	
Vit D Intake In Pregnancy			
None	31 (23%)	18.6±9.3	[3]0.4
Irregular	26 (19.3%)	18.9±9.1	
Regular	20 (14.8%)	20.6±7.4	
Vit D Intake In Infancy			
None	5 (3.7%)	12.3±3.9	[3]0.04
Irregular	15 (11.1%)	16.4±8.8	
Regular	74 (54.8%)	20.3±9	
Daily Sunlight Exposure			
No	50 (37%)	17.5±8	[1]0.03
Yes	85 (63%)	20.8±8.6	
25(OH)D Level by Seasons			
Winter	43 (31.9%)	17.4±8.4	[3]0.01**
Spring	46 (34.1%)	18.2±9.3	
Summer	26 (19.3%)	23.7±5.4	
Fall	20 (14.8%)	22.2±7.9	

**Statistically significant at 0.05; ***Significant at 0.01; [1]Two-Sample T-test, [2]One-way Analysis of Variance, [3]Kruskal-Wallis*

Table 2. Laboratory results about categories of vitamin D status

Variables	Deficient	Insufficient	Sufficient	P
Number of children *(n, %)*	31 (23%)	42 (31.1%)	62 (45.9%)	
25(OH)D *(ng/mL)*	9.3±1.8	15.9±2.3	27.2±5.6	[1]0.01*
Ca *(mg/dL)*	9.4±0.5	9.5±0.3	9.5±0.4	[1]0.9
P *(mg/dL)*	4.5±0.7	4.5±0.5	4.6±0.9	[1]0.8
ALP *(U/L)*	165.4±41	181±37	189.4±45	[1]0.06

**Statistically significant at 0.01; [1]One-way analysis of variance*

Vit-D deficiency is still an important public health problem in Turkish children, despite support programs for babies and pregnant women. In 2005, Hatun et al. found the incidence of vit-D deficiency to be 21.7% among adolescent girls (17). In another study, Ölmez et al. drew attention to undulating prevalence patterns of insufficiency among adolescent girls, being 59.4% during the end of winter and 15.6% during the end of summer (18). In 2010, Arıca et al. reported that 37.5% of children aged 0-36 months had serum 25(OH)D levels of ≤10 ng/mL (19). In 2011, Andıran et al. reported the prevalence of vit-D deficiency as 25% among children and adolescents 0 to 16 years of age (20). In 2015, Doğan et al. reported a vit-D deficiency in 35% of children aged 1 month to 17 years, with a higher incidence of 41% among children aged 4 to 7 years (21). In 2016, Demiral et al. found that 51.5% of pediatric patients presenting to the endocrinology clinic had serum 25(OH)D levels of <12 ng/mL (22). Despite the use of varying cut-off values, these studies show that the rates of vit-D deficiency remain high among the pediatric population. In our study, the mean serum 25(OH)D level was within the range of deficiency (19.6±8.5 ng/mL) and the incidence of vit-D deficiency was 23%. Previous studies primarily focused on the vit-D status of risky groups such as infants and adolescents. In this study, we evaluated only preschool children (2-7 years of age) owing to limited data for this age group. Our results demonstrated a high incidence of vit-D deficiency and insufficiency in preschool children.

The primary source of vit-D is sun exposure because it is primarily produced in the skin through exposure to ultraviolet radiation. Therefore, the factors causing limited sun exposure have been implicated as the risk for a deficiency, including increased skin coloring, use of sunscreens, a covered dress style, winter season, living at higher latitudes, and increased daily time spent indoors (1, 3). As for seasonal variations, Karagüzel et al. reported the incidence of insufficiency as 93% throughout spring and as 71% throughout autumn among school children 11 to 18 years of age (23). Using serum samples obtained at the end of winter, Ölmez and Erol et al. reported vit-D insufficiency in 59.8% of female adolescents and 80% of children and adolescents, respectively (18, 24). In our study, 25(OH)D was significantly lower in winter serum samples than in fall and summer samples. An advanced level of daily sunlight exposure was also associated with significantly higher 25(OH)D levels.

Concerning other risk factors, children with a higher socioeconomic status and a higher mother education level had higher 25(OH)D levels and vice versa. A study from Belgium reported a significantly lower incidence of vitamin D deficiency among pregnant women with a higher education level (25). In a study, El Koumi et al. showed that low socioeconomic status was related to maternal vitamin D deficiency, which in turn had a significant adverse effect on neonatal vit-D status (26). Several studies from Turkey also found adverse effects of low socioeconomic status and a low educational level on the vit-D status of mothers and their newborns (18,24,27). Impaired vit-D status of children related to the low socio-economic status and low educational levels may be attributed to the fact that these risk factors directly influence individual lifestyles, among which are nutritional habits, clothing, and insufficient time-spending outdoors. Concerning nutritional habit, we also evaluated

serum 25(OH)D levels of children in terms of consumption of nutrients wealthy in Ca and vit-D, which, albeit not significant, showed higher 25(OH)D levels in children having adequate consumption. This finding supports the major role of sunlight in vit-D status as well as the relatively small role of dietary intake.

There are some limitations to our study. Especially, this study was conducted in a localization where the majority of the population consists of families with low-medium socio-economic status. Second, PTH levels could not be measured. Therefore, many further studies are needed to fully elucidate the results associated with vit-D status in preschool children.

Conclusion

There is growing evidence from recent studies that the problem of vit-D deficiency in children is far to abate. The data in the present study advise that vit-D deficiency is a significant problem not only in risky age groups but also in the preschool period. Therefore, considering relevant and amendable risk factors, preventive strategies such as preschool support programs or expanded support program continuing in babies may be considered to ensure the health of future generations.

Conflict of Interest

The authors do not report a conflict of interest for the present study.

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ORIGINAL ARTICLE OPEN ACCESS

Organ Transplant Center Management and Patient Monitoring During Severe Acute Respiratory Syndrome Coronavirus Type 2 (SARS-CoV-2/COVID-19) Pandemia

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Introduction: COVID-19 is a viral infectious disease that affects more than 200 countries in the world and has been declared a pandemic by the World Health Organization. This article provides some strategies for the management of the organ transplant unit in the COVID-19 outbreak or other respiratory infections.
Materials and Methods: Organ transplantation clinics, services or intensive care units are potential sources of transmission during this pandemic. Therefore, it is imperative for hospitals and organ transplant centers to organize management strategies for organ transplant patients during the COVID-19 outbreak. The study shared our special precautions and protocols created by a clinic that conducts over 1300 liver and 1500 kidney transplants.
Results: Five live donor liver transplants were performed in our clinic. A total of 8 patients are in our service and in the intensive care unit. Two patients who underwent live donor liver transplantation stay in the intensive care unit, and 2 patients with Post-op 2 donor and transplant preparation. COVID-19 infection was not detected in patients who underwent live donor solid organ transplantation and were followed up in our service.
Conclusion: It is important to have an arrangement to prevent and control the transmission of COVID-19 and to manage the organ transplant clinic during this period. A method that can protect both the hospital staff and the medical team and patients should be determined. Liver transplantation with live donors increases donor risk; on the other hand, it also provides important advantages such as planning the operation time, insulating the transmitter and providing sufficient and repeated tests. We think that we performed liver transplantation in the safest possible environment by following the guidelines adopted in our clinic.

Keywords: Organ transplant, acute respiratory syndrome, coronavirus, pandemia

Introduction

Coronavirus disease 2019 (COVID-19) is a viral infectious disease mainly transmitted by the respiratory system; it is highly infectious and has been declared an epidemic and a global public health emergency by the World Health Organization (WHO)(1). Turkey, in terms of absolute numbers, is one of the world's most affected countries (2). Worldwide research is ongoing to better understand the transmission dynamics and clinical disease spectrum. Due to the associated immunosuppression, solid organ transplant patients are included in the high-risk group for their follow-up and treatment; also, due to the co-morbidity present, their follow-

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up, diagnosis and treatments differ slightly from the healthy population (3). Given the early pandemic, information and case reports on COVID-19 and its effect on solid organ transplant patients are very limited to expert discussions (3-4). There is still an insufficient information about the natural history of COVID-19 regarding live donors and recipients, given the current diagnostic tests and the lack of a common approach plan in treatment (5).

Previous experience with SARS-CoV and MERS-CoV has shown that the solid organ transplant recipients may be exposed to long-term viral transmission and potentially increase infectiousness, morbidity and mortality (6,7).

There are several ways organ transplant centers can approach the COVID-19 outbreak to reduce risk for solid organ transplant live donors, prospective candidates and recipients. In particular, the centers can restrict access based on urgency and donor transplant can limit depending on the exposure risk. COVID-19 transplant centers can change the evaluation and monitoring practices of solid organ transplant patients may develop treatment protocols for screening algorithm for suspected cases and confirmed cases. However, there is currently no evidence-based guide to inform these practices (8).

A cadaveric or live donor with an unidentified COVID-19 infection can also spread the virus to recipient patients. In live donor transplants, the close monitoring and isolation of the donor in quarantine during COVID-19 incubation is predicted to eliminate this risk (9, 10). In this study, it was aimed to share the pre-operative preparation and postoperative follow-up of solid organ transplant patients with live donors during the COVID-19 pandemic by the way applied in our own clinic.

Materials and Methods

Ethical Statement

Ethics committee approval was obtained from the Local Ethical Committee of Sisli Memorial Hospital. The study was conducted according to the criteria of the Declaration of Helsinki and the Declaration of İstanbul. The study was performed as a retrospective study with anonymized data analyses.

Live Donor Liver Transplantation Preparation and Planning

Between 2004-2020, 1300 liver transplants with live donors were performed at the Şişli Memorial Hospital Organ Transplantation Center. This is a clinical study that shows how to prepare staff training, recipient and donor surgery, and follow-up of outpatient clinic patients during the COVID-19 pandemic period in our center with live donor and cadaveric solid organ transplantation.

Organ Transplant Service Management Strategy

Since the beginning of COVID-19 pandemic, the process has been closely monitored by our clinic, and a protocol has been established with the opinion of the Ministry of Health on the subject (2), the Turkish Surgical Association (10) and the pandemic board views of our hospital. In the execution of this protocol, the organ transplant center manager, infectious diseases specialist and infection control nurse take over the task of control.

To train the staff in our organ transplant center, relevant information and basic methods were determined in the prevention and control of COVID-19. Each was given theoretical and practical basic training. Clinical skills such as hand hygiene, masking and the use of goggles and protective face screens were taught repeatedly online through the video.

Organ Transplant Service Management

With the decision of the Ministry of Health and the hospital pandemic board, access control management was strengthened, and visiting hours were suspended. The patients will not be accompanied in the preoperative period. In the postoperative period, an adjustment was made to accompany each patient. Basically, companions were not allowed to leave their rooms, where they had been waiting outside in certain special circumstances. In this case, trainees were given training to leave their rooms, to inform the nurses and to conduct hand hygiene and face hygiene when they return to their rooms. No visitors were allowed during the epidemic. Our clinic offers visitors remote video access to reduce emotional impact and to minimize the risk of potential infections among family members.

In our hospital, 6 beds in the intensive care unit and 20 beds in the service were reserved for organ transplant patients. During COVID-19 pandemic, patients can be presented with many symptoms, and it is reported that the majority of patients can asymptomatically progress (2). Therefore, donors and recipients who applied for live donor transplantation were accepted as COVID-19 and hospitalized in isolated rooms. Doctors and other healthcare professionals dealing with the treatment of patients acted in accordance with approach determined by the Ministry of Health (2).

Epidemiologically, travel history and virus exposure were examined and recorded. Live donors and patients were evaluated for coronavirus symptoms, and a PCR test was performed for COVID-19. Detailed blood and radiological examinations were performed to eliminate asymptomatic patients. Recognizing that the symptoms of the disease may appear within 14 days on average (2), the PCR test was repeated before surgery. The two tests, consecutively negative, and the donors were operated on, assuming that COVID-19 was not. In this process, plans were made to direct suspicious patients to the COVID-19 service.

Outdoor Patients Management

For patients who were followed-up after the transplant, the time of control was contacted through the e-doctor application of Memorial Hospital. Predicting that the COVID-19 clinic may be different in immunosuppressive patient (12), the control of patients without complaints and the need for medical support was delayed to a later date (13). Psychological support was provided to patients in need. Four patients in need of medical support were hospitalized in our service with the protocol applied to donors and recipients before the surgery, and their treatments were arranged.

Personnel Management

It was recommended for personnel to use public transport as much as possible. Everyone was guided to monitor and report health status daily, including fever, cough, shortness of breath and other abnormal conditions. If there are any signs or symptoms related to COVID-19, this information should be reported to the outbreak prevention and control team as soon as possible. The service personnel were tested and recorded their body temperature twice each day with contactless detector. Meanwhile, the detail of the transportation between the personnel's home was recorded. In addition, according to the condition of the patients in the service, flexible work planning was made for the personnel who need to work in the service in order to minimize the risk of contamination. Meanwhile, emergency arrangements have been strengthened for the transplant clinic.

Operating Room and Intensive Care Standards

Novel studies have shown that endotracheal intubation, tracheostomy, ventilation and fiber optic bronchoscopy increase the risk of SARS- and MERS-CoV transmission (13, 14). According to the "health institutions for the prevention of suspicious new coronavirus infection and control of temporary guidelines" announced by WHO (14), healthcare personnel performing tracheal intubation, tracheostomy intubation, airway lavage and bronchoscopy microscopic lavage should act with a double preventive (e.g., standards such as hand hygiene, use of personal protective equipment, respiratory health, injuries of injuries, cleaning of medical equipment and medical waste treatment). Also, suspected or approved cases of COVID-19 should undergo droplet isolation and contact isolation. Isolation is also required for air used for aerosol-producing medical operations. It consisted of a room equipped with a negative pressure in the operating room as well as interconnected rooms where only the entrance section and anesthesia induction chambers had negative pressure. A special transport ventilator was used for patients coming from the intensive care unit (10).

Patient Selection

We recommend to stop your organ transplant program before the COVID-19 is completely eliminated. Only necessary operations can be performed on suspicious or confirmed patients that may be life-threatening or significantly affect prognosis. In the 6 transplants we performed during this period, the mean PELD score of one pediatric patient was 24.2, and the average of MELD score of other adult patients was 19.2. Considering the etiology of the disease, patients with diseases such as budd-chiari, hbv+hcc, hbv+primary sclerosing cholangitis and idiopathic decompensated liver failure with esophageal variceal hemorrhage were included in the transplant program during the COVID-19 pandemic period.

For patients who need liver transplantation and donors, firstly, routine blood tests at the outpatient clinic, chest direct radiography or CT scan as well as CRP, hemogram and COVID-19 PCR examinations were performed if necessary. Recipients and donors who did not have COVID-19 suspicion were insulated with 14 days of hospitalization. In 14 days, donors and recipients who had a negative radiological, clinical, and repeat PCR tests underwent operations. Infection specialists should be consulted for the treatment plan of patients suspected or confirmed in the outpatient clinic examination and, if necessary, transferred to the infection department for further treatment.

Isolated patients and donors tend to be alone with fear, anxiety, anger, sleep disturbances and other problems. Therefore, it is necessary to psychologically evaluate the patients correctly. To patient and donor, necessary psychological support should be given according to emotional reactions and behavioral changes. Health personnel should also provide patients with more emotional support and accurate information to reduce mental problems.

Results

After 1 March 2020, five live donor liver transplants were performed in our clinic. A total of 8 patients are in our service and in the intensive care. Two patients who underwent live donor liver transplantation stay in the intensive care unit, and 2 patients with Post-op 2 donor and transplant preparation. One patient was discharged on the post-op 2nd month, one patient post-op on the 45th day, the other patient did not complete the

post-op 30th day and outpatient follow-ups are being performed. COVID-19 infection was not detected in patients who underwent live donor solid organ transplantation and were followed up in our service.

It is important to have an arrangement to prevent and control the transmission of COVID-19 and to manage the organ transplant clinic during this period. A method that can protect both hospital staff and medical team and patients should be determined. Liver transplantation with live donors increases donor risk; on the other hand, it also provides important advantages such as planning the operation time, insulating the transmitter and providing sufficient and repeated tests. We think that we performed liver transplantation in the safest possible environment by following the guidelines adopted in our clinic.

Discussion

The potential COVID-19 outbreak after the flu epidemic of 1918 represents the largest global public health crisis of this generation. The speed and volume of clinical trials initiated to investigate potential treatments for COVID-19 emphasize both the need for production and its ability. No treatment has been shown to be effective to date (11).

During the COVID-19 pandemic, a significant decrease in all elective surgical procedure activity was detected in our country. In addition, successful and evidence-based treatment paradigms have been created as well as the solid organ transplant Covidien-19 diagnostic algorithm, which are likely to improve the reduction in the number of transplants. Due to the lack of consistent and highly reliable testing practices or treatment mechanisms, COVID-19 pandemic should be considered a major threat to solid organ transplant programs. In the general population, although the reported case fatality rate is low, most fatal cases are patients with advanced age or those with one ore more underlying medical co-morbidities. Therefore, careful monitoring of the high-risk population is required. Although it is thought that patients with solid organ transplantation and using immuno suppressants in the first place will be in this risky group, the number of reported cases is limited (12). We think this may be because patients know how to isolate themselves because of the immunosuppressants they use.

In countries where cadaveric donor use is predominant, patients waiting for the cadaveric organ list require high MELD scores. They also suffer from poor clinical availability and transplant when an appropriate organ occurs. Considering the mortality of liver diseases and the appropriate organ, transplantation seems to be a risk that can be taken even in the time of the pandemic. In contrast, in countries where live donor liver transplantation is intense, it also brings the risk of getting infected in a donor who has undergone major surgery as well as the risk of becoming infected. In liver transplantation with live donors, where donor safety is paramount, conducting patient selection in the pandemic environment will decrease both morbidity and mortality from donor and recipient.

Conclusion

In addition to all these risks, the superiority of solid organ transplant with live donors emerged during COVID-19 pandemic period. Removal of organs in cadaveric transplantation and limited time for transplantation may increase the risk of infectious disease and transplantation to the infected recipient, whereas in live donor transplantation, the recipient and donor have the advantage of

being isolated during the incubation of the disease. With the protocol we apply, we see that solid organ transplantation with live donors can be performed safely during the pandemic period.

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CASE REPORT OPEN ACCESS

Acute Nonsuppurative Sialadenitis After Contrast Material Administration For Computed Tomography Angiography

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Introduction: By the use of iodine-containing intravenous contrast agents, sialadenitis rarely occurs with a sudden expansion of the salivary glands. Its pathogenesis is uncertain, but it appears to be the result of an idiosyncratic reaction or accumulation of iodine in the ductal system of the salivary gland. In this article, we aimed to present a case of contrast-induced sialadenitis after an iodine-containing contrast agent.
Case Presentation: A sixty-six-year-old male with a history of the abdominal aortic aneurysm was referred for CT angiography. The patient had referred to emergency service with mildly tenderness, pain, and swelling in the bilateral submandibular region. Physical examination also revealed mildly enlarged bilateral submandibular glands, with no fever. There was no erythema, ulcer, and no symptom in both oral and oropharyngeal mucosa. The US performed bilateral diffuse homogeneous expansion of the submandibular salivary glands. In the light of clinical and radiological findings, contrast-induced non-suppurative sialadenitis was considered.
Conclusion: Non-suppurative sialadenitis is a rare adverse effect of contrast material administration, which true incidence is unknown. Chronic kidney failure and repeated dose of contrast material are risk factors for this complication. We think that this adverse effect deserves more attention, and follow-up, and prevention for recurrence because its long-term importance is unknown yet.

Keywords: Nonsuppurative sialadenitis, contrast material. computed tomography, angiography

Introduction

Owing to the use of iodine-containing intravenous contrast agents, sialadenitis rarely occurs with a sudden expansion of the salivary glands. Contrast-induced sialadenitis (iodide mumps) was first described in 1956. The actual incidence is unknown, and fifty-two cases have been reported until 2015. Its pathogenesis is uncertain, but it appears to be the result of an idiosyncratic reaction or accumulation of iodine in the ductal system of the salivary gland (1, 2). In this article, we aimed to present a case of contrast-induced sialadenitis after an iodine-containing contrast agent.

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Case Presentation

A sixty-six-year-old male with a history of the abdominal aortic aneurysm was referred for Computed Tomography (CT) angiography. The patient had long-term arterial hypertension and chronic renal failure (stage 2 chronic kidney disease). Before this administration, he had a contrast-enhancement CT evaluation twice within 5 years. He had no history of allergy before. CT angiography of thoracic and abdominal aorta was performed by 90cc Iopromide (Ultravist300,ScheringAG, Germany), a non-ionic low-osmolar contrast material containing about 300mg iodine/ml. No adverse effects were experienced during administration and the stay at the radiology department. Around 8-10 hours after administration, the patient had referred to emergency service with mildly tenderness, pain, and swelling in the bilateral submandibular region. Physical examination also revealed mildly enlarged bilateral submandibular glands, with no fever. There was no erythema, ulcer, and no symptom in both the oral and oropharyngeal mucosa. He had a regular heart rate of 80bpm and blood pressure of 140/80 mmHg. There was no feature on the other system's examinations. On the laboratory tests, there was no feature, the patient had no leukocytosis, and his C-reactive protein was in a normal range. Ultrasonograph y(US) was preferred to evaluate submandibular glands. It performed bilateral diffuse homo geneous expansion of the submandibular salivary glands (Figure 1). There was no abscess and sialolithiasis. Bilateral level 1 (nearly sub mandibular gland) lymph nodes of the cervical region were normal. Doppler US performed a mildly increase in central vascularisation. The parotid glands were normal, and no sialolithiasis was seen. In the light of clinical and radiological findings, contrast-induced non suppurative sialadenitis was considered.

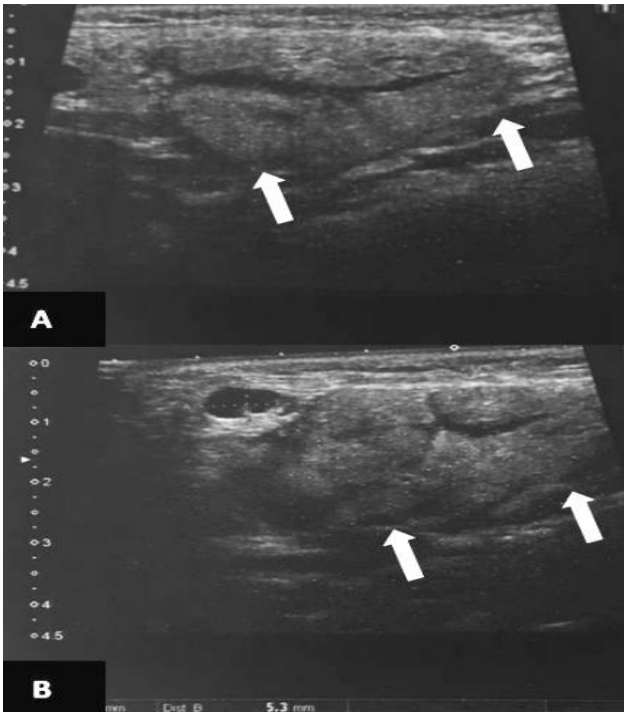

Figure 1. Ultrasonography shows widespread edema, and intraglandular hypoechoic tubular structures in bilateral submandibular glands (A: right submandibular gland; B: left submandibular gland) (white arrows). Accompanying sialolith structures are not seen.

Then, the patient was treated conservatively, with analgesic/anti-inflammatory drugs. There was complete resolution of submandibular glands swelling in 3days after administration. On follow-up, sialadenitis was not repeated (Figure 2). A written patient consent is present.

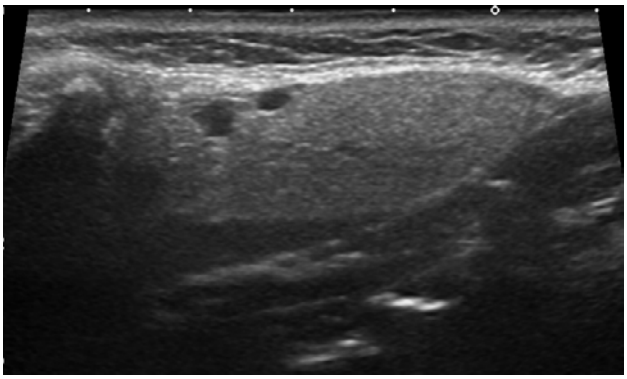

Figure 2. The submandibular gland is seen normally in the ultrasonographic examination after treatment

Discussion

Adverse reactions to intravascular contrast material are not frequent. Recent estimates of adverse reactions to iodinated contrast material range from 1 to 12%, with severe reactions comprising only 0,01 to 0,2% of total reactions. Katayama et al. (3) reported 337,647 cases of adverse reactions to ionic and nonionic contrast material are about 12% and 3%, for all that no cases of sialadenitis reported in that study. Adverse reactions are generally classified as either idiosyncratic or chemotoxic.

Idiosyncratic (anaphylactoid reactions) occur unpredictably and independently of the dose or concentration of the agent. Conversely, chemotoxic-type effects relate to dose, the molecular toxicity of each agent, and the physiologic characteristics of the contrast agents (i.e., osmolality, viscosity, hydrophilicity, calcium-binding properties, and sodium) (4).

Sialadenitis is a rare adverse effect of iodinated contrast material. The mechanism of this complication is not well known but it is believed to be caused by iodine contrast, as an idiosyncratic (i.e., anaphylactoid) reaction or due to its toxic accumulation which causes the inflammation of mucous membranes and duct obstruction due to the concentration through the sodium iodide symporter of salivary gland tissue.5 98% of iodine is excreted by the kidney and the remaining 2% by the salivary sweat and lacrimal glands. Increasing iodine concentration creates a risk of iodine accumulation in salivary glands after the administration of iodinated contrast material (6). As known, substantially of reported cases participate in chronic renal failure or repetitive exposure to iodinated contrast material. Therefore, it is supposed that chronic kidney disease (as also our patient had) could be a risk factor for the development of this complication. The incidence of this adverse effect is not known. Until now, there were many extensive studies about acute and reverse effects of contrast administration but they had no cases of contrast-induced sialadenitis (2, 3, 5, 6). McCullough et al.(7) reported that 18 of 1381 patients who had i.v. contrast administration had parotitis of which had unilateral in the most of the cases in their study. It supports that although we have few cases in the literature clinically or radiological for this adverse effect, the true incidence is more than generally assumed.The course of postcontrast sialadenitis (iodide mumps) is substantially benign. The symptoms occur within a few minutes to up to 5 days after the administration. Complete resolution always occurs, in most cases within 2 or 3 days (8).

There were no fatal reactions associated with iodide mumps that have been reported in the literature until today. Facial paralysis, pancreatic mumps-global enlargement of the pancreas-, localized erythema, enlargement of the thyroid gland and the lacrimal glands, conjunctivitis, photophobia, coryza, generalized puffiness of the face, vague abdominal pain, slight dysphagia, and mild stridor and dyspnea reported association with iodide mumps in the literature (8, 9). Our presented case had no additional symptoms with sialadenitis.

The US might help us for diagnosis; diffuse homogenous size increasing, dilate hyper echoic ducts, and increasing central vascular activity of the submandibular gland could be seen. There must be no abscess, sialolithiasis, and superlative lymphadenopathy in the US. The US also contributes to the differential diagnosis of other causes that enlarge the submandibular gland. US findings can be unilateral or bilateral. In our case, US findings

were bilateral. Increased vascularization in the central part of the gland in Doppler US indicates hyperemia in the acute stage. Cross-sectional imaging methods such as CT and MRI can also be used in diagnosis. Especially in CT, the decrease in the density of the submandibular glands indicates the presence of edema (1, 9). In the differential diagnosis, Sjogren's syndrome, sialadenosis, sarcoidosis, and other causes of infectious sialadenitis should be thought (2,5,9). Treatment is conservative, however sialadenitis complete resolution within 2 or 3 days without treatment, patients can be supported by analgesic on demand. There is no evidence of the benefits of steroid and antihistamine (1,4,5).

Conclusion

In conclusion, nonsuppurative sialadenitis is a rare adverse effect of contrast material administration, which true incidence is unknown. Although non-ionic and low-osmolar agents are more reliable than ionic and high-osmolar ones, as our case had, there were few cases reported iodide mumps after nonionic low-osmolar agents. Chronic kidney failure and repeated dose of contrast material are risk factors for this complication. We think that this adverse effect deserves more attention, and follow-up, and prevention for recurrence because its long-term importance is unknown.

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Conflicts of interest

The authors declare no conflict of interest.

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LETTER TO THE EDITOR OPEN ACCESS

Acclaimed African Immunity or Resistance to Sars-Cov-2: Explore or Ignore?

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Dear Editor,

Towards the end of the year 2019 and early 2020, the world was thrown into chaos as a result of a monster virus that is ravaging the world, called severe acute respiratory syndrome 2 (SARS-CoV-2) or Coronavirus disease 2019 (COVID-19). There is nearly no country in the world that the claws of this most dreaded coronavirus SARS-CoV-2 have not made an impact, although to a varying degree. Over 63 million infected cases and more than 1 million deaths have been recorded across the globe as of 30th November 2020 (1). On January 30, 2020, this virus was pronounced a Public Health Emergency of International Concern by the World Health Organization (WHO) and afterward pronounced pandemic on March 11th, 2020 by the same body (2).

The negative impact of this virus on the 'giant' countries of the world was alarming. Despite their well-developed health care delivery system, the influx of infected patients was overwhelming and the mortality rate was traumatic (3). As of November 30, 2020, the United States of America (USA) has recorded 13,750,608 COVID-19 infected cases with 273,077 deaths; Brazil 6,314,740 infected cases with 172,848 deaths; India 9,432,875 infected cases with 137,177 deaths and United Kingdom (UK) 1,617,327 infected cases with 58,245 deaths among other giant countries of the world (1). Hospitals in the USA and other developed Western countries were overwhelmed to the extent that corpses were abandoned to putrefy in homes and by the roadsides (4).

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COVID-19 in Africa

As the rates of infection and mortality become geometric especially in the developed countries, series of predictions were made that Africa, a continent with many developing countries will suffer the most from the damaging effect of the COVID-19 pandemic (4). These predictions were based on dilapidated health care delivery system and the high poverty level that are endemic in the region (3,4). Surprisingly, Africans seem to have watered down these predictions as the claws of the virus seem to have spared them as compared with other continents (4,5). While countries like the USA, Brazil, India are recording millions of infected cases and the death toll in hundreds of thousands, the whole African continent with about 54 countries have only recorded 2,177,263 infected cases and 51,831 deaths as of mid-November 2020 (1). The question is, are Africans truly immune or resistant to SARS-CoV-2 as acclaimed?

Acclaimed Immunity by Africans

One of the reasons for the acclaimed Africans immunity to COVID-19 is as a result of the low reported cases of infection and mortality. This is coupled with several myths and misinformation concerning the COVID-19 pandemic in Africa which have made most Africans negligent on COVID-19 safety measures and a contributory factor to the increase in the infection rate. Besides, the corruption surrounding COVID 19 in developing countries of Africa makes an average citizen believe COVID-19 is a 'rich or white man's disease' and the figures of reported cases were jacked up for the government's selfish gain from the international organization. Notwithstanding, the continent's acclaimed immunity should not be dismissed without solid scientific proof.

First, the testing capacity for COVID-19 by African countries must be evaluated. This is one of the claims by the international communities as it is visible to the blind that African countries' testing capacity for COVID-19 is too low when compared with other continents. As of 16th November 2020, an African country like Nigeria with a population of over 200 million had tested just 756,237 of its population (less than 1%) (1). Egypt, where the index case in Africa was detected, had tested just one million out of the over 100 million population (about 1%). South Africa is the country with the highest record of testing as it has conducted over 5 million tests out of about 60 million population (close to 10%) (1). Combining these records in the three African countries mentioned, their testing capacity is less than 4% of the COVID-19 tests conducted in the USA alone with a record of over 190 million tests out of about 331 million population (about 50%) (1). Until the testing capacity of African countries is satisfactory enough, the true state of Africans as regards SAR-CoV-2 infection cannot be ascertained. Nonetheless, according to the WHO, the period for SARS-CoV-2 incubation is on an average of 5-6 days but might be up to 14 days. Therefore, if truly the testing capacity in Africa is below par and the period for SARS-CoV-2 incubation is between 1-14, then, dead bodies should have littered the streets of Africa as predicted or there should have been a surge in hospitalization as seen in the USA (4). Besides, the index case of the COVID-19 patient in Africa was recorded on 14th February 2020 in Egypt; Nine months down the line, the COVID-19 infection, and the death rate are still very low in Africa when compared with other continents. Hence the source of the acclaimed immunity or resistance to SARS-CoV-2 is worth exploring.

Moreover, before the roll-out of some vaccines for SARS-CoV-2, one of the recommended drugs by the US Food and Drug Administration (USFDA) and some researchers in treating people with COVID-19 is chloroquine (CQ) and hydroxyl chloroquine (HCQ) (5). Although the World Health Organization has kicked against this that the drug causes other heart-related problems and USFDA also revoked its earlier directive on the usage of CQ and HCQ as research is ongoing (5,6). Notwithstanding, Prodromos and Rumschlag argued in their recent research that early administration of HCQ seems to be consistently effective for the treatment of SARS-CoV-2 (7). Prodromos et.al also supported their argument with the fact that HCQ is protective to the heart by reducing cardiac mortality. It helps to reduce thrombosis and cholesterol in the case studied (8).

Going down memory lane, chloroquine is a very familiar drug in Africa. This drug has been used in treating malaria disease caused by mosquitoes such as Plasmodium falciparum that is endemic in the region for decades (5). Malaria is an age-long familiar or common sickness in Africa but might be life-threatened in some climes. Could the age-long usage of Chloroquine have provided a form of immunity or resistance for Africans? This needed to be explored as well. Kewel and Tanuj (5) reinforced their argument that the carrier of sickle cell hemoglobin, which is an inherited pair of a mutated hemoglobin cell (homozygous, HbSS) is also resistant against Plasmodium falciparum malaria (5). This type of sickle hemoglobin is rare in the white race but peculiar in Africans. Exploring the relationship between chloroquine, malaria disease, sickle cell resistance, and SARS-CoV-2 might give a better insight into the low frequency of SARS-Cov-2 in Africa.

Furthermore, research also revealed that Vitamin D is among the supplement used in treating SARS-CoV-2 patients (9). According to a case study, over 80% of the 200 COVID -19 infected patients studied had vitamin D deficiency leading to an increase in blood inflammatory markers (9). Vitamin D helps in adjusting the immune system by suppressing dangerous cytokine production. During the 1918-1919 influenza pandemic, the death rate seems to have been lowered as a result of sunlight and vitamin D (9). Could it be that exposure of Africans to sunlight might have given them some shots of Vitamin D when compared with cold continents where the coronavirus death toll is alarming?

As a result of the dilapidated healthcare delivery system in Africa, Africans have developed other alternatives to care for themselves, especially through the use of traditional medicine. Self-medicating with traditional medicine may not necessarily be an accurate cure for the particular disease but might have helped in boosting the immune system. Fortification of the immune system through the daily routine of traditional medicine could also help to fight major infections as minor. This has been an African heritage, although the scientific community has undermined their traditional medicine as the correct dosage measurement is poorly developed.

Conclusion

As some Western countries are planning to go on a second lockdown owned to the second wave of SARS-CoV-2, African countries are yet to record a quarter of what was experienced in the first wave by some states in the Western countries. Some scientists have even argued that the strain of the coronavirus in Africa is weaker and less lethal strains without concrete

proof. Understanding Africans genetic make-up, history of past epidemics and pandemics, alternative or traditional medicine, and weather conditions could be of great help towards managing and producing a more effective SARS-CoV-2 vaccine. Unless proven otherwise, the self-acclaimed immunity or resistance to SARS-CoV-2 by Africans should be explored and not ignored. Researching into every issue raised here will go a long way in combating the COVID-19 pandemic globally. COVID-19 is real and might have come to stay pending when the vaccines will be readily available and accessible globally. Personal hygiene and other safety protocols must be taken with high consciousness as we navigate through the COVID-19 pandemic.

Disclosure of interest

The author declares no competing interest.

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