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Articles

AIMS

EURAS Journal of Health (EJOH) is a peer-reviewed international scientific open access periodical published in accordance with independent, unbiased, and double-blind peer-review principles. It publishes two issues per year. The publication language of the journal is English. The journal is the official publication of the Eurasian Universities Union (EURAS).

EJOH aims to contribute to the literature by publishing manuscripts of highest scientific level in all fields of health including medicine with clinical and basic fields, nursing, physiotherapy, audiology, nutrition and dietetics, dentistry, public health, epidemiology, and all relevant disciplines.

SCOPE

The journal targets all healthcare professionals in all health disciplines and publishes original experimental and clinical research articles, case reports, reviews of experts in a specific field, letters to the editors and brief reports on new methods or techniques or preliminary results of original studies. The journal conforms to the Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice).

From The Editor

Welcome to the EURAS Journal of Health (EJOH)!

From the beginning, we underlined the multidiciplinary frame of our journal in the health field and this current issue presents a good example of our intention. Thanks to all our authors, reviewers and editors.

We continue to invite and welcome works from our international colleagues and look forward to the future submissions.

Thanks for joining us.

With best regards,
Prof. Zeynep Çiğdem KAYACAN, M.D.

Determination of Obesity Tendency in Individuals During COVID-19 Pandemic: An Observational Study in Istanbul, Turkey

Beyza TAĞRAF[1], Indrani KALKAN[1 *], Gülgün ERSOY[1]

Abstract

Objective: Lockdown-period and confinement at home during COVID-19 pandemic is suggested to have changed the eating habits and physical activity in adults globally.

Materials and Methods: This study was conducted to determine obesity tendency in adults, during COVID-19 pandemic, by examining the changes in anthropometric measurements, eating habits (meal numbers/day), and physical activity as per statements provided by them through online questionnaire method inquiring anthropometric measurements, eating habits and physical activity before and during the pandemic. SPSS (Statistical Package for Social Sciences for Windows) version 21.1 was used for statistical analyses. Descriptive statistics as frequency (n), mean ($\overline{X}$), standard deviation (SD), percentages (%) were calculated. Fisher's chi-square test was conducted to determine the difference between groups (95% confidence limit; $p<0.05$).

Results: Seven hundred and twenty-five (725) individuals (414 females, 311 males) aged between 18-65 years ($\overline{X}$=37±11.8) participated in the tof body mass index (BMI), there was an increase in obese and pre-obese categories during COVID-19 pandemic ($p<0.05$). On determining metabolic risk based on WHO waist circumference (WC) criteria, a significant increase in the high-risk category was observed during pandemic period ($p<0.05$).

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Conclusion: The study indicated that individuals were prone to obesity during COVID-19 pandemic due to changes in eating behaviors (meal numbers/day), physical activity and were at a risk from general health perspectives as well as pre-disposition to COVID-19. Nutritional counseling should be given to individuals by dietitians or other health professionals during pandemic periods, in order to decrease death rates, enable quick discharge from hospitals and increase health recovery rates.

Keywords: COVID-19, Coronavirus, Obesity, Pandemic.

Introduction

The Coronavirus Disease 2019 (COVID-19) caused by Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) first appeared in Wuhan Hubei province China in December 2019 and since then has spread rapidly all over the world (1). As of March 2022, the global outbreak, has infected approximately 469 million and resulted in the death of more than 6 million individuals, (2) whose main clinical symptoms include fever, dry cough, fatigue, muscle pain, shortness of breath, respiratory tract infection and gastro-intestinal symptoms as vomiting and diarrhea.

Obesity characterized by abnormal or excessive fat accumulation has been defined by World Health Organization (WHO) in terms of BMI value of 30 kg/m^2 or more. Obesity is considered as an increasing global epidemic and increases the risk of chronic diseases as well as leads to the development of certain respiratory diseases. Studies have shown that obesity or being overweight adversely affect outcomes of COVID-19 infection and the potential mechanism has been suggested as chronic inflammation, comorbid complications, immune dysregulation, endocrine dysfunction, respiratory compromises and so on (3-5). Studies indicate that with the increase in BMI, severity of COVID-19 diseased condition worsened and there were complications in treatment as prolonged stay in intensive care unit, being intubated or connected to mechanical ventilation for a longer period. Therefore, obese individuals may be considered as a vulnerable and risk group for COVID-19 disease. Since chronic diseases as diabetes, hypertension, cardiovascular heart diseases, certain cancers are related to obesity and such diseases would be a risk factor in COVID-19 patients; it is necessary to control obesity in order to alleviate the course of the disease and ease the treatment period (4-6).

The quarantine period in the COVID-19 pandemic caused social restrictions and significantly affected the nutritional habits, diet patterns and physical activity level of individuals in general (7). Psychological factors such as depression, fear and stress during the pandemic period resulted in an increase in the desire to consume fast food, refined sugar and sugary drinks for hedonic reasons (7, 8). Decreased physical activity during quarantine was reported to cause positive energy balance leading to unwanted body weight gains and obesity during this period (9).

Understanding the changes in life style and obesity during the pandemic period would be helpful in controlling COVID-19 and increasing awareness among individuals. This study was conducted to examine the changes in BMI and waist circumference, which is also an important indicator of abdominal obesity of adults and to evaluate the tendency of obesity during the COVID-19 pandemic period.

Materials and Methods

This cross-sectional study was conducted in Istanbul between May-June 2021 on a total of 725 Turkish adults aged between 18-65 years. Considering the adult population in the city, sample size was calculated by means of G-power analysis (95% power, effect size 0.1 and α=0.05) and minimum sample size was determined to be 385. However, all participants who volunteered to participate in the study were included. Individuals who did not qualify the defined range of age, who had eating disorders, had clinical cachexic conditions as cancer, or had dementia were excluded from the scope of the study. Ethical approval was obtained from Istanbul Medipol University Ethics Committee dated 25.05.2021 with approval number E-10840098-772.02-2388.

An online questionnaire comprising of questions related to anthropometric measurements (height, weight, waist circumference), eating habits and physical activity of participants before and during the pandemic was applied to the participants. BMI was calculated from the height and weight data provided by the participants (weight in kg/height in m^2). The participants were given instructions by means of you a tube video clip tutorial regarding the measurement of waist circumference (waist circumference measured while standing and placing the measuring tape around the waist

just above the hipbone) (10). The height and waist circumference were recorded in cm and weight in kg. BMI and metabolic risk classification categories based on waist circumference was based on WHO classification. WHO defines the metabolic risk category based on waist circumference as <94 cm as low risk, 94-102 cm as moderate risk and >102 cm as high risk. For females, waist circumference of <80 cm is considered as low risk, 80-88 cm as moderate and >88 cm as high risk (11).

SPSS (Statistical Package for Social Sciences for Windows) version 21.1 was used for statistical analyses of the data obtained. Descriptive statistics as frequency (n), mean ($\overline{X}$), standard deviation (SD), percentages (%) upper lower limits were calculated. Fisher's chi-square test was conducted to determine the difference between groups. The results were evaluated at 95% confidence interval, at the $p<0.05$ significance level.

Results

Seven hundred and twenty-five (725) adult Turkish individuals who voluntarily agreed to participate in the online questionnaire were included in the study. The participants were between 18-65 years of age and 57.1% (n= 414) were females and 42.9% (n= 311) were males. Of the participants, 65.4% were between 31-60 years, 31.4% were between 18-30 years, 3.2% were between 61-65 years. The mean ($\overline{X}$) age of the participants was 37±11.8 years.

Regarding medical history of the participants, majority (75.9%) stated that they did not have any disease, while 24.1% had at least one chronic disease but under control (diabetes, hypertension, cardiovascular diseases and/or others).

Regarding pre-pandemic BMI values, as per WHO categorization, 43.2% of the participants were normal, 35.9% were pre-obese, 13.2% were obese, and 0.3% were super-obese. The pre-pandemic BMI average value of the participants was 24.7 kg/m^2 (not shown in the tables). However, during pandemic (date of survey) the mean BMI value had risen to 25.14 kg/m^2 (not shown in the tables) which was statistically significant ($p<0.05$).

Table 1. Demographic and anthropometric characteristics of participants during COVID-19 pre-pandemic and pandemic period

	Frequency (n)	Percentage (%)
Gender		
Female	414	57.1
Male	311	42.9
Age (years)		
18-30	228	31.4
31-60	474	65.4
61+	23	3.2
BMI Category (Pre-pandemic)		
Underweight	54	7.4
Normal	313	43.2
Pre-obese	260	35.9
Obese	96	13.2
Super obese	2	0,3
BMI Category (During pandemic)		
Underweight	46	6.3
Normal	293	40.4
Pre-obese	282	38.9
Obese	100	13.8
Super obese	4	0.6
WC Category (Pre-pandemic)		
Normal (no risk)	439	60.5
Risk (moderate)	131	18.1
High Risk	155	21.4
WC Category (During pandemic)		
Normal (no risk)	424	58.5
Risk (moderate)	124	17.1
High Risk	177	24.4

On evaluating the change on BMI categories during COVID-19 pandemic, a fall in numbers was noted in underweight and normal category whereas an increase in numbers were noted in all the obese (pre-obese, obese, super-obese) categories (Table 1). Regarding risk categories based on waist circumference; during COVID-19 pandemic, there was a decrease in number of individuals in no risk category (n=15) and moderate risk category (n=07) and an increase in the number of individuals in the high-risk category by 3% (n=22) which was found to be significant ($p<0.05$). (Table 1)

Table 2. Other life style parameters of participants during COVID-19 pandemic

	Frequency (n)	**Percentage (%)**
Meal Numbers/day		
Decreased	105	14.5
No change	402	55.4
Increased	218	30.1
Chronic Diseases		
None	550	75.9
Hypertension	34	4.7
Diabetes	16	2.2
Hypercholesterolemia	6	0.8
PCOS	6	0.8
Non-alcoholic fatty liver disease	3	0.4
Other	110	15.2
Physical activity (During pandemic)		
Decreased	506	69.8
No change	146	20.1
Increased	73	10.1
Sleeping duration (During pandemic)		
0-6 hours	152	21.0
6-9 hours	438	60.4
9-12 hours	0	16.7
12 hours+	14	1.9

While 69.8% of the participants stated that their physical activity level decreased, 20.1% stated that their physical activity status did not change, and 10.1% stated that their physical activity status increased during the pandemic process. Although the sleeping duration for majority of the individuals (60.4%) was between 6-9 hours; however, 21% reported to have slept for less than 6 hours during pandemic period (not shown in the table).

Of the participants, 14.5% stated that the number of consumed meals in a day decreased, 55.4% stated no change, and 30.1% reported that the number of consumed meals in a day increased during the pandemic process (Table 2).

In Table 3, waist circumference was evaluated for both genders separately for pre-pandemic period as per statements provided by the participants.

Table 3. Waist circumference according to gender (pre-pandemic period)

	Category			
Gender n (%)	**Normal (No Risk) n (%)**	**Moderate Risk n (%)**	**High Risk n (%)**	**Total n (%)**
Female 414 (100)	271 (65.4)	55 (13.3)	88 (21.3)	414 (100)
Male 311 (100)	168 (54.0) *	76 (24.4) **	67 (21.5) **	311 (100)

* Chi square test, significantly lower $p<0.05$

** Chi square test, significantly higher $p<0.05$

When metabolic risk in this study group was evaluated according to waist circumference and gender in pre-pandemic period; 65.4% of females were found to have no risk, 13.3% were at moderate risk and 21.3% were at high risk. Among males, 54.0% were at no risk, 24.4%, were at moderate and 21.5%. were at high-risk group. As per chi-square analysis, it was observed that during pre-pandemic period, males had a significantly higher metabolic risk compared to females in terms of waist circumference ($p<0.05$). (Table 3)

Table 4. Waist circumference according to gender (during pandemic period)

	Category			
Gender n (%)	**Normal (No Risk) n (%)**	**Moderate Risk n (%)**	**High Risk n (%)**	**Total n (%)**
Female 414 (100)	268 (64.7)	51.0 (12.3)	95 (22.9)	414 (100)
Male 311 (100)	156 (50.1) *	73 (23.4) **	82 (26.5) **	311 (100)

* Chi square test, significantly lower $p<0.05$

** Chi square test, significantly higher $p<0.05$

When metabolic risk was evaluated according to waist circumference and gender during pandemic period; 64.7% of females were found to have no risk, 12.3% were at moderate risk and 22.9% were at high risk. Among males, 50.1% were at no risk, 23.4%, were at moderate and 26.5%. were at high-risk group. As per chi-square analysis, during pandemic period, males had a significantly higher metabolic risk compared to females in terms of waist circumference ($p<0.05$) (Table 4).

In both genders, there was an increase in the number of individuals in the high-risk group during COVID -19 pandemic; the increase was more in case of males (Table 3 and 4).

Discussion

This study indicates a weight gain among the participants during COVID-19 pandemic, evaluated as an increase in the body mass index. Based on the provided statements, in 30.1% of participants there was an increase in meal frequency. On the other hand, 69.8% of the participants stated that their physical activity level decreased (Table 2). In a study performed on 727

adults in USA, 40% of participants stated to have gained weight post-lock-down period and change in BMI was found to be significant ($p<0.01$). The participants stated that they engaged in much less physical activity and craved snacks and ultra-processed food items (12). When inquired about their sleeping duration, approximately 21% stated that they slept less than 6 hours which is below the recommendations for adult individuals, by American Academy of Sleep Medicine (AASM) and Sleep Research Society (SLS) (13). It may be suggested that factors such as lack of physical activity, increased frequency of meals, increased sleeping hours may lead to weight gain and obesity during lock-down period. Increased sleep time may be related to increased stress and poor quality of sleep-in individuals. In a study conducted on 1959 adults in Poland during COVID-19 lockdown period, 57% of the participants reported moderate and 29% high stress levels respectively. Moreover, 64% of them reported poor quality of sleep. It was found that increased physical activity improved the quality of sleep in the participants (14). Stress, anxiety and poor-quality sleep have also been related to weight gain and obesity due to uncontrolled and emotional eating attitudes, consumption of highly processed sugary foods and beverages (15).

Several studies indicate that an obesity state promotes chronic inflammation, vitamin D deficiency, hinders immunity and causes mechanical lung compression. These increase susceptibilities to COVID-19 infection, complications including the requirement of invasive ventilation. Existing co-morbidities enhances these complications (16-18). In this study, less than 25% of individuals stated to have been diagnosed with at least one disease and few stated to have been diagnosed with chronic diseases as hypertension, diabetes, cardio-vascular heart diseases. Globally, approximately one in three of all adults suffer from multiple chronic conditions (MCCs) with prevalence rates from 16% to 58% in UK, 26% in US and 9.4% in urban south Asians (19). Some of the major chronic diseases have been related to obesity and therefore indirectly related to eating habits, physical activity, stress and sleep (20).

COVID-19 pandemic situation poses a risk for individuals with chronic diseases and puts them into the higher risk category for hospitalization and mortality and irrespective of positive or negative alterations in their

anthropometric measurements, their nutrition and physical activity status should be followed professionally.

In this study, the BMI values of the individuals were found to increase significantly during COVID-19 lockdown period ($p<0.05$). On comparing the waist circumference risk categories, the male participants were significantly at a higher risk as compared to females during pre-pandemic as well as pandemic periods ($p<0.05$). There were more individuals in the high-risk category based on waist circumferences in both genders (indicated in the form of metabolic risk categories) during pandemic period (Table 3 and 4).

In a study conducted on 14,382 adult males and 11,484 adult females (19-75 years), waist circumference was found to be related to age and after the age of 40-45 years, approximately 34.9% of females and 51.1% of males were at moderate and high risk based on WHO waist circumference metabolic risk category classification (21). Prevalence of Obesity in Turkey is approximately 32%, and obesity related chronic metabolic diseases as hypertension, type 2 diabetes mellitus, cardiovascular heart diseases, non-alcoholic fatty liver diseases have shown 2-3-fold increase in the last 15-20 years (22). COVID-19 pandemic has increased the prevalence of obesity related health risks in the world including Turkey.

Limitations and Drawbacks of the Study

This study has certain limitations. Data regarding anthropometric measurements, meal frequency, physical activity, sleeping habits before and during pandemic, of the participants were based on statements provided by them. Meal frequency is not sufficient to understand the food habits of participants during pandemic. Food frequency chart or food consumption records would have been useful; however, these could not be applied due to the online questionnaire conducted on participants from variable spheres of the society with different educational levels.

Conclusion

The results of this study indicated that COVID-19 lockdown period resulted in changes in eating habits, physical activity and sleeping hours leading to weight gain and predisposition to obesity. Obesity on the other hand

increases vulnerability and risk of chronic diseases as well as COVID-19 incidences with severe outcomes as hospitalization, ventilation, slow recovery and increased mortality. In line with all this information, nutritional status should not be ignored during pandemic periods, it is necessary to provide dietitian support for risky groups if necessary. Finally, it is recommended that nutritional follow-up of individuals be done on a routine basis and public awareness be created in this aspect.

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Anti-proliferative Effect of Niaouli and Elemi Essential Oils on MDA-MB-231 Breast Cancer Cell Line

Ayşe AK [1, *], Ebru ÖNEM [2], Ahu SOYOCAK [3]

Abstract

Objective: Recently, there has been an increasing number of studies investigating the role of essential oils in cancer treatment. In our study, we aimed to investigate the cytotoxicity of Niaouli and Elemi essential oils against human breast cancer MDA-MB-231 cells.

Material and Methods: The breast cancer cell line MDA-MB-231 was used and cells were cultured in Dulbecco's modified eagle medium (DMEM) in an incubator with 37°C and 5% CO_2. Cells were treated with essential oil at different concentrations (100, 210, 430 and 650 µg/mL for Niaouli and 105, 210, 420 and 630 µg/mL for Elemi) for 24 hours. After that, cytotoxicity was determined by the MTT test.

Results: In our study results showed that Niaouli and Elemi essential oils had cytotoxic activity for the breast cancer cells line. For Niaouli and Elemi essential oils, 50% inhibition concentration (IC_{50}) values were determined as 280±1.29 µg/mL and 214±1.01 µg/mL, respectively.

Conclusion: As a conclusion, it is thought that there is a need for research using molecular analyzes to discover which molecular signaling pathways are involved and *in vivo* studies to support these activities.

Keywords: Niaouli, Elemi, Essential oils, Breast cancer, Cytotoxicity, MTT

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Introduction

Breast cancer is among the most common causes of death in the world, and the need to support conventional treatment and develop new agents for treatment continues (1,2). To date, we know 3000 essential oils, but only 300 of them are consumed as pharmaceuticals, cosmetics, and food (3). The fact that different essential oils obtained from aromatic medicinal plants have many bioactive makes them among the candidates that can be used in the treatment of breast cancer (2). Studies about plants have demonstrated that essential oils have better activities than intact plants (4). Studies have reported that essential oils have antioxidant, antimicrobial, antifungal, antiviral, and anticancer properties (2,5–7). Phytochemical compounds in essential oils lead to protective and cytotoxic effects against diseases (2,7,8). These compounds have been shown to increase the effectiveness of commonly used chemotherapy drugs (7). Niaouli essential oil is extracted from *Melaleuca viridiflora* which is a plant in the myrtle family Myrtaceae and elemi essential oils extracted from *Canarium luzonicum* plant have industrial importance and are used for medicinal purposes (9–11). 3-(4,5-dimethylthiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay is the most common method used to determine whether an active substance has anti-cancer properties. This method is a colorimetric method and gives results of cell viability according to the color change (12). In this study, it was investigated of Niaouli and Elemi essential oils' cytotoxic activities in MDA-MB-231 breast cancer cells.t

Materials and methods

Cell culture

The breast cancer cell line MDA-MB-231 (The American Type Culture Collection (ATCC) was used and the cells delivered as a cold chain were thawed and grown in a cell culture medium. Cells were grown using a medium containing Dulbecco's modified eagle medium (DMEM) (Capricorn Scientific), 10% heat-inactivated fetal bovine serum (FBS) (Sigma Aldrich), and 1% penicillin-streptomycin antibiotic solution (Sigma Aldrich). $7x10^3$ cells per well were seeded in 96-well plates to apply essential oils and form experimental groups. 24 hours after cell cultivation, control and essential oil groups were exchanged with normal medium and medium

containing essential oil at different concentrations (100, 210, 430, and 650 µg/mL of Niaouli and 105, 210, 420, and 630 µg/mL of Elemi). Essential oils were dissolved in %0,5 DMSO. To evaluate the acute effect of essential oils, cells were incubated for 24 hours in an incubator (37°C, 5% CO_2). At the end of the experiment, cell viability/cytotoxicity was determined based on the mitochondrial activity by the MTT test. DMEM, FBS, and penicillin-streptomycin antibiotic solution (Wisent-Canada), MTT, (Sigma-Aldrich, Germany), and essential oils used in the experiments were commercially available.

Cell viability/cytotoxicity analysis

The tetrazolium ring of MTT (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide), a tetrazolium salt, is cleaved by the succinate-dehydrogenase enzyme in the mitochondria of living cells, resulting in water-insoluble formazan salts. As cell proliferation increases, the absorbance value increases due to the formation of formazan salt. Cell viability/cytotoxicity depending on mitochondrial activity is determined by this method. In our study, mitochondrial activities of breast cancer cells treated with essential oils were evaluated with this method. At the end of the experiment, the medium was removed from the cells in the 96-well plates incubated with essential oils. Then, 100 µL of fresh medium and 10 µL (5 mg/mL) of MTT solution were added to the wells and incubated for 3 hours. At the end of the incubation period, 100 µL of DMSO was added to each well and absorbance values were determined at 570 nm in an ELISA microplate reader (Multiskan GO-Thermo).

Statistical analysis

The data obtained from the study were evaluated with the IBM SPSS 21 package program. The normal distribution of the data was determined by the Shapiro-Wilk test. Comparisons between groups were made with One-way ANOVA, one of the independent sample tests. Results were given as mean ± standard deviation (mean ±SD). The statistically significant level of $p<0.05$ was accepted.

Results

In our study, the cytotoxic effect of Niaouli essential oil on MDA-MB-231 breast cancer cells was evaluated, and it was determined that all concentrations of Niaouli decreased viability significantly compared to the control group ($p<0,001$) (Figure 1). It was found that there was no statistical difference between the group treated with DMSO used as a solvent and the control group ($p=0,719$). When the groups administered Niaouli essential oil were compared with each other, there was no statistical difference between N1 and N2 ($p=0.069$), but a statistically significant difference was found between N1 and N3 ($p=0.000$) and N4 ($p=0.000$). It was determined that N3 ($p=0.008$) concentration decreased cell viability less than N2 concentration, and a statistically significant difference was observed between these two concentrations. There was no statistical difference between the N2 and N4 ($p=0.019$) groups and between the N3 and N4 ($p=0.999$) groups.

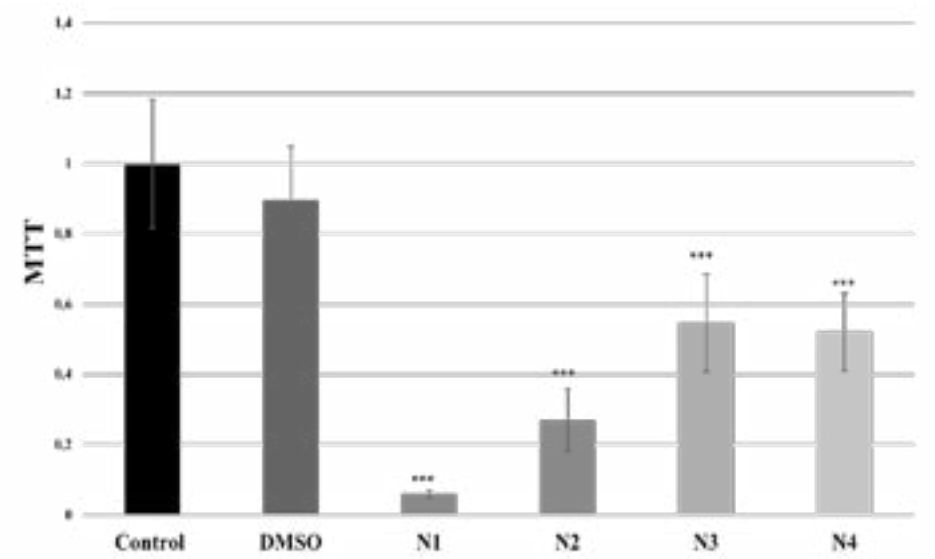

Figure 1. *The effect of Niaouli essential oil applied to MDA-MB-231 breast cancer cells. N1=650 µg/mL N2=430 µg/mL N3=210 µg/mL N4=100 µg/mL. p<0.001****

Similar to Niaouli essential oil, Elemi essential oil has been found to have cytotoxic effect on cell viability of MDA-MB-231 breast cancer (p<0,001) (Figure 1). There was no statistical difference between the group treated with DMSO as a solvent and the control group (p=0,601). When Elemi essential oil groups are compared with each other, it was found that there was no statistical difference between E1 and E2 (p=1,000) groups, and there was a statistically significant difference between E1 and E3 (p=0.000) and E4 (p=0.000) groups. Compared to the E2 group, viability decreased less in the E3 (p=0.000) and E4 (p=0.000) groups. However, it was determined that there was a statistically significant difference between the concentrations. It was found that cell viability decreased less in the E4 (p=0.000) group compared to the E3 group, and there was a statistically significant difference between the groups.

According to the cytotoxic effects of Niaouli and Elemi essential oils, 50% inhibition concentration (IC_{50}) values were calculated and determined as 280±1.29 µg/mL and 214±1.01 µg/mL, respectively.

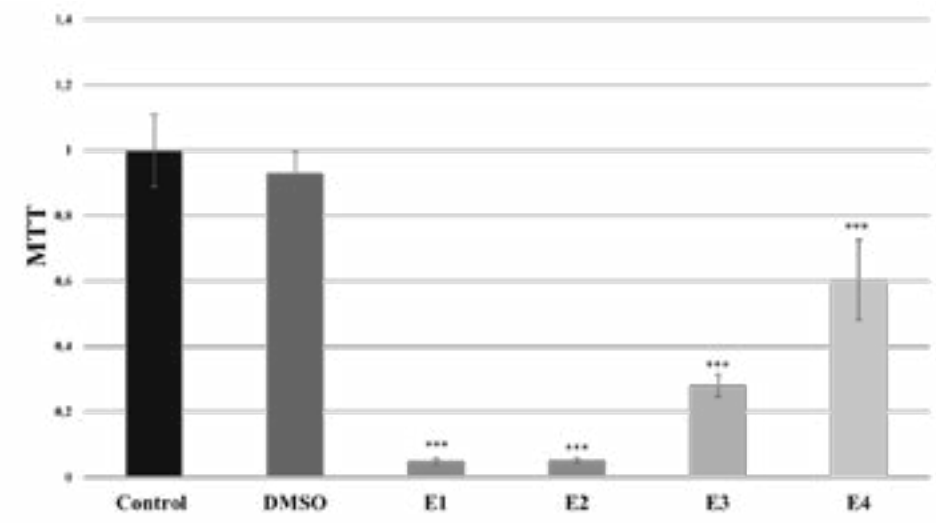

Figure 2. *The effect of Elemi essential oil applied to MDA-MB-231 breast cancer cells on proliferation. E1=630 µg/mL E2=420 µg/mL E3=210 µg/mL E4=105 µg/mL. p<0.001****

Discussion

Essential oils have been shown to have antioxidant, antibacterial, antifungal, antiviral, and anticancer effects in studies. Essential oils contain phytochemical components that have both preventive and cytotoxic effects against diseases. These compounds have been demonstrated to improve the efficacy of commonly used chemotherapy medicines (2,5–8). In comparison to synthetic medications, plant-derived products are believed to cause less adverse effects. The number of new cancer therapy options based on endemic plants is growing every day. Essential oils are lipophilic, meaning they pass the plasma membrane to affect the cell. Many studies have shown that essential oils can contribute to cancer treatment due to their anti-cancer effects (13–15). Biological activity varies depending on the way the oil is obtained, its content and usage doses. It also varies according to the cell line used (16). For example, essential oil is not cytotoxic in a normal fibroblast cell but may have cytotoxic activity in a cancerous cell line (3,16). Tea tree oil (TTO) obtained from *Melaleuca alternofiolia*, which belongs to the same genus as the plant from which Niaouli is obtained, has been studied intensively and according to the results obtained, it has been determined that it causes both digestive problems and skin irritation when used in high concentrations (17). However, it does not cause genotoxic effects in cancer cells and human lymphocytes (17,18). In our study, the concentration of Niaouli that caused the death of 50% of cells was 280 mg/mL and had a relatively higher concentration than Elemi's concentration (214 mg/mL). The dose range in which TTO caused 50% cell death of various cells (HeLa, K562, CTVR-1, Molt-4, Hep G2, HL-60, fibroblast, and epithelial cells) was determined as 20-2700 mg/mL (16). Aromatic essential oils are oils that can be applied especially on the skin by massage. In an *in vivo* study, topically applied TTO essential oil has also been reported to have antitumoral properties (3,19). The other essential oil obtained from *Canarium luzonicum* is a valuable oil that can be used for human health with its ingredients (20). It has been reported that Elemi has low cytotoxic activities in B16 melanoma cells, but can suppress melanogenesis, therefore, elemi can be used as a depigmentation agent for the cosmetic industry (21). In our study, we have found that Elemi essential oil also had cytotoxic activity for breast cancer cells.

Conclusion

Taken together, the results obtained in the present work suggest that the Niaouli and Elemi essential oils have cytotoxic effect on cell viability of breast cancer for 24h.

It may be possible to develop drugs with high efficacy and less side effects by using essential oils. However, it was concluded that there is a need for studies to determine which molecular signaling pathways affect by using molecular analyzes and to support these activities with *in vivo* studies.

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Disregarding Study Design of the Primary Studies Produces Misleading Meta-analyses

Lukman THALIB [1]

Meta-analyses use statistical methods to combine the findings from several studies that address similar research questions. The basic premise of the meta-analysis is that the combined results from a group of studies produce a more precise estimate of an effect than the individual studies when testing a hypothesis. In the era of evidence-based medicine, meta-analyses play a vital role as the findings from meta-analyses are considered to produce higher level of evidence. As such, results of some of the meta-analyses have led to major changes in clinical practice over the past decades.

The level of certainty of the pooled estimates, however, depends largely on the quality of studies that is being aggregated (1). In particular, meta-analyses have the potential to produce seriously misleading results when it is not done correctly. As the Cochrane methods group argues that "*for the meta-analyses to be reliable, appropriate attention should be given to formulating the review question; specifying eligibility criteria; identifying and selecting studies; collecting appropriate data; considering risk of bias; planning intervention comparisons; and deciding what data would be meaningful to analyse"* [2]. As such, a wrongly done meta-analyses can provide very misleading results, particularly, when the specific study designs, within-study biases, variation across studies, and reporting biases were not carefully considered (2).

In this commentary we aimed to demonstrate how meta-analyses can produce misleading findings when the design structure of the primary studies

is ignored, while extracting data for synthesis. This is an area that is not well discussed in the literature, yet it is vitally important, if we were to avoid producing misleading conclusions. We will demonstrate this with an example of a recently published meta-analysis.

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The meta-analysis in question was carried out by Pardamean et al (3) who attempted to quantify the increase in mortality after contracting COVID-19 among patients with schizophrenia. They claimed to compare the mortality experience of Schizophrenia cases with that of the general population. In their review, the authors presented what they called the solid evidence to support the idea that risk of death after COVID in patients with schizophrenia is 2.2 times higher than in the general population.

However, their conclusion is based on wrong data extraction ignoring the study design and as such their meta-analysis ended up falsely finding significant association between mortality and schizophrenia while the primary studies were showing no such associations. In other words, while the primary studies indicated non-significant or lack of association between mortality and schizophrenia, the authors attributed significant association to those non-significant studies.

This happened as the data extraction ignored the study design structure and the hypothesis that authors were testing was different to the aim and objectives of the individual studies that they synthesized. We are presenting this as an example so that the systematic review and evidence-based community becomes aware of such issues in critically evaluating meta-analytical findings for their clinical practice applications.

Pardamean et al. (3) have included 10 primary studies that were predominantly, retrospective cohort and case-control studies. The brevity of this communication will not permit us to discuss each of these 10 papers included in their review to demonstrate how most of the papers included were not possibly even eligible to explore the hypothesis that authors were testing. However, the wrong relative risk attributed to individual studies possibly the most serious. That led to incorrect pooled estimates and led the authors to wrongly conclude that there are significantly more deaths among patients with schizophrenia, compared to the general population.

To demonstrate this point, we have taken one individual study they included as an example to highlight the issue. Tyson et.al (4) is one of the papers they included in their meta-analysis. Tyson and colleagues were attempting to find the predictors for mortality among hospitalized COVID patients in general. They carried out a case-control study with 75 COVID

patients who died in-hospital and an age-gender matched group of 75 COVID patients who survived after hospitalisation. In the whole paper, only one place Schizophrenia is mentioned that was in their first baseline characteristics table, in fact, as the last item in the long list of baseline characteristics. History of schizophrenia was not statistically different in their study. Only 1 patient in the survival group and 5 patients in those who died had any history of schizophrenia. They also provided a bivariate p-value to compare all the baseline characteristics and reported a p-value of 0.096 for differences in the history of schizophrenia between those who died and survived after COVID.

As Tyson et.al (4) were looking for predictors for COVID mortality and schizophrenia was not considered in the multivariate models as they were not different at crude level. Unfortunately, in the meta-analyses of Pardamean et al. (3), a relative risk (RR) of 1.71 (along with a significant 95% confidence interval of 1.15 – 2.55) were attributed to Tyson et.al (4). How did they get such an estimate? In my view, this happened as the way data were extracted to form the 2 X 2 table to create the forest plot. Tyson et.al (4) reported 5 patients among the cases (died) and 1 in the control group (survived) to have Schizophrenia. Rather than using the correct fraction of 5/75 (cases) compared to 1/75 (control), they instead computed the risk of death among Schizophrenia as 5/6 and compared to 70/144 as non-schizophrenia controls. As though the study took 6 patients with schizophrenia as cases and compared to 144 controls, completely ignoring the age-matched case-control study of 75 cases of those who died out of COVID in-hospital compared to 75 controls who did not die after COVID! While Tyson et.al (4) found other major predictors to be significantly associated with COVID mortality at univariate and multivariate levels and schizophrenia was not a predictor at crude or multivariate levels, the meta-analysis by Pardamean et al. (3) attributed Tysen et.al a significant association between schizophrenia and mortality after COVID.

Ignoring the study design also created a serious selection bias. The meta-analysis of Pardamean et al. (3) was based on mortality experience of about 2 million COVID patients, but Schizophrenia subjects consisted of less than 1.5% of their study population, i.e., over 98.5% of the study population had no Schizophrenia. Moreover, 8 out of 10 studies had less

than 5% of people with any history of Schizophrenia, indicating a serious selection issue. This is important as they predominantly pooled retrospective cohort and case-control studies where 2,773 Schizophrenia cases were compared to 193,159 control patients. This happens as the more balanced case-control and retrospective cohort structures were ignored.

Ignoring the design structure while extracting data can happen when there is paucity of primary studies that review authors wish to evaluate. When there is lack of primary studies that focuses on the hypothesis that a researcher is interesting in exploring there is not much a systematic review can do when there are insufficient data to synthesize. Systematic reviews and meta-analyses are simply meant to summarize the best evidence related to the hypothesis that is being tested. Lack of primary studies does not permit one to resort to include inappropriate studies with inappropriately extracted data to synthesize the evidence. Evidence synthesized in such manner would not be of any use as they will not be the best evidence to answer the query at hand. Authors who are pooling observation studies should also pay attention to pooling estimates that are appropriately adjusted for potential confounders.

Given the importance of maintaining the design structure in extracting data for meta-analyses from primary studies, this commentary is meant to warn the evidence-based research community on an issue that is probably wide-spread, yet not well discussed in the literature.

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Rare Presentation of Myeloid Sarcoma: Nasopharyngeal Myeloid Sarcoma

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Ayşegül BATIOĞLU KARAALTIN[1]

Abstract

Myeloid sarcoma is a rare tumor of extramedullary immature myeloid cells and very few cases of nasopharyngeal involvement have been documented. Despite being most common in skin, bone and lymph nodes; it can be seen in any region with nasopharynx being one of the rare sites. In this article, we aimed to present a case of nasopharyngeal myeloid sarcoma. 58 – year – old male who was previously diagnosed with AML transformed from MDS and treated with chemotherapy, presented with complaints of hearing loss in the right ear and swelling in the right side of the neck. He had a mass lesion in the right half of the nasopharynx in the endoscopic examination performed upon the presence of hearing loss and serous otitis findings. Imaging findings revealed a mass lesion starting from the right half of the nasopharynx extending to the posterior of the carotid space and paravertebral area. Patient was diagnosed with acute myeloid leukemia (AML) transformed from myelodysplastic syndrome (MDS) and was treated with chemotherapy regime consisting of cisplatin and concomitant radiotherapy. Since myeloid sarcoma is a rare tumor seen in 3-8% of patients with AML, high suspicion and immunohistochemical analysis are important in the diagnosis of this tumor.

Keywords: *nasopharynx; myeloid sarcoma; acute myeloid leukemia.*

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Introduction

Myeloid sarcoma is a pathological definition expressing the extramedullary proliferation of blasts in one or more myeloid series. It usually occurs as an isolated extramedullary leukemic tumor or as a relapse of AML(1). Although this tumor most commonly occurs in the skin, bone and lymph nodes; it can be seen in many regions such as the CNS, oral and nasal mucosa, breast, genitourinary tract, chest wall, pleura and mediastinum(2) . The most common clinical findings are severe pain due to the mass effect of the tumor and abnormal bleeding. Treatment options such as chemotherapy, radiotherapy, hematopoietic stem cell transplantation and targeted therapy are available. The prognosis is worse for those that occur in presence of AML(3).

In this article, we aimed to present a case of nasopharyngeal myeloid sarcoma, which is a rare presentation of myeloid sarcoma.

Case Presentation

A 58 –year – old male patient, who was previously diagnosed with AML transformed from MDS and treated with chemotherapy, presented with complaints of hearing loss in the right ear and swelling in the right side of the neck. Endoscopic examination revealed serous otitis findings in the right ear and a submucosal mass lesion in the right half of the nasopharynx. Cranial and cervical computed tomography (CT) and magnetic resonance imaging (MRI) of the patient revealed a mass lesion starting from the right half of the nasopharynx extending to the posterior of the carotid space and paravertebral area, surrounding the internal carotid artery by 360 degrees, penetrating into the carotid canal (Figure 1). In the PET-CT scan of the patient, a hypermetabolic mass lesion involving bilateral nasopharynx, being more prominent on the right was thought to be due to the primary disease (Figure 2). In complete blood count, white blood cell count was 7900/microliter with %52.1 neutrophils, %37.5 lymphocytes, hemoglobin was 15.1 g/dl and platelet count was 222000/microliters. While nasopharyngeal carcinoma was the most possible diagnosis, histopathology and immunohistochemistry of the punch biopsies taken from the nasopharyngeal mass revealed myeloid sarcoma. For the treatment, chemotherapy regime consisting of cisplatin and concomitant radiotherapy were initiated by the

medical oncology and radiation oncology departments but the patient died 1 week after the beginning of the treatment regime.

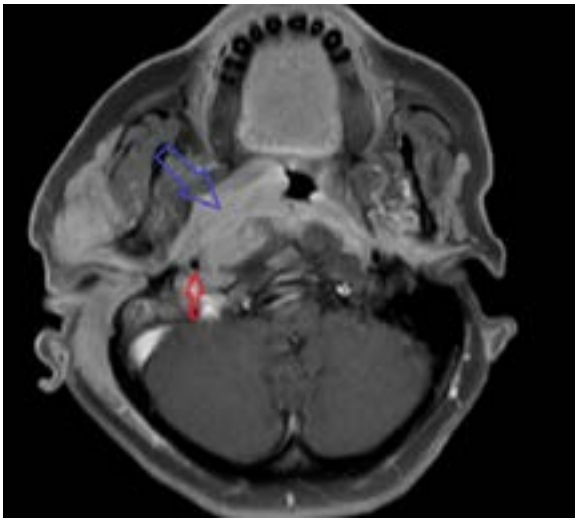

Figure 1. *Contrast-enhanced neck magnetic resonance imaging axial section revealed a mass lesion starting from the right half of the naso-pharynx (indicated by blue arrow) and surrounding the internal carotid artery (indicated by red arrow) by 360 degrees.*

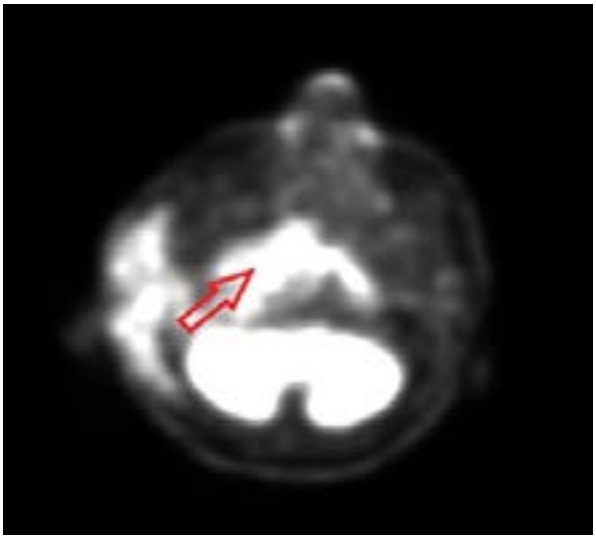

Figure 2. *PET-CT scan axial section indicated a mass lesion at the skull base, involving bilateral nasopharynx (indicated by red arrow).*

Discussion

Myeloid sarcoma is the extramedullary proliferation of blasts in myeloid series, usually occurring as an isolated extramedullary leukemic tumor or in association with other myeloid disorders (mostly AML, myeloproliferative diseases especially CML and MDS) (1). It is a rare tumor seen in 3-8% of patients with AML. Despite being most common in skin, bone and lymph nodes; it can be seen in any region with nasopharynx being one of the rare sites (2). Myeloid sarcoma is usually misdiagnosed initially due to its clinical presentation with nonspecific symptoms (4). Patients can present with sore throat, jaw pain, sinus pain, skin lesions (papules, nodules or rash), tonsillar enlargement or lymphadenopathy. In the oral cavity, authors have emphasized that myeloid sarcoma could mimic pyogenic granuloma, abscess or other inflammatory processes, thereby delaying biopsy and diagnosis (5).

The characteristic microscopic appearance of myeloid sarcoma is Indian mesh pattern. It is classified as granulocytic, monoblastic and myelomonocytic according to the most common cell type. The most common positive immunohistochemical markers are CD68 and CD13 (CD68 for macrophages and CD13 for granulocytes); myeloperoxidase (MPO) and CD117 (markers for myeloid differentiation) ; lysozyme (a marker of monocytic lineage), CD43 (on the myeloid cells as well as the T cells and the B precursors) , CD34 and TdT (expressed on immature cells) (1,3). Initially, nasopharyngeal carcinoma was suspected in our case due to the clinical findings and localization. The main symptoms of our case were unilateral hearing loss which was explained by serous otitis media and unilateral neck mass, which were highly consistent with nasopharyngeal carcinoma. However, submucosal appearance of the lesion was suggestive for diagnoses other than nasopharyngeal carcinoma. Hence, our case was diagnosed with myeloid sarcoma, in the context of AML history, the presence of lymphoepithelial component and the presence of CD117 positive cells.

In the case of nasopharyngeal myeloid sarcoma reported by Raphael et al., although high grade lymphoma is suspected primarily for a 73-year-old man presented with a right-sided nasopharyngeal mass confirmed by imaging; absence of expression of lymphoid markers and myeloperoxidase

lead the final diagnosis of MS. The patient was treated with conventional induction AML therapy. The clinical outcome was favorable, the PET-CT scan showed no abnormal FDG uptake, the bone marrow and blood count were normal except for a megakaryocytic hypoplasia. However, three months later, another bone marrow examination revealed an overt acute myeloblastic leukemia and the patient was enrolled in a phase I trial with the Etoposide. After 2 cycles there was no response, the treatment was stopped and patient died from disease progression (6). In another case report, further work-up was suggested to a 73 years old male patient who presented with one month history of dysphagia and noisy breathing. Hypertrophy of the lingual and palatine tonsils was detected in the endoscopic examination. Biopsies taken from both tonsils were compatible with myeloid sarcoma and the patient opted for treatment with palliative chemotherapy but he died due to the rapid progression of the disease (7)where a mass (tumour. In an article three cases of myeloid sarcoma were presented: (1) A 17-year-old boy with AML-M4 presented with sudden bilateral facial paralysis, sudden hearing loss, vision disturbance and a rapidly growing mass in bilateral postauricular area (2). A 17-year-old girl presented with ill-defined soft mass measuring 5 cm in diameter in the right preauricular area (3). 33-year-old man presented with multiple masses on his skin, bilateral cervical and axillary lymphadenopathy. All patients undergone chemotherapy and immunotherapy with interleukin-2 was also added for case 3. However, all of them relapsed and died (8). For our case, a second regime of chemotherapy consisting of cisplatin and concomitant radiotherapy was initiated as the treatment; yet patient died one week after.

Although there are different approaches in the treatment of the disease, such as chemotherapy, radiotherapy, hematopoietic stem cell transplantation and targeted therapies (imatinib, gemtuzumab, etc.), no clear consensus or recommendation exists in the literature. The prognosis seems more dismal than overt AML although it remains controversial. In the absence of leukemia, surgical removal of the tumor followed by local radiotherapy may be performed; because granulocytic sarcomas are generally regarded as radiosensitive tumors (9). Local radiotherapy may also yield better local tumor control if residual disease persists or the tumor relapsed after initial chemotherapy. Also, high-dose chemotherapy followed by stem cell

transplantation may be associated with a higher probability of survival or cure(10). 9 of 497 AML patients with isolated myeloid sarcoma retrospectively reviewed by Lee J.Y. et al. and the most common site for MS was head and neck region, with 4 patients evolving into AML in a median time of 13.4 months. Patients achieving complete remission after first-line treatment was higher in the "local treatment with or without systemic treatment (LS)" group than in the "systemic treatment only (S)" group and while all patients in the LS group survived, all those in the S group died (P=0.012) (11). Patients with myeloid sarcoma combined with AML have a poor prognosis. Even after chemotherapy with or without radiotherapy, as many as 85% of the patients relapse within 1 year (12) and these relapses are usually the cause of death (6–8).

Myeloid sarcomas are related with worse prognosis especially in the presence of AML. So, early treatment initiation is crucial, which makes differential diagnosis more important. For a nasopharyngeal mass, it should be highlighted that submucosal localization was not consistent with nasopharyngeal carcinoma and suspicious for other pathologies. In addition, history of hematological diseases such as myelodysplastic syndrome or myeloblastic leukemia is also suggestive for myeloid sarcoma. If myeloid sarcoma is one of the possible diagnoses, it is important to communicate with the pathologist to lead the diagnosis to a more accurate direction, in order to prevent waste of time.

Conclusion

In conclusion, myeloid sarcoma is a rare tumor seen in 3-8% of patients with AML and extremely rare when speaking of nasopharyngeal myeloid sarcoma in particular. High suspicion and immunohistochemistry are important for the diagnosis of this tumor having a poor prognosis. Local treatment options such as surgical removal and radiotherapy could be considered in isolated tumors in the absence of leukemia, but systemic chemotherapy would be the treatment of choice in patients with leukemia.

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WRITING RULES

Manuscript Preparation

General Rules

Articles should be organized according to the ICMJE recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals updated in December 2019 (http://www.icmje.org/icmje-recommendations.pdf). CONSORT (www.consort-statement.org) for randomized trials, STROBE for observational studies (http://strobe-statement.org/), PRISMA for systematic reviews and meta-analyses (http://prisma-statement.org/), STARD for studies of diagnostic accuracy (http://www.equator-network.org/reporting-guidelines /stard/), ARRIVE for experimental animal studies (https://arriveguidelines.org/), and TREND for non- randomized public behavior (https://www.cdc.gov/trendstatement/) are other guidelines to help authors design their articles.

The upper limit of plagiarism determined by the editorial board for the journal is 20 percent. The filtering options in the plagiarism detection program are set to neglect references, quotes, and text sections of less than five words.

If there is an institution that supports the study, the last word of the article title should have an asterisk (*) and the information on the same page should be given as a footnote.

Manuscript Format

Title page

A separate title page should be submitted and this page should include:

- The full title of the manuscript, as well as a short title (running title) up to 50 characters,
- Name(s), affiliations, highest academic degree(s) and ORCID ID(s) of the author(s),
- Grant information and detailed information on the other sources of support,
- Nameandaddress,phone(includingthemobilephonenumber) number and email address of the corresponding author,
- Acknowledgment of the individuals who contributed to the preparation of the manuscript without fufilling the authorship criteria.

Abstract Title

An English abstract should be submitted with all kind of manuscripts with the exception of Brief Reports and Letters to the Editor. The abstract of an Original Article should be

constructed with subheadings (Objective, Methods, Results, and Conclusion). All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text. The abbreviation should be provided in parentheses following the definition. Please refer to Table 1 below for word count specifications.

Keywords

All manuscripts except Brief reports and Letters to the Editor must be accompanied by a minimum of three to a maximum of six keywords at the end of the abstract. Keywords should be selected from Medical Subject Headings (MeSH) of Index Medicus (https://www.nlm.nih.gov/mesh/MBrowser.html) Keywords will be used for subject indexing.

Table 1. *Limits in Manuscript Types*

Article Type	Text Words	Abstract Words	Keywords	References	Tables	Figures/ Images
Original Article	7.500	300	5	30	10	10
Review Article	10.000	250	5	50	10	20
Case Report	1.500	200	3	20	1	10
Brief Report	2.000	200	3	20	1	10
Letter to the Editor	1.000	No abstract	No keywords	10	1	No figures/ images

Manuscript Evaluation

Authors may send their articles, which are prepared in accordance with the below stated publishing and editorial principles, together with the "article presentation form" via e-mail to the provided addresses. Providing the permissions of all authors is obligatory. EJOH Editorial Board is authorized to decide whether or not to accept articles through international peer-reviews. Following the Section Editors' preliminary reviews, the articles which are in accordance with the EJOH publication rules are sent to two reviewers determined by the Editor in Chief, for evaluation. In case of disagreement between the assigned reviewers, the manuscript is sent to a third reviewer. The articles which are sent back to the authors for further improvement, correction or revision should be edited accordingly and delivered back to the journal within one month at the latest. The results of corrections or revisions of the authors are re-examined by the reviewers and their decisions are reported to the editor. Manuscripts designated as appropriate for publication by the reviewers are sent to the statistical editor and if approved, the publication process begins. The articles which

are found to be conflicting with this guideline, will be rejected and will not be issued.

Manuscript Types

The aim of the EURAS Journal of Health is to publish original research papers of qualified scientific value on health issues. Reviews to highlight relevant outstanding topics and developments, as well as case reports and brief reports or short notes to provoke research and discussion are also within the scope. The EURAS Journal of Health encourages and enables health professionals in the primary, secondary and tertiary health services to publish their research and reviews.

Original Article: Research article is the most important type of manuscript because it provides new information as a result of original research. The main text of original articles should be structured in detail with Introduction, Materials and Methods, Results, Discussion, and Conclusion subtitles and sections. Conclusions are supported by statistical analysis which is mainly necessary. Statistical analyses must be in accordance with international statistical reporting standards (Altman DG, Gore SM, Gardner MJ, Pocock SJ. Statistical guidelines for contributors to medical journals. Br Med J 1983:7;1489–93).

Information on statistical analyses should be supplied in a separate subheading under the Materials and Methods section and the statistical software that was used during the process must be included. Units should be prepared in accordance with the International System of Units (SI).
Limitations, drawbacks, and the shortcomings of original articles should be mentioned in the Discussion section before the conclusion paragraph.

Review Article: It is prepared by experts who have extensive knowledge in a particular field and whose intensive scientific background is translated into numeros publications with high impact potential. Experts should describe and evaluate the
current level of knowledge of a topic and guide future studies in the field. EJOH may also invite submissions from such authors. The main text should contain Introduction, Research Consequences, and Conclusion sections.

Case Report: Rare or challenging cases which are considered to be interesting and educative and those offering new therapies or revealing knowledge not included in the literature also are accepted for publication. Case Report should include the Introduction, Case Presentation, Discussion, and Conclusion sections.

Brief Report: Brief reports are similar to original research in that they build up with same structure such as content, format and guidelines but are designed for small scale scientific

research outcomes that may contain preliminary data and initial findings that indicate need for further investigation. Brief reports are shorter than manuscripts and must contain significant data as in original research articles.

Letter to the Editor: It discusses important or neglected aspects of a previously published article for educative purposes. The text should be unstructured. Abstract, Keywords, and Tables, Figures, images, and other media should not be included. The manuscript that is being commented on must be properly cited within this manuscript.The manuscript that is being commented on must be properly cited within the Letter.

Writing Rules

Page Layout: Standart A4-sized format. Margins: top 3.5; down 2.5; left 2.5; right 2 cm with 170 mm X 240 mm overall text space.

Font: Times New Roman style and 11 pt. font size are used for the whole text. All article should be justified. Single line spacing should be used throughout the main text and between the paragraphs.

Title: Bold capital letters in 14-pt must be used fort he main title. Subtitles should be written in bold and 11 pt,. After the title, author names, author ORCID numbers and e-mail addresses should be stated in 11 pt font size, with two lines of space.

Abstract: Single paragraph in 11-pt, including subsections for Objective, Materials and Methods, Results and Conclusion sections are needed.

Keywords should be in italic, bold type and 11 pt.

Figures and Images: Figures, graphics and photographs should be submitted as separate files (in TIFF or JPEG format). The files should not be embedded in a Word document or the main document. Images should not be labeled (a, b, c, etc.) to indicate figure subunits. When there are figure subunits, the subunits should not be merged to form a single image. Each subunit should be submitted separately through the submission system. As requested for the whole submission, the figures should also be blinded. Any information within the images that may indicate an individual or institution should be blinded. The minimum resolution of each figure should be 300 DPI. Figure legends can be supported by thick and thin arrows, arrowheads, stars, asterisks, and similar marks can be used on the images. Figure legends should be listed at the end of the main document. All submitted figures should be clear in resolution and large in size in order to prevent delayed evalutaion process.

When a drug, product, hardware, or software program is mentioned within the main text,

product information, including the name of the product, the producer of the product, and city and the country of the company (including the state if in USA), should be provided in parentheses in the following format: “Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)”

Tables

- The number of tables allowed for each type of manuscript is stated in Table 1.
- The tables should effectively display the information about desired levels of detail, so that the length of the text is also shortened. Each table should be printed on a separate page with double spacing.
- The table numbers should be given according to their order in the text, and each one should contain a short title. Authors should make explanations in footnotes, not in titles. All non-standard abbreviations should be explained in footnotes. The following symbols should be used for footnotes, respectively (*,†,‡,§,||,¶,**,††,‡‡).
- Statistical measurements of the variation (i.e. Standart error, standart deviation) should also be included. If data from another published or unpublished source are used, the cited source should be fully informed before its permission is obtained.
- Tables with a lot of data (backup) take up a lot of space and can only be suitable for electronic publications or they can be provided directly to the readers by the authors. In such a case, an appropriate expssion should be added to the text. Such tables should be submitted with the article for the evaluation by reviewer.

References: References are numbered and listed by their order of appearance in text; the text citation is followed by the appropriate reference number in parentheses. All references should be cited in text. While citing publications, priority should be given to the most up-to-date publications and accuracy of references is the responsibility of the authors. The DOI number should be provided ıf an ahead-of-print publication is cited. Journal titles should be abbreviated in accordance with the journal abbreviations in Index

Medicus/ MEDLINE/PubMed. All authors should be listed if there are six or fewer authors in the study. In case of six or more authorship, the first six authors should be listed followed by “et al.

Journal Article: Blasco V, Colavolpe JC, Antonini F, Zieleskiewicz L, Nafati C, Albanèse J, et al. Long-term outcome in kidney recipients from donors treated with hydroxyethylstarch 130/0.4 andhydroxyethylstarch 200/0.6. Br J Anaesth 2015;115(5):797-8.

Book Section: Philips SJ, Whisnant JP. Hypertension and Stroke. Editors: Laragh JH, Brenner BM. Hypertension pathophisiology, diagnosis, and management. 2nd ed. New York: Raven Press, 1995: 465–78.

Book with a Single Author: Jarvis C. Physical Examination and Health Assessment. 3rd ed. Philadelphia: W.B. Saunders Company; 2000.

Book with editor: Breedlove GK, Schorfheide AM. Adolescent pregnancy. Wieczorek RR, editor. 2nd ed. White Plains (NY): March of Dimes Education Services; 2001. p: 32-47.

Editor(s) as Author: Huizing EH, de Groot JAM, editors. Functional reconstructive nasal surgery. Stuttgart-New York: Thieme; 2003.

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Short Report: Cusick M, Chew EY, Hoogwerf B, Agrón E, Wu L, Lindley A, et al. Early Treatment Diabetic Retinopathy Study Research Group. Risk factors for renal replacement therapy in the Early Treatment Diabetic Retinopathy Study (ETDRS), Early Treatment Diabetic Retinopathy Study KidneyInt: 2004. Report No: 26.

Thesis: Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

Manuscripts Accepted for Publication, Not Published Yet: Slots J. The microflora of black stain on human primary teeth. Scand J Dent Res. 1974.

Epub Ahead of Print Articles: Cai L, Yeh BM, Westphalen AC, Roberts JP, Wang ZJ. Adult living donor liver imaging. DiagnIntervRadiol. 2016 Feb 24. doi: 10.5152/dir.2016.15323. [Epub ahead of print].

Manuscripts Published in Electronic Format: Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) 1995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL: http:/www.cdc.gov/ncidodlElD/cid.htm

Webpage: Author. Title. Available at: URL. Accessed Access Date, Access Year.

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Manuscripts are judged on the significance of the contribution to the literature, the quality of analysis and the clarity of presentation. Papers are expected to demonstrate originality

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