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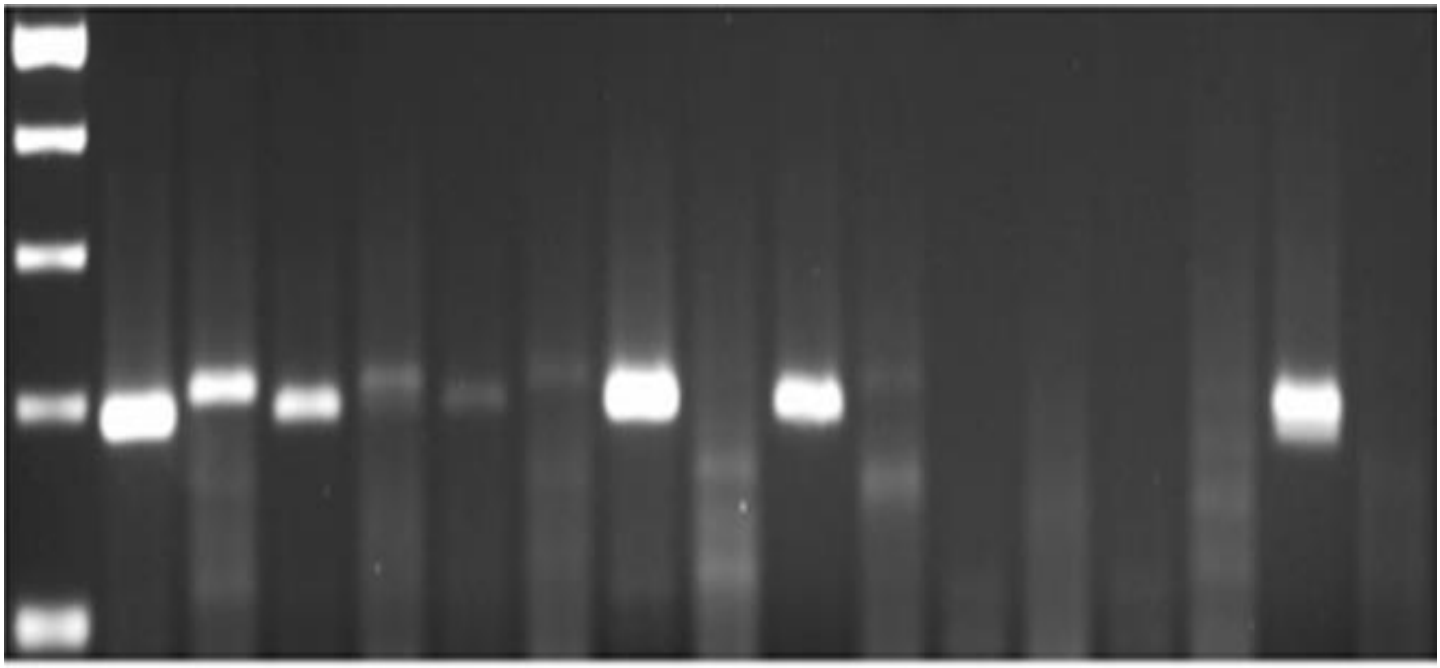

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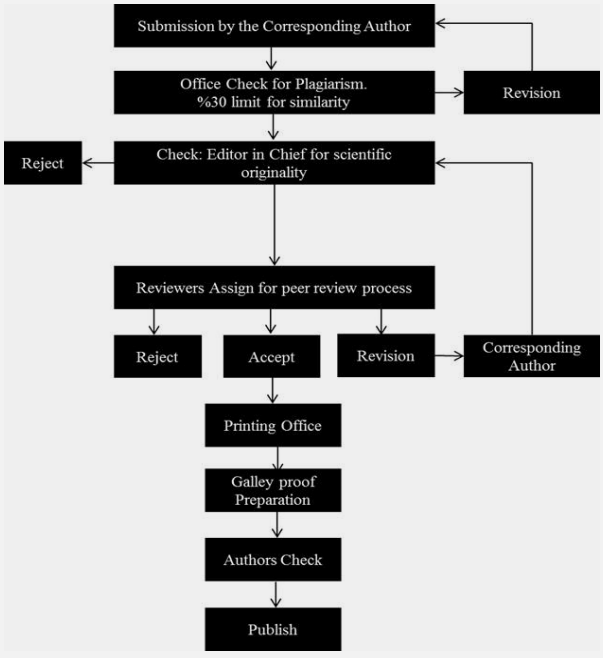

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Medical Science and Discovery, August 2021, Vol.8, No.8

Contents

Review Articles

Breast Cancer: Treatment Effects on Fertility and Subsequent Pregnancy Outcomes
Antigoni Sarantaki, Kyriaki Perisaki / 442-447

Research Articles

Clinical evaluation of intravenous sedation in pediatric endoscopic procedures: A retrospective observational study
Nermin Kilicarslan, Muruvvet Dayioglu, Ayşe Neslihan Balkaya, Kaan Demiroren / 448-452

Epigenetic Regulation of the Tumour Suppressor RASSF1A in Bone Cancer Cells: DNA Methylation Study
Amal Majed Alenad / 453-459

Effect of intermittent hypoxic intervention on aerobic and anaerobic performance of the elite athletes
Ali Eroğlu / 460-464

The status of anatomy education in scope of the national core education program 2020: The case of Turkey
Emre Atay, Abdülkadir Bilir, Tolga Ertekin / 465-469

Acute pancreatitis and low ascites-serum albumin gradient ascites caused by Brucellosis
Mesut Aydin / 470-474

The effect of different doeses of aspirin application on oxidative stress in ovarian tissue
Deniz Dirik, Ahmet Ufuk Komuroglu / 475-479

Do depression, anxiety, or stress have any effect on pain scores in patients undergoing colposcopy?
Muzaffer Seyhan Cikman, Ismet Gun / 480-484

A retrospective comparative cohort study on the routine pre-engraftment use of granulocyte colony-stimulating factor in allogeneic hematopoietic stem cell transplantation
Muruvvet Seda Aydin, Esra Cengiz, Ahmet Kursad Gunes, Mehmet Ali Ucar, Funda Ceran, Gulsum Ozet, Simten Dagdas / 485-491

Case Report Articles

One Disease, Two Approaches. Acute Post-Streptococcal Glomerulonephritis – A Case Report of Two Young Patients
Tudor Lungu, Kinga Krisztina Nagy / 492-497

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Breast cancer: Treatment effects on fertility and subsequent pregnancy outcomes

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ABSTRACT

Objective: Breast cancer is the most common cancer type in women of reproductive age. Given that most women postpone childbearing, breast cancer occurrence possibly perplexes their plans for starting a family. The treatment for breast cancer can affect their fertility and have adverse effects on a pregnancy that occurs during that period. The aim of this narrative review is primarily to explore the influence of breast cancer therapy on the ability of a woman diagnosed with breast cancer to gestate. Moreover, to determine the safer timing for childbearing after being treated for breast cancer and investigate the pregnancy outcome when conception is succeeded.

Childbearing after treatment for breast cancer is considered safe and pregnancy outcomes are favorable if conception happens one year after chemotherapy or at least two years after chemotherapy and radiation therapy. Counseling is of great significance, and fertility preservation methods should be thoroughly discussed with women diagnosed with breast cancer, even prior to commencement of the treatment.

Keywords: breast cancer, fertility, pregnancy, treatment, review

Review Article

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INTRODUCTION

Breast cancer is the most common type of cancer in women of reproductive age, as about 10% of the cases are diagnosed before the age of 40, with the mean age of diagnosis being 33 years (range 23-47) (1). However, 60% of the cases can be successfully treated with early diagnosis, surgery, and adjuvant systematic therapy (2).

The breast is an organ with great plasticity which undertakes numerous and complex developmental changes throughout a woman's life, capable of permanently altering the mammary gland by promoting oncogenesis or preventing it. Breast oncogenesis mimics mechanisms happening most commonly during gestation, such as "augmented cell proliferation, decreased cell apoptosis, altered gene expression and extracellular matrix modifications" (3)

Breast Cancer and fertility

As noted previously, most women diagnosed with breast cancer are of reproductive age. The therapies usually used for breast cancer treatment have an impact on women's ability or even the circumstances to become pregnant. Factors such as ovarian insufficiency, delay of childbearing due to therapy administration, inability to breastfeed, even future fertility concerns constitute major hindrances for those women (4).

The fact that contemporary women delay childbearing enhances the importance of matters such as fertility preservation after breast cancer diagnosis and subsequent pregnancy (5). In the paragraphs below the effect that substances used for breast cancer treatment have on fertility is described. Once breast cancer is diagnosed, a multidisciplinary approach should be taken. This includes obstetricians, surgeons, medical and radiation oncologists, and breast cancer counsellors (6).

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Even though radiation scatter to the uterus and ovaries while treating breast/axillary cancer should be minimal, it is recommended that the pelvic area be shielded during the procedure and becoming pregnant should be delayed until after the completion of radiation therapy (4). Furthermore, any fertility treatment (e.g. collecting or fertilizing eggs for future use) should commence before radiation treatment in case the tiny amounts of radiation that reach the eggs that begin maturating affect them. Nonetheless, this amount of radiation is not likely to affect immature eggs inside the ovaries (7).

Chemotherapy

With young age being a poor prognostic factor, women of this age group are often advised (neo)adjuvant chemotherapy (8). One of the more frequent side effects of chemotherapy in women of reproductive age is changes in the menstrual period -such as premature menopause and impaired fertility- which may arise or be permanent. Not all chemo-drugs have the same likelihood to provoke those side effects (9).

American Cancer Society (9) advises women who undertake chemotherapy treatment and want to become pregnant, to refer to their doctor about this matter before starting the treatment.

Endocrine Therapy

Even though pre-menopausal women are most likely to be diagnosed with hormone receptor-negative breast cancer, two-thirds of them will still have estrogen receptor-positive breast tumors (5). In these cases, adjuvant endocrine therapy should be considered (10).

Tamoxifen or aromatase inhibitors therapy has been proven beneficial as adjuvant anti-hormonal therapy for premenopausal women with hormone receptor-positive breast cancer. This type of cancer is characterized by the long-term risk of recurrence and for that reason, 10-year duration of tamoxifen treatment is recommended for many patients (8). Such a long treatment period could lead to natural ovarian reserve decline as a result of the woman's age (10). What's more, tamoxifen's teratogenic effect being well-known, concerns have been risen with regard to fertility and pregnancy and so, tamoxifen treatment is often discontinued or not initiated at all (4).

The ovarian function should be monitored when tamoxifen is used after chemotherapy, in case of unnoticed ovarian function recovery, to avoid teratogenesis or unplanned pregnancies (8).

Fertility preservation

Cancer survivors' fecundity levels are lower than the general population's. As precedently mentioned, chemotherapy is usually suggested as part of treatment for breast cancer to women of younger age, which can lead to premature ovarian insufficiency and cause impaired fertility, a fact that may affect the therapy plan of choice (10). Currently, there are options to consider for preserving fecundity, hence detailed counseling is of grave importance so women diagnosed with breast cancer can make the most optimal decision for them (8).

According to the European Society of Clinical Oncology (ESCO) (11), the recommended solutions for the preservation of fertility are embryo/oocyte cryopreservation for future use. In the case of breast cancer, GnRHa administration is a feasible choice in order to avoid chemotherapy-induced ovarian insufficiency. Ovarian tissue cryopreservation for future transplantation can be chosen to be performed immediately and without ovarian stimulation.

Prevention of ovarian deficiency could be preferred on fertility preservation grounds over freezing oocytes, embryos or ovarian tissue. Despite the usage of Gonadotropin-Releasing Hormone Analogues has been presumed as an agent for protecting the gonads, the mechanism resulting in the gonad protection is not fully understood (8).

Pregnancy after breast cancer treatment

Women longing for a child after the diagnosis of breast cancer, have two main concerns: the impact of treatment on fertility and a subsequent negative consequence of the pregnancy on prognosis. Women with breast cancer history have the lowest pregnancy rate among other types of cancers, namely a 67% reduction chance for pregnancy due to gonadotoxic treatments provoking ovarian reserve damage, but also due to patient and provider concerns about a pregnancy affecting negatively the evolution of breast cancer, the latter being a hormonally driven disease. Withal, according to available evidence, the prognosis of a woman treated for breast cancer won't be negatively impacted by becoming pregnant and so childbearing should be considered safe and should not be discouraged (10).

Knowing about the effects of chemotherapy and antihormonal therapy on fertility, proper information of patients should be mandatory. Up to 40% of women receiving chemotherapy for breast cancer experience a chemotherapy-induced amenorrhea (CIA) (12). Incidence depends on age and treatment regimen. In general, younger patients are less likely to suffer from CIA. A treatment by CMF (cyclophosphamide, methotrexate, 5-fluoruracil) seems to induce more amenorrhea than a regimen with ACT (doxorubicin, cyclophosphamid, paclitaxel) or AC (doxorubicin, cyclophosphamid). It has also been shown that the anti-Müllerian hormone level decreases significantly during chemotherapy (13). Nevertheless, it is actually impossible to predict individual fertility after chemotherapy. For this reason, all women who did not complete child bearing at diagnosis, should be informed about the different possibilities of fertility preservation including GnRH analogues, cryoconservation of oocytes or ovarian cortex. For the choice of the method for fertility preservation the individual prognosis, impact of therapy on ovarian function and delay of treatment should be considered. The appropriate interval between cancer and pregnancy remains unclear. The main concerns are the fear of recurrence and the interruption of antihormonal treatment. Patients should be counselled individually regarding tumor biology and prognosis. In patients with hormone-receptor negative breast cancer a delay of 2–3 years according to prognosis should be respected. Despite the contemporary data and knowledge, the time period demanded between the completion of therapy and conception is not totally clear. Experts suggest the timing should be decided considering the age of the patient and her ovarian reserve, the time of completion of previous therapies and the individual risk for relapse (10).

Concerning chemotherapy, the recommendation is that women postpone pregnancy for 6 to 12 months after chemotherapy treatment, so they do not conceive with an oocyte that was maturing during treatment. Hartnet et al. (14) refer to chemotherapy being known to kill rapidly dividing cells so, "it might damage the oocytes being recruited for ovulation, resulting in higher risks of miscarriage and birth defects in pregnancies conceived soon after treatment" (14).

When using anti-HER2 therapy (e.g. trastuzumab and pertuzumab) it is recommended that childbearing should be delayed for at least 7 months after completion of the therapy, due to its teratogenic effect (4).

Tamoxifen's effects on the fetus and gestation are adverse and the epithelial changes observed resemble those of diethylstilbestrol (DES) (2) so it is recommended that women who undergo tamoxifen treatment use non-hormonal barrier contraception which should continue up to 3 months afterwards (15).

Pregnancy outcome

Pregnancy outcome, pertaining to the neonatal well-being, is not different from the neonatal outcome of the general population. Howbeit, the rates of induced abortion observed succeeding breast cancer diagnosis, reach 30% and the incidence of birth complications -such as caesarean section, preterm birth, low birth weight neonates- is higher in breast cancer survivors than in general population. Overall, pregnancy in such cases should be closely monitored (10).

Findings of population-based studies indicate that the birth outcome of women who have breast cancer history may correlate with 50% increased risk of preterm birth or low birth weight compared with the general population (8). Immunosuppression, being one of chemotherapies side effects, has been observed to increase the risk for the conditions mentioned above (14), which are much more likely to occur to women who received chemotherapy or gave birth within two years of diagnosis (8).

In the for mentioned research (14) is described how the preterm birth risk was double, in comparison with women who had not been associated with a cancer diagnosis in the same time of birth, for chemotherapy without radiation and 2.4 times higher for chemotherapy with radiation. The highest risk for preterm birth and low birth weight was linked to pregnancies that started within one year of starting treatment. Conversely, the risk for a preterm infant, low-birth-weight infant or small for gestational age infant was the same to women with no cancer history if the pregnancy was conceived at least one year after starting chemotherapy without radiation, or two years or more, after chemotherapy with radiation. Last but not least, 3 out of 9 of women whose first pregnancy after treatment ended in stillbirth, had conceived within one year of starting chemotherapy.

A different study aiming to determine pregnancy outcome to women in Korea after treatment for breast cancer had the following findings: 992 women became pregnant out of 33,761 women included in the study. 622 (67.5%) out of 992 women who became pregnant had a successful delivery, while the remainder 370 women failed to deliver. Those who successfully delivered were younger (mean age 30.6 years vs. 33.9 years of those who failed to deliver) and had lower frequencies of chemotherapy (29.4% vs 41.9%, p=0.012). Furthermore, they were more likely to have become pregnant >2 years after surgery for breast cancer treatment (17.7% vs 34,1%, p<0.001), compared with those who failed to deliver (16).

BRCA mutations

Pregnancy after breast a cancer diagnosis, even in patients with hormone-positive disease, is considered safe. Yet, data concerning women with BRCA mutations (12% of breast cancers in women younger than 40 years of age) are very limited. On those grounds, an international, multicenter, hospital-based, retrospective cohort study was conducted and the results presented favorable fetal outcomes. While 20 of 195 women experienced a miscarriage (10.3%) and 16 women undertook an abortion (8.2%), 150 women gave birth, and from the total of 170 neonates, pregnancy complications occurred in 13 (11.6%) and congenital anomalies in 2 (1.8%) of the cases (11).

Cryopreservation

The first live birth after cryopreserved oocytes associated with an oncologic indication was reported in 2007. Ter Welle-Butalid et al. (8) have summarized the results of studies concerning embryo cryopreservation. Even though the oncology indications were different, the purpose was fertility preservation. The number of women who actually had an embryo transferred after preceding oocyte preservation, ranged from 0-5%. Overall, 23% of 614 women underwent at least one embryo transfer and 40% of them had a live birth. It should be noted that return rates are influenced by the general advice to abstain from becoming pregnant for at least two years after diagnosis. It is understood that return rate depends on the longer or shorter observation time and other factors such as maintained ovarian function after chemotherapy or even reconsideration on family planning.

In recent studies, it has been revealed that pregnancy does not have a negative impact on prognosis (17). Knowledge about the different options of fertility preservation is expanding. The relatively new technique of cryo conservation of ovarian cortex shows promising results, but data of long-term follow-up remain necessary. Besides, the appropriate interval between cancer and the following pregnancy needs to be defined. Improvements in local and systemic treatment, along with earlier diagnoses through breast awareness and screening, have led to increases in survival and a decline in breast cancer (BC) recurrence. Women with BC should be informed about the subsequent adverse effects of BC and its treatments on conception. With the increasing trend for women to defer childbirth to later in life, provision of fertility-related information, access to fertility preservation, and fertility-related psychosocial support should be offered to women of a reproductive age before they begin BC treatment (18). When proven fertility preservation methods are not feasible, and in the setting of young women with breast cancer, GnRHa may be offered to patients in the hope of reducing the likelihood of chemotherapy-induced ovarian insufficiency. GnRHa should not be used in place of proven fertility preservation methods (19).

An improvement in the survival rates of cancer patients and recent advancements in assisted reproductive technologies have led to remarkable progress in oncofertility and fertility

preservation treatments. Currently, for adults and post-pubertal girls, oocyte or embryo cryopreservation is an established method. The field of ovarian tissue cryopreservation is advancing quickly and may evolve to become standard therapy in the future (**20**).

Nowadays, giving hope for a family after cancer diagnosis and treatment should be considered a crucial ambition in cancer care. Therefore, oncofertility care has become a priority and a mandatory component of the management of young women with newly diagnosed breast cancer. Increased awareness by all health care professionals in oncology is needed to make sure this topic is always discussed at diagnosis and women can make fully informed decisions about the proposed anticancer therapies and their potential interest in accessing the available strategies for ovarian function and/or fertility preservation. (**21**).

CONCLUSION

Breast cancer is the most common type of cancer occurring to women of reproductive age. Pregnancy after breast cancer is actually still a rare entity but the incidence is increasing. Thus gynecologists, obstetricians, and oncologists may be confronted with this complex situation more often and should be familiar with the management possibilities. Women can therefore be reassured and interruption of pregnancy is not generally indicated. Women who intend to get pregnant after a diagnosis of breast cancer, also need special information. It has now grown to be clear that becoming pregnant after treating breast cancer is safe. There are ways of preserving fertility during the treatment, such as oocytes and/or embryo cryopreservation. Pregnancy is contraindicated for at least two years after chemotherapy and radiation treatment completion or for one year after chemotherapy treatment completion, since this timing of conceiving showed more promising pregnancy outcomes.

Preterm birth, low-birth-weight neonates or small for gestational age neonates, were associated with becoming pregnant within one year of starting breast cancer treatment. Counseling on childbearing should be provided before the initiation of breast cancer treatment and women should be informed about all the available choices for preserving their future ability to gestate.

A number of options are available to preserve fertility in female BC patients, and these fertility preservation procedures should be discussed and preferably introduced, before the patient starts systemic therapy. A patient's age at diagnosis, the type of adjuvant treatment given, time available before the start of treatment, and the delay in childbearing required after treatment should be considered when counseling patients about the effects of treatment. Clinicians should provide patients with support and detailed information regarding the reproductive risks after BC treatment to improve their overall physical and emotional recovery. Women with breast cancer welcome the option to discuss and explore the fertility and pregnancy options available to them prior to commencement of their treatment.

In fact, this is essential to promote women's well-being and can increase treatment compliance later on. Additional age-related issues should be considered when managing breast cancer in young women and effective communication is at the forefront of this approach. Health professionals working with women diagnosed with breast cancer, should be encouraged to approach fertility issues at the outset of cancer treatment. Early referral of patients with breast cancer and incomplete family planning to a specialist, to discuss fertility preservation options, is of paramount importance to female cancer patients.

Building a positive rapport surrounding this issue can help and facilitate each individual woman's cancer journey. Considering the rising trend in delaying childbearing and the suboptimal knowledge of health care providers towards these survivorship issues, further awareness and education towards enhancing the oncofertility counseling of young women with breast cancer need to be prioritized.

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Clinical evaluation of intravenous sedation in pediatric endoscopic procedures: A retrospective observational study

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ABSTRACT

Objective: The main benefits of sedation are to reduce the patient's anxiety and discomfort, to improve their tolerability. The aim of study was evaluate intravenous sedation for pediatric gastrointestinal endoscopic procedures

Materials and Methods: We analyzed patients' data, who underwent gastrointestinal endoscopic procedures in our pediatric endoscopy unit, retrospectively. All gastrointestinal endoscopic procedures were performed by a pediatric gastroenterologist and sedations were managed by an anaesthesia team, including two staff anesthesiologists.

Results: During the study period, 530 gastrointestinal endoscopic procedures were performed. 461 (87%) were esophagogastroduodenoscopy, 56 (10.6%) were both esophagogastroduodenoscopy and colonoscopy and 13 (2.5%) were percutaneous endoscopic gastrostomy. Propofol was given all of the patients either as a single drug (6 patients, 1%) or in combinations (77.4% with midazolam; 12.3% with ketamine and 9.2% with fentanyl). Overall adverse event rate due to sedation was 19.6%, but no serious side effects were documented. The most frequent side effects were injection pain (10.4%), and nausea (7.5%). Allergic reactions were experienced in 1.3% patients and resolved with methylprednisolone and antihistaminic medications. Respiratory depression was observed in only two girls (3 and17 years old) and did not need advanced interventions to control the problem. Seven patients' gastroscopies were interrupted by gastroenterologist due to gastric content in order to prevent vomiting and aspiration.

Conclusions: Intravenous sedation for pediatric gastrointestinal endoscopic procedures can be applied safely and successfully with a trained team and organized endoscopy unit.

Keywords: child, anaesthesia, intravenous agents, sedation, endoscopic procedures

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INTRODUCTION

Anesthesia procedures outside the operating room have increased as a result of increasing number and kind of procedures in the other fields of the medicine. Sedation is often used during gastrointestinal endoscopic procedures (GEP) for the diagnosis and management of the patients. The main targets of sedation are to reduce the patients' anxiety and discomfort, to improve their tolerability. Additionally, in children it is important to modify behavior to provide immobility to allow the safe completion of the procedure.

Different combinations of medications have been used for pediatric sedation including propofol, ketamine, midazolam, fentanyl, and pethidine (1). In our pediatric endoscopy unit, midazolam, ketamine, propofol and fentanyl have been used as alone or in combinations.In this study, we aimed to evaluate our intravenous sedation (IVS) administrations for pediatric GEP and based on these evaluations, to discuss the issue in the light of current literature.

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MATERIAL and METHODS

This is a retrospective observational study. This study was approved by the institutional non-invasive clinical research ethics committee (Approval Date and Number: 26.06.2019, 2011-KAEK-25 2019/06-04). Informed consent was obtained from the parents of all children prior to endoscopy, and the study was conducted according to the tenets of the Declaration of Helsinki.

Patients

A total of 530 pediatric patients, given IVS, between1.10.2018 and 1.6.2019were included in the study. Patients' data were collected from anaesthesia records and hospitals software database. Patients' age, gender, American Society of Anesthesiologists (ASA) status, type of procedure, anaesthesia time, procedural time, recovery time, medications were recorded. Adverse events were also recorded from anesthesia complication charts, including respiratory depression (defined as SpO2 <90%), allergic reactions, injection pain, nausea and vomiting. Serious adverse event means that a deep sedation not easily treated with basic interventions, causes hypoventilation, laryngospasm, pulmonary aspiration, and needs to antidotes or endotracheal intubation.

Anaesthetic procedures

According to routine operating procedures in our clinic anesthetic procedures were managed by two anesthesiologists. Patients were evaluated for the risks of anaesthesia before any procedural intervention and obtained a written consent from patients and/or parents. In elective cases patients and/or parents informed about gastric emptying time, but in urgent or emergent situations where complete gastric emptying is impossible, we did not delay sedation based on fasting time alone. There was no premedication prior to the procedure.

In line with our clinical follow- up protocol, all medications were given to achieve moderate to deep sedation as defined by ASA (2). Patients monitoring included continuous electrocardiogram, heart rate and oxygen saturation. All patients received supplemental oxygen at 2 L/min via nasal cannula according to ASA recommendations. After the procedure, patients were observed and monitored by an anaesthesia nurse in the recovery room until they are near their baseline level of consciousness and no longer at cardiac risk for cardiorespiratory depression according to ASA guideline (2).

An emergency kit which contains the necessary age and size-appropriate equipment, emergent medications and antidotes of the sedation drugs for managing unintended deeper sedation, was always ready in the endoscopy unit and checked before every procedure.

Statistical analyses

Statistical analyses were conducted with SPSS 22.0 (IBM Corporation, Armonk, New York, United States). Results with variable data were expressed as mean ± SD. Results with categorical data were expressed as percentage (%).

RESULTS

Out of 530 children underwent GEP with IVS, 326 (61.51%) were girls and 204 (38.49%) were boys. Patients' descriptive values were listed in (**Table 1**).

There were three main endoscopic procedure types: 461 (86,98%) patients underwent esophagogastroduodenoscopy (EGD), 56 (10.57%) both EGD and colonoscopy, and 13 (2.45%) percutaneous endoscopic gastrostomy (PEG).

Propofol, midazolam, ketamine, fentanyl and their combinations were used for sedation. Propofol was the main agent and used in all patients: Except six (1.13%) patients, propofol was used in combination with another drug. For 410 (77.35%) patients, midazolam was added to propofol, 65 (12.26%) ketamine, 49 (9.24%) fentanyl.

As for the side effects, only two (3 and17years old girls) patients, represented respiratory depression (0,37% of all patients) were given propofol plus midazolam combination. Fortunately, none of them was serious and controlled with basic intervention (encourage or physically stimulate to breathing deeply). The most frequent adverse event was injection pain (55 patients, 10.37%) and followed by nausea (40 patients, 7.54%). Allergic reactions, which were only represented with urticarial lesions, were seen in 7 (1.32%) patients. Methylprednisolone and antihistaminic medications were enough to controlled the situations in all cases (**Table 2**).

Patients were divided subgroups according to their ages, considering that age can change the side effect profile. Respiratory depression (1.36 %) was frequent in 0-3 age group. Injection pain, nausea and allergic reactions were recurring in 11-18 ages group (**Table 3**).

All of the procedures were completed successfully except for seven EGDs. In these seven patients, underwent EGD, the procedure was stopped due to gastric content, without any complication. Five of these seven procedures were elective, and the other two were emergent, but procedures were delayed in order to ensure gastric emptying time.

In this retrospective study, we did not find any document about other possible adverse events, like laryngospasm, hypersalivation, hemodynamic instability.

Table 1. Descriptive values (*min= minutes)

Variable	Overall (n= 530)
Age (year) (mean ± SD)	10.7 ± 5.2
Gender (male-female; %)	204-326 (38.5%-61.5%)
Timing (elective-emergency; %)	506-24 (95.5%- 4.5%)
ASA pysicalstatus (I, II, III, IV; %)	437, 83, 9, 1 (82.5, 15.7, 1.7, 0.2 %)
Duration of procedure (min*)	15.89± 6.7
Duration of recovery (min*)	21.01± 2.7

Table 2. Incidence of side effects

Side effects	n (%)
Injection pain	55 (10.37 %)
Nausea/ vomiting	40 (7.54 %)
Allergic reactions	7 (1.32%)
Respiratory depression	2 (0.37%)

Table 3. Incidence of side effects by age groups

Side effects	0-3 years old (n=73)	4-11 years old(n=170)	11-18 years old (n=287)
Injection pain	9 (12.3%)	15 (8.82%)	31 (10.80%)
Nausea/ vomiting	3 (4.10%)	16 (9.4%)	21 (7.31%)
Allergic reactions	1 (1.36%)	2 (1.17%)	4 (1.39%)
Respiratory depression	1 (1.36%)	0	1 (0.34%)

DISCUSSION

During the past 40 years pediatric GEPs have become important and effective procedures for the diagnosis and treatment of gastrointestinal tract and needed optimal sedation conditions. Especially young children can be uncooperative and tend to have psychological trauma as a result of separation from their parents and pain due to the procedure. Endoscopic sedation is intended to reduce patients' anxiety, minimize psychological trauma, maximize the potential for amnesia and improve tolerability with minimizing discomfort and pain. Of course it also provides optimal conditions for the endoscopist.

″ Sedation and analgesia″ comprise a continuum of states ranging from minimal sedation (anxiolysis) through general. Both American Academy of Pediatrics, American Academy of Pediatric Dentistry and ASA suggested that the practitioner must be sufficiently skilled to rescue a child with cardiorespiratory complications, a well-trained support personel must be accompanied with him/her and must be preset in the room, as unintended level of sedation may be occurred (**3,4**). Studies demonstrated that, when a pediatric sedation team involving an anesthesiologist was attended, successful sedation rates were 100% and adverse events ranged 1.7-5% (**5**). In our hospital sedations were managed by anesthesiologists and support personals were well-trained anesthesiology nurses. Our pediatric endoscopy unit is well designed and fully equipped just in case.

To date, data are limited to have a conclusion about the best sedation regimen (**6, 7, 8**). Midazolam, propofol, ketamine and fentanyl are used alone or in combinations. As a first line sedative agent, midazolam is considered safe but midazolam alone often provides inadequate sedation so usually opioids or ketamine are used together (**9**). Midazolam also used as premedication in many anesthetic situations (**10**). Ketamine, a NMDA receptor antagonist, is in common use in pediatric patients as a safe and effective agent (**11**). Both propofol and midazolam are effective in reducing ketamine's hallucinogenic emergence reactions (**12**). Propofol is a rapid onset sedative-hypnotic agent and is commonly used to relieve anxiety and to sedate children who undergo therapeutic or diagnostic procedures such as cardiac catheterization, endotracheal intubation, emergency orthopedic procedures, dental procedures, and radiological imaging (**13**). Sunhee Kim et al. performed a systematic review and meta-analysis of randomized controlled clinical trials to evaluate propofol's safety for pediatric procedural sedation. They concluded that propofol sedation had advantages in recovery time compared with other drugs, without excessive adverse events and suggested propofol as a safe sedative for pediatric procedures as an option that is comparable to other alternatives (**1**).

Propofol also has antiemetic effects, explained by its interactions with the

dopaminergic and the serotoninergic systems (**15,16**). Fentanyl is a potent lipid-soluble analgesic with sedative properties. It is often combined with propofol or midazolam to provide sedation for outpatient procedures (17). However, studies showed that propofol based sedation provides a safe sedation profile (**14,18**), combinations with midazolam, ketamine or fentanyl are used in order to provide effectiveness and quality (**8, 19, 20, 21**).

The use of more than two drugs has been identified as a risk factor, the addition of fentanyl or midazolam to propofol significantly reduced the individual dosage of drugs and minor adverse events were observed. Our retrospective analyses demonstrated that propofol is the main drug for our team to provide sedation. Safety and efficacy level of propofol alone are satisfactory and reported with rare complications. Only six patients were given propofol alone, which refers to combinations were preferable. In that context, the most popular combination drug was midazolam, which was followed by ketamine and fentanyl (**22**).

The incidence of respiratory problems is predominant in pediatric patients compared to cardiovascular adverse events. Apnea, laryngospasm, bradycardia, hypotension, aspiration and vomiting are also described. Younger age, higher ASA status, female sex and intravenous sedation have been reported as the main risk factors for procedural sedation (22). In our study, only two children represented respiratory depression, controlled with basic interventions, and this is account for 0.37% of the patients. Both of them have ASA III physical status and this finding is consistent with previous data.

The most frequent adverse event was injection pain, which is more likely to be with propofol injection and can be controlled with lidocaine or opioid injection before propofol (**23**). Unfortunately, our team did not prefer this intervention. Nausea was seen 7.54% of the patients and none of them experienced vomiting, the patients whose procedure stopped because of the gastric content, not excepting. We attributed this rare and light emesis complication to the antiemetic properties of propofol. Urticarial lesions were significantly rare and could be handled with medications. In our study, overall adverse event rate was 19.62 %. In the literature, adverse events in various rates were reported (**8, 14, 24, 25**). Therefore, it is more important to compare serious adverse events. Amorniyotin et al. reported 0.6% serious adverse effects in a cohort study (**24**). Also, in a study performed by Barbi et al., desaturation was 3%, and major desaturation was 0.7% (**25**).

Although we used pulse oximetry, it is not sufficient enough to detect the respiratory complications for sedation (**5**). End-tidal carbon dioxide may be used for early detection of hypoventilation and apnea, but it was not an opportunity for

our team. We believe that close clinical monitoring of the breathing pattern is the best way to detect such complications.

In our clinical practice all patients were ordered according to ASA practice guidelines for preoperative fasting and the use of pharmacologic agents to reduce the risk of pulmonary aspiration (2). Nevertheless, in seven patients, gastroenterologist saw gastric content during endoscopy and then stopped procedure, in order to prevent vomiting and aspiration. This finding indicates that one should be careful about gastric residual volume, even if preoperative fasting period is over. Pre-procedural gastric ultrasonography may be suggested as a solution. Fortunately, these patients did not experienced vomiting, the procedure ended without any complication.

The results of the publications, described the use of propofol for paediatric sedation by providers other than anesthesiologysts are contradictory, due to definition differences, especially (26, 27, 28). For example, Wehrmann and Riphaus(27) showed lower incidence of adverse events, but they restricted the definition of adverse events, bag-mask ventilation, intubation and intensive care administration. In this study, definitions of adverse events are undisputablymoredelicate. Although propofol was administered by specially trained pediatricians, Barbi E et al. (28) pointed out that, constant and immediate availability of anesthesiological support continues to be mandatory. The work of Amornyotin S et al. (24) also high lightened that pediatric GEP procedures could be safely and effectively performed with anesthesiologyst and basic monitoring.

The limitations of the present study were as become a retrospective study and there were some data collection deficiencies because of improper chart documentations for example, it was not possible to obtain the total drug doses from the records. Drugs were given with titration to achieve the target sedation level.In addition, as a consequence of our clinical practice, in which, one member of the team continuously palpate radial pulse and observe the patients' respiratory pattern, the data did not contain blood pressure, so we could not demonstrate hypo/ hypertension.

CONCLUSIONS

In conclusion, although we used different combinations, propofol and fentanyl became more favorable regimenand IVS for pediatric GEP can be applied safely and successfully by the well- trained team with an anesthesiologist and a well-designed, fully- equipped endoscopy unit.

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Ethical approval: This is a retrospective observational study. This study was approved by the institutional non-invasive clinical research ethics committee (Approval Date and Number: 26.06.2019, 2011-KAEK-25 2019/06-04). Informed consent was obtained from the parents of all children prior to endoscopy, and the study was conducted according to the tenets of the Declaration of Helsinki.

Conflict of interest: Nonedeclared.

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Epigenetic Regulation of the Tumour Suppressor RASSF1A in Bone Cancer Cells: DNA Methylation Study

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ABSTRACT

Objective: Osteosarcoma is a bone cancer that affects children and adolescents. The RASSF1A is a tumor suppressor capable of mediating the regulation of cell cycle arrest, migration, including apoptosis. It is the most continually silenced gene that contributes to human cancer. Furthermore, RASSF1A functions as a scaffold protein that can regulate microtubules network and bind apoptotic kinases MST1 and MST2 via the Sav-RASSF-Hippo domain. Epigenetic inactivation of genes by DNA methylation is a key factor regulating gene expression and genomic stability. Our aim was to study the RASSF1A gene promoter methylation in three osteosarcomas (U2OS, Saos-2, and MG-63), two Ewing Sarcoma (A-673 and SK-ES-1), and one-fibrosarcoma (HT-1080) cell lines.

Materials and Methods: Three osteosarcomas (U2OS, Saos-2, and MG-63), two Ewing Sarcoma (A-673 and SK-ES-1), and one-fibrosarcoma (HT-1080) cell lines were used to study RASSF1A gene promoter methylation, using bisulphite conversion of DNA, followed by methylation-specific polymerase chain reaction (PCR)

Results: The RASSFIA's gene promoter methylation was established as a frequent event. Hypermethylation of RASSF1A promoter, was detected in five out of six studied cell lines.

Conclusions: These results demonstrated that altering the Sav-RASSF1-Hippo may be accomplished through hypermethylation of RASSF1A and may play an essential role in Ewing's sarcoma and Osteosarcoma. The methylation pattern of Sav-RASSF1-Hippo tumor suppressor pathway in human bone cancer along with RASSF1A expression with its effector proteins merits further investigation. This may reveal how the RASSFIA has a physiological signal transduction, including how the process of its deregulation can contribute to transformation of the cell, eventually leading to the incorporation of novel therapeutic options with improved prognosis for bone cancer.

Keywords: RASSF1A, Osteosarcoma, DNA Methylation, Epigenetics

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INTRODUCTION

A primary malignant tumor of the bone known as Osteosarcoma, results in formation of immature bone tissue by tumor cells. According to the World Health Organization, bone tumors are classified into central and surface tumors (1). Almost 90% of all osteosarcoma cases are represented with central high-grade primary Osteosarcoma, which is the third most common type of neoplasia after leukemia and lymphoma, among children and adolescents (2). Treatment mainly involves conducting standard chemotherapy before surgery and after completion and then subjecting it to radiation with a 5-year survival rate of 60–70%. However, the survival rate of patients with locally advanced or metastatic tumors remains low (~20%), with the median survival time of only 23 months (3). Evidence from osteosarcoma studies suggests that Osteosarcoma could be a result of failure in the differentiation program of the cells of origin mesenchymal stem cells (4). Moreover, chromosomal instability (CIN) is a hallmark of Osteosarcoma, which represents a high level of genomic instability (5). However, the mechanisms underlining osteosarcoma metastasis are still unidentified. Thus advances in identifying biomarkers for osteosarcoma metastasis and therapeutic regimens are urgently required.

Both genetic and epigenetic events control cancer initiation and progression. Epigenetics is defined as altering gene expression while maintaining the DNA sequence (6). The epigenetic aberrations are reversible, which is a great potential to develop epigenetic therapies to treat cancer (7). The most important epigenetic mechanisms are MicroRNA, histone modifications, and DNA methylation (8, 9). DNA methylation is defined as the covalent addition of a methyl group to the nitrogenous base cytosine at CpG dinucleotides on the DNA strand (10, 11). Methylation of CpG dinucleotides result in inactive promoter which associate with a "closed" chromatin structure, inaccessible by the transcription factors (12). Altered methylation pattern leads to genomic instability, which result in the initiating of tumorigenesis and persistence in the malignant state of cancer cells (7, 13). DNA methylation represses the tumor suppressor's gene expression and therefore removes regulatory proteins required for normal cell growth and development and thus causing cancer (14, 15).

Evidently, RASSF1A is a compelling tumor suppressor, and considered as one of the most perturbed genes that contributes to human cancers. Interestingly, RASSF1A functions as an upstream regulator of Hippo pathway, importantly, the Hippo/ mammalian STE20-like protein kinase MST1and MST2 pathway has a connection with its tumour suppressor activity. Though, the Hippo pathway comprises of a large network of kinases cascade that control cell proliferation and cell death (16). The kinases consisting of MST1 and MST2 cascade include large tumor suppressor LATS1 as well as LATS2, including the adaptor proteins Salvador homologue 1 (SAV1) (16). Activation of MST1 and MST2 results in phosphorylation of LATS1, LATS2 and downstream cell cycle regulation and apoptosis (17). RASSF proteins are critical in the regulation of the MST1 and MST2 by preventing forming a complex with a protein called RAF1, and cleavage of this inhibitory protein is actively regulated by RASSF1A which activates the hippo pathway (18). Sav1 interacts with MST1/2 forming a complex that phosphorylates the LATS proteins. When phosphorylated, LATS1 and LATS2 activate Yap1 protein which leads to its nuclear relocalization and binding to p73 and induces apoptosis (**Figure 1**) (18, 19).

It is worth noting that, RASSF1A is a tumor suppressor gene that assumes the role of upstream regulator of Hippo pathway initiating kinase cascade that phosphorylates and negatively regulates transcription by transcriptional coactivators. Hyper methylation of RASSF1A results in the loss of function of the Hippo pathway which triggers Osteosarcoma. When RASSF1A restores the MST2 and interaction with LATS1 leads to phosphorylation of LATS1 by MST2, resulting to YAP1 phosphorylation as well as its nuclear translocation therefore, inhibiting YAP1 oncogenic function. Eventually, YAP1-p73 complex is formed and induce apoptosis, when the pathway is activated by RASSF1A.

The process of CpG methylation islands, associated with the promotor regions of RASSF1A, results in reduced expression of the functional protein and loss of the tumor suppressor activity (20, 21, 22, 23). The role of the hippo pathway in cancer has been extensively studied, with events such as mutations and methylation of the promoters present in many different cancers and correlating with poor prognosis (24). To our knowledge, with regard to primary bone tumors, limited studies from the past to current publications have synthesized the presence of changes in methylation for several genes, combining RASSF genes. Due to the fact that methylation changes frequently appear during the early stages of the disease, the detection of hypermethylated genes may help in the identification of tissues derived from patients with increased risk.

The aim of this study is to identify the prevalence of RASSF1A promoter hypermethylation that may alter (Sav-RASSF-Hippo) pathways, thus contributing to the cancer phenotype. Investigating the potential activation of the Sav-RASSFIA-Hippo tumor suppressor pathway at the pathogenesis of bone cancer involved the analysis of hypermethylation of RASSF1A in bone cancer cell lines. The process involved the bisulfite modification of DNA and then the methylation-specific PCR.

MATERIAL and METHODS

Cell culture

All the six cell lines namely, U-2-OS, A-673, Saos-2, SK-EK-1, HT-1080 and MG-63 were assessed and purchased from American Type Culture Collection (ATCC) (Manassas, VA, USA). All media for cell cultures were acquired from ATCC, whereas supplements such as fetal bovine serum (FBS) and trypsin-EDTA were obtained from Gibco (Gaithersburg, MD, USA). The brief characteristics of cell lines and protocol for cell culture and maintenance are given below.

Cell lines

U-2 OS: Cell line U-2 OS is altered in chromosomal way, having chromosome counts in the hypertriploid range. This cell line was obtained from a moderately differentiated sarcoma of the tibia of a girl aged 15 years. For culturing, vials were softened or melted by introducing a gentle agitation at a temperature of 37°C water bath and decontaminate using a spray containing 70% of ethanol. The content of vial was then kept in a centrifuge tube containing 9.0 ml of an entire culture medium and was spun at approximately 125xg for 7 minutes. The cell pellet was suspended in component of McCoy's 5a Medium (ATCC) having 10% FBS (Gibco) and transferred into a culture flask calibrated at 75 cm2. The cells were kept warm at 37^0C using an incubator containing 5% CO_2.

A-673: The A673 cell line was obtained from a patient that had a probability of primary rhabdomyosarcoma. Such kinds of cells have a history of producing several growth factors with oncogenic potential, including cell growth-inhibitory factors. Hence the cell lines were made to grow using the Dulbecco's Modified Eagle's Medium containing 10% FBS.

Saos-2: Saos-2 is derived from the primary Osteosarcoma of an 11-year-old Caucasian girl. For culturing, cells were subjected to growth using McCoy's 5a Medium that was made of 15% FBS.

SK-ES-1: SK-ES-1 is a human Ewing sarcoma (anaplastic Osteosarcoma) cell line that displays epithelial morphology and grows in adherent. This cell line was established in 1971 from a bone biopsy in an 18-year-old Caucasian male with

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Ewing's sarcoma. For culturing, cells were subjected to the process of growth in McCoy's 5a Medium having 15% FBS.

HT-1080: The cell line is derived from tissue taken in a biopsy of a fibrosarcoma present in a 35-year-old human male. Briefly, vials were softened gently at a temperature of 37°C water bath and decontaminate and then sprayed using 70% ethanol. The contents of vials were then poured into a centrifuge tube containing 9.0 ml of complete culture medium and were spun at approximately 125xg for 7 minutes. The cell pellet was suspended in Eagle's Minimum Essential Medium (EMEM) containing 10% FBS and poured into culture flask with a capacity of 75 cm2. The cells were kept warm at a temperature of 37 ^{0}C and by using an incubator containing 5% CO_2.

MG-63: MG-63 cell line is derived 14-year-old Caucasian male suffering with an osteosarcoma. These cells were grown in EMEM containing 10% heat-inactivated FBS.

Protocol for the Process of Subculturing

The process began by removing the culture medium followed by rinsing of the flask using 0.25% -EDTA solution (0.03%). Subsequently, an additional 2 ml of trypsin-EDTA solution was added and the flask was maintained at a temperature of 37^{0}C until the cells detached. This followed by an addition of fresh culture medium and the cells and dispensing of the cells into new culture flask with subcultivation ratio of 1:4.

Freezing cells

Back-up cells were made using Cryopreservation for future studies. The medium was separated from flask; the cells were cleaned and trypsinized as well as using trypsin-EDTA solution. Once cells are detached, 5-10 ml media was added to the flask and the contents are transferred in to a 15 ml centrifuge tube. The cells were pelleted by spinning down at 1500 rpm for approximately 5 minutes and then followed by removal of the medium. Cells were then re-suspended in enough in a medium of a freezing state (Complete culture medium + 5% DMSO), creating a cell suspension and aliquot of about 1ml were made into storage vials. Cells were immediately transferred to -20°C for one hour, followed by -80°C overnight before permanent storage in liquid nitrogen.

DNA extraction

The cells were harvested and were transferred into 1.5ml centrifuge tubes. The Genomic DNA was obtained using Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). Briefly, according to the manufacturer instruction, the cells were lysed using nuclei lysis buffer, and RNase digestion step was included at this time. The cellular proteins are then removed by a salt precipitation step, which precipitates the proteins but leaves the high molecular weight genomic DNA in solution. Finally, the genomic DNA is concentrated and desalted by isopropanol precipitation. The DNA concentration and purity (260/280) were checked using Nano-drop spectrophotometer.

Bisulfite conversion

Bisulfite conversion of DNA is possible due to the presence of bisulfite-mediated and chemically conversion of unmethylated cytosine residues into uracil. Evidently, there was no change in Methylated cytosine residues. Sequence-specific PCR primers can distinguish unmethylated genomic regions after bisulfite conversion (**24**).

The other approach involved subjecting Aliquots of 2 µg of each DNA sample in bisulfite modification by employing the Methyl Edge Bisulfite Conversion System (Promega) following the manufacturer instructions. The next stage involved a brief mixing of 2 µg of DNA with 130 µl containing bisulfite conversion reagent. Then, there was bisulfite conversion using an effective thermal cycling program: denaturation at 98°C for 8 minutes followed by another warm maintenance at 54°C for 60 minutes. After re-using, the bisulfite-converted DNA purification on a spin column and eluted in a volume of 20 µl and stored at -20˚C.

Methylation analysis

At this level, methylation-specific PCR (MS-PCR) was used in the analysis of the methylation status of supporter areas of RASSF1A. One hundred nanograms of bisulfate preserved DNA were PCR improved in 20 µl reaction buffer containing 10x Taq hot start master mix (New England Biolabs, Ipswich, MA).

Primer sequences for Methylation-specific primer forward (MSP-F): CGAGAGCGCGTTTAGTTTCGTT;

Methylation-specific primer reverse (MSP-R): CGATTAAACCCGTACTTCGCTAA;

Unmethyaltion specific forward (USP-F): GGGGGTTTTGTGAGAGTGTGTTT;

Unmethyaltion specific reverse (USP-R): CCCAATTAAACCCATACTTCACTAA.

PCR reactions for all the genes were performed using Touchdown (TD) PCR method.
TD-PCR provides modest and fast methods to enhance PCRs, aggregating specificity, compassion, and yield, without extending the length and design of the primers. Any alteration in Tm between the right and wrong annealing will result in an exponential benefit of the twofold per cycle (**25**). The cycling program for TD-PCR comprises two discrete stages. Stage 1 is the landing point that consists of ten cycles with a galvanizing temperature above the melting temperature (Tm) of the primers being used and changes to a less annealing temperature over the course of consecutive cycles. Phase 2 is a generic intensification period of 20 or 25 cycles using the last galvanizing temperature stretched in Phase 1. Phase 1 annealing temperature of 63 ^{0}C to 53 ^{0}C for both MSP & USP primers, for 10 cycles followed by phase 2 with annealing temperature of 53 ^{0}C for 30 cycles. The extension was performed at 68 ^{0}C for 30 sec. PCR products were unglued on 2.5% Agarose gel and envisioned with ethidium bromide.

The findings of abnormal supporter methylation of the RASSF1A gene in all six studied cell lines have been recurrent six times to promote the accuracy of the results.

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RESULTS

The results of aberrant promoter methylation of RASSFIA gene in all six studied cell lines have been repeated six times to ensure robust outcomes.

Methylation Analysis of RASSF1A

Genomic DNA of cell lines was treated with bisulfite alteration to illuminate the position of RASSF1A hypermethylation in sarcoma cell lines. Thereafter, we analyzed the status of RASSF1A promoter CpG Island methylation by MS-PCR. The PCR was conducted using two diverse mechanisms and galvanizing temperatures to prevent the probability of a non-specific PCR product. The use of a relatively great difference (50 ^{0}C) in Tm of the primers utilized, descent PCR procedure (highest galvanizing temperature 63 ^{0}C and lowest at 53 ^{0}C) was also tracked to prevent the occurrence of non-specific PCR products.

Two cell lines (lines (SK-ES-1 and HT-1080) from the six investigated were thoroughly methylated while three cell lines (U-2-OS, A-673 and Saos-2) were incompletely methylated and no cell line was left unmethylated. However, as demonstrated by (**Figure 2**) no methylation was found with MG-63.

MS-PCR was used to analyze the methylation position of the RASSF1A promoter area in sarcoma cell lines. MSP uses both Methylation-specific (m) and unmethylation-specific (u) primers, which were set on 2.5% agarose gels with the addition of a marker (100 bp). The expected PCR product for MSP is 192 bp and for USP is 204 bp. Touchdown PCR, annealing temperature (53 ^{0}C). N denotes normal DNA from leucocytes.

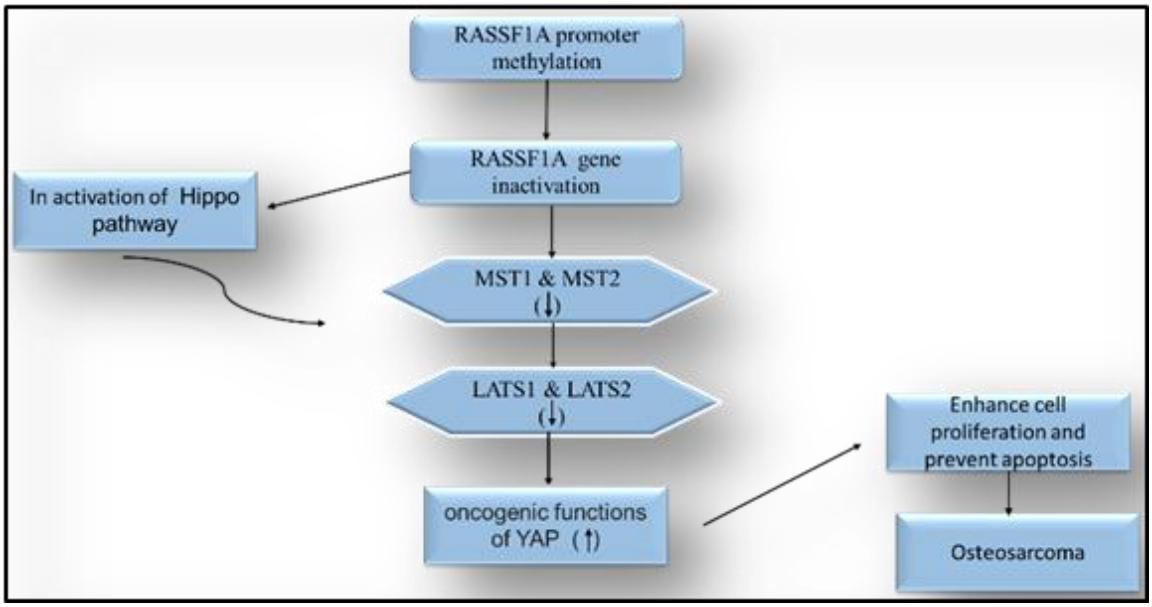

Figure1. Schematic diagram of RASSF1A protein signal and modulation of hipo pathway components. It is worth noting that, RASSF1A is a tumor suppressor gene that assumes the role of upstream regulator of Hippo pathway initiating kinase cascade that phosphorylates and negatively regulates transcription by transcriptional coactivators. Hyper methylation of RASSF1A results in the loss of function of the Hippo pathway which triggers Osteosarcoma. When RASSF1A restores the MST2 and interaction with LATS1 leads to phosphorylation of LATS1 by MST2, resulting to YAP1 phosphorylation as well as its nuclear translocation therefore, inhibiting YAP1 oncogenic function. Eventually, YAP1-p73 complex is formed and induce apoptosis, when the pathway is activated by RASSF1A.

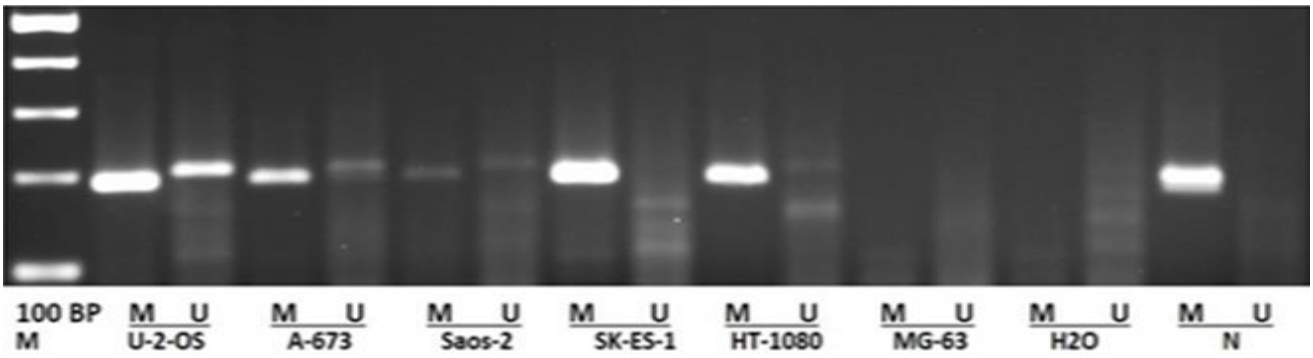

Figure 2. MS-PCR of the RASSF1A CpG Island. MS-PCR was used to analyze the methylation position of the RASSF1A promoter area in sarcoma cell lines. MSP uses both Methylation-specific (m) and unmethylation-specific (u) primers, which were set on 2.5% agarose gels with the addition of a marker (100 bp). The expected PCR product for MSP is 192 bp and for USP is 204 bp. Touchdown PCR, annealing temperature (53 ^{0}C). N denotes normal DNA from leucocytes.

DISCUSSION

Osteosarcoma is a malevolent tumor of the bone mostly found in children. Although there has been tremendous therapy to increase the rate of cancer survival, it is still the same place it was decades ago. One of the basic inactivation methods is silencing tumor suppressor genetic factors by epigenetic alteration. It reduces the impacts of cancer-related genetic factors in the pathogenesis of human tumors. Hypermethylation plays a significant function in suppressing tumor genes (26).

According to Jones and Baylin, RASSF1A is a vital tumor reducer gene that is involved in trials controlling cell cycle seizure and apoptosis. RASSF1A inactivation has been identified in various malignancies (26). Moreover, it has been inactivating at various high frequencies to produce positive outcomes. Aberrant methylation of RASSF1A was often perceived in many cancers. As demonstrated by Li et al., (27), RASSF1A methylation is found in 80% of minor cell lung tumors, in 90% of hepatocellular carcinomas, and more than 70% of prostate tumors. Moreover, RASSF1A was revealed to be a cancer suppressor in Osteosarcoma and presented to be methylated in Ewing sarcoma. The results of the study conquer with the previous report as they also show that RASSF1A was absolutely methylated in 2 Ewings sarcoma (SK-ES-1 and A-673) cell lines (28). Lim et al., also carried out a study on the manifestation of RASSF1A in key osteosarcomas and cell lines. The authors demonstrated a lack of RASSF1A manifestation in 4/10 key and 5/6 cell lines (29). Moreover, a recent study by Malpeli et al. showed that the methlytion of two CpG islands situated at RASSF1A promoter controls its manifestation. Although physiological suppression of RASSF1A manifestation by methylation remains unknown, it is believed to be through cell gesturing procedures (30). RASSF1A promoter hypermethylation leads to the alteration of its composite gesturing network that is essential for tumor growth (31). Moreover, studies have found that the hypermethylation of the RASSF1A promoter causes the transcription of its merging isoform RASSF1C that may bind to RASSF1A downstream effector (32, 33). This splicing also avoids the regulation of diverse biological purposes, including cell cycle seizure, apoptosis, migration, control of the microtubule network, and autophagy (34, 35). Demethylation agent (5-aza-2′ -deoxycytidine) was used to treat RASSF1A-negative cell lines and RASFF1A manifestation was upregulated, signifying that RASSF1A promoter methylation can be a probable strategy for the transcriptional suppression of RASSF1A (36, 37). The current study also established that the RASSF1A is methylated in 2/3 bone cancer cell lines (U2OS and Saos-2) to reduce the development of cancer. In contrarily, Harada et al. (38) demonstrated that the two cell lines (SAOS-2 and A-673) did not present methylation of the RASSF1A. Therefore, we had to repeat the experiment more than three times due to this contradiction. The experiments were conducted more than two times to prevent false results and conclusions. However, we found that the results were reproducible. Our results are supported by the work of Lim et al. (29) because they also found that a relationship between osteosarcoma and RASSF1A hypermethylation. Moreover, Dammann et al. also found that RASSF1A hypermethylation suppressed cancer genes. They demonstrated that out of eight of the pancreatic carcinoma, five cell lines (PATU-S, PATU-T, PATU-2, PaCa-2, and CAPAN2) were fully methylated, while two cell lines (HUP-T3 and HUP-T4) were partly methylated and only CAPAN1 was unmethylated (39).

Hippo is a preserved gesturing trail, first recognized in drosophila, and is significant in controlling organ growth, cell propagation, and cell loss. Conspicuously, the hippo/MST trail has been statistically related to the cancer suppressor action of RASSF1A. According to Aruna et al., primary elements that have been studied include LATS1, LATS2, MST1, MST2, and Sav1. Furthermore, DNA methylation was found to diminish MST1 appearance in glioblastoma and sarcoma (40). MST1 and MST 2 are the mammalian orthologues of the drosophila hippo protein and an existing primary element in the hippo trail. Aruna et al., (40) found that RASSF1 interrelates through its C-terminal domain with MST1and thus controls MST1 arbitrated apoptosis. Nevertheless, it is not yet determined whether a similar approach exists in Osteosarcoma. Recent evidence revealed that RASSF1 is participating in the crossroad of a compound gesturing network, which includes key regulators of cellular homeostasis, for instance, MST2/Hippo, Ras, p53, and death receptor pathways. Moreover altered methylation and expression of RASSF1A is one of the greatest actions in solid cancers (41).

Accruing proof backs the utilization of RASSF1A hypermethylation as a prognostic biomarker that relates with deprived prognosis, and demonstrates that its inactivation has a primary task in tumor growth. For instance, studies investigating neck, head, and renal cancers revealed that cancers with high levels of RASSF1A methylation not only have a negative outcome but development to metastatic illness significantly earlier than other cancers (42, 43, 44). Therefore, the initiation of RASSF1A is an effective mechanism for treating numerous cancer types.

CONCLUSIONS

RASSFs suppress protuberant cancer and control microtubule steadiness, apoptosis, and cell cycle. Although the specific process of the RASSF biological task is complex, comprehending it is highly beneficial because it may lead to the discovery of effective anticancer drugs. The results of the study demonstrate epigenetic dormancy of RASSF1A through methylation was evident in Osteosarcoma, fibrosarcoma, and Ewing's sarcoma. RASSF1A gene methylation analysis can serve as the foundation of cancer diagnostic tests. Classifying different methylated CpG investigations between the osteosarcoma cell lines helps identify novel epigenetic biomarkers that can probably provide optional methods of recognizing cancer subtypes. Future studies on methylation data analysis will determine whether there exists a correlation between tumor stages, metastasize, and survival rate.

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Ethical approval: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by Local Ethical Committee.

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Effect of intermittent hypoxic intervention on aerobic and anaerobic performance of the elite athletes

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ABSTRACT

Objective: The use of hypoxic training has increased to improve the performance of endurance athletes in recent years. Due to not having the suitable conditions and environment for each athlete and team, intermittent hypoxic training has been noted. The purpose of this study is to investigate the effect of intermittent hypoxic training on aerobic and anaerobic performance of elite athletes.

Materials and Methods: A total of 40 elite distance athletes were taken into our study and divided into two groups as hypoxia and normoxia. While using the intermittent intervention for the hypoxic group 5 minutes intervals for a total of 1 hour per day, 3 days per week for a-4 week period, the same normoxic training protocol was used for the normoxic group. Aerobic and anaerobic performance parameters were measured with venous blood samples of the athletes in the first three days before and after hypoxic intervention.

Results: When the hypoxia and normoxia groups were evaluated before and after intermittent hypoxia, there was no statistically change in aerobic and anaerobic performance values ($p>0.05$).

Conclusion: We observed that there was not a statistical change of intermittent hypoxic intervention for the performances of hypoxic group. However, the more dose and the duration of hypoxic training, the more amount of performance gain can be achieved.

Keywords: Intermittent hypoxic intervention- aerobic performance-anaerobic performance

INTRODUCTION

The use of hypoxia training has been increasing in recent years among athletes to increase performance. However, since not every athlete or team has the appropriate geographical environment and conditions for hypoxia training, the use of various hypoxic training methods has been considered. While there is a significant amount of studies investigating the effects of adaptation and response mechanisms that depending on due to the acute and chronic hypoxia on sportive performance, studies on the effects of intermittent hypoxic training are limited (1-4). In general, authors using hypoxic training modalities evaluate that responses to acute or chronic hypoxia differ individually (6-8). The purpose of hypoxic training methods is to increase the performance of the hypoxic adaptation gained (5-8). Due to the difficulties of hypoxic training due to high altitude (such as suitable equipment, cost, and time constraints), various alternative methods and equipment have been developed to gain similar adaptations. While designing hypoxic training equipment and protocols, it is taken into consideration to provide the athlete with the least possible discomfort and maximum performance increase (10). Although the method and protocol to be used for Intermittent Hypoxic Training (IHT) are important, the type of athlete to be used and the dose and duration of hypoxia exposed are also important (9). In the studies of Bonetti and Hopkins, it was evaluated that intermittent hypoxic training could be effective in sub-elite or recreational athletes rather than elite athletes (9). In our study, it was aimed to investigate the effects of intermittent hypoxia application on aerobic and anaerobic performance of elite athletes.

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MATERIAL and METHODS

Participants

A total of 40 athletes, 20 from the Gendarmerie Athletic team and 20 elite male long-distance runners from the Turkish Athletics Federation were included in our study.

Twenty of the athletes were included in the hypoxia group, while the other 20 were included as the normoxia group. It was learned from the participants that similar training programs were used before and after the competitions. The height and weight of the athletes were measured with Tanita bc 418 body analysis machine. Following the detailed anamnesis, musculoskeletal examination, ECG, and routine laboratory tests of the athletes recorded, patients with acute respiratory tract infection, a chronic febrile illness, a family history of sudden death, and a chronic sports injury were not included in the study. All athletes volunteered to take part in this study. Written informed consent form was obtained from each participant, and ethical approval was obtained from the Human Research Ethics Committee of the Institution (15/02/11-168).

Intermittent Hypoxic Intervention

In the study, athletes were divided into two groups as hypoxia and normoxia. The intermittent hypoxic intervention(IHI) procedure used by Hamlin MJ et al. was adapted to our study (**18**). Hypoxia was applied to the hypoxia group for four weeks, three days a week, for 60 minutes a day, for a total of 6 hours. Five minutes of normal ambient air (normoxia) followed by five minutes of hypoxia (15% FiO_2) were applied during each 60-minute hypoxic condition. The amount of hypoxia was applied by starting with 15% FiO2 and decreasing it by 1% every week to 12% FiO_2 oxygen concentration and 90-95% O_2 saturation during the 4th week. It was observed that there was a correlation between FiO_2 application and weekly O_2 saturation.

Normoxic group was ventilated with ambient air (21% FiO_2) at five-minute intervals. Subjects were instructed to remove their masks during the normoxia period following each hypoxia period. During the hypoxic intervention, the arterial oxygen saturation and heart rate variability of the individuals were monitored by a portable pulse oximeter from the fingertips. The hypoxic environment was created with the GO_2 Altitude hypoxicator (Biomedtech, Victoria, Australia) device that can mimic the oxygen density in the range of 9-21% with a mouth nose mask. The most important advantage of the GO_2 altitude hypoxicator device used in the study is that diluting the oxygen gas in the normal air, providing real-time monitoring of the hypoxic environment within very narrow limits during the application session, as well as an automatic biofeedback system that provides the status of hypoxia. Individuals who could not attend more than two of the 12 total sessions throughout the study were excluded from the study. At the beginning of the study, both groups were told not to train for 1 hour before and after the session.

Before the aerobic and anaerobic performance tests, all the athletes were informed about the study protocol. For the VO_2 aerobic performance test, the bicycle ergometer that the athletes would sit on, was specially adjusted for the athlete. ECG electrodes were placed in the appropriate chest leads. Then, the mouth and face mask were settled on the athlete's head in such a way that they could not get air. During the test, the blood pressure monitor, which was connected to the system, was attached to the right arm and blood pressure was monitored on the monitor along with the pulse (Figure-1). Before the aerobic performance test, the athletes were adapted and warmed up for 2 minutes at a workload of 50 watts with 50-60 rpm. In order for the athletes to reach the maximum exercise intensity, the system was automatically increased by 25 watts every 3 minutes. During the test, the exhaled air in each expiration was evaluated by the Viasys metabolic measurement analyzer with the breath by breath method and automatically recorded to the computer. The criteria for reaching maximal exercise intensity were that the athlete could not continue the test, reaching the maximal heart rate, and falling the pedal speed below 60 Rpm. The test was ended in the presence of two of the three criteria. Oxygen consumption and carbon dioxide production graphs against time were continuously monitored during the test. The test was ended when the athlete showed dizziness, chest pain, severe dyspnea, pallor, bruising, confusion, and paroxysmal supraventricular tachycardia, prominent left bundle branch blocks, and a horizontal depression of more than 0.2 mm in the ST segment on the EKG. The test acceptance criteria were considered to be that reaching the 95% of the athlete's maximal heart rate (220-age) or the Respiratuar Quotient (RQ) value was above 1.0.

In our study, anaerobic capacity was measured by using the Wingate anaerobic performance test. The personal, physical characteristics and the contact information of the athletes were recorded. The seat height of the bicycle ergometer was adjusted to create a flexion angle of 15-200 at the athlete's knee joint, and a weight of 75 gram per kilogram of body was loaded onto the weight pan of the ergometer (Figure-2). First, the participants were asked to pedal the bicycle with all their strength without any weight loading. The maximum number of revolutions was noted. After a two-minute rest interval, the test was started. During the test, when the maximum speed was reached, the weight on the pan was reflected on the wheel as resistance. Athletes were asked to pedal against this resistance for 30 seconds using all their strength. As a result of the test, the maximum, average, and minimum power were automatically determined and recorded by the software of WAnT.

Hematological Measures: Participants were required to visit our laboratory to provide a blood sample from the forearm vein. Blood samples were obtained 2-3 days before the intervention (IHI) and 7-10 days post-intervention . Blood was analyzed for hemoglobin (Hb), ferritin, erythrocytes and reticulocytes.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences software version 17.0 (SPSS Inc., Chicago, IL, USA). Mean, median, standard deviation, minimum and maximum values were used to define the data. Evaluation of the data was done non-parametrically. A two-way repeated-measures ANOVA was performed to determine the effect of different treatments (IHI or NORMOXI) over time (pre/post) on all measured variables.

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In the comparisons between groups, Student-T test was used for data with normal distribution, and Mann-Whitney-U test was used for data that did not. Student-T (Paired samples T test) was used for dependent groups for within-group comparisons. Pearson correlation test was used to evaluate the relationships between variables and a $p<0.05$ value was considered significant in statistical evaluations.

RESULTS

Physical characteristics of athletes

The ages, heights and weights of the athletes in the hypoxia and normoxia groups are given in **Table 1**, respectively. There was no statistically important difference between hypoxia and normoxia groups in terms of physical characteristics ($p>0.05$).

Hematological parameters of athletes before/after intermittent hypoxic intervention

The hematological parameters of the athletes before and after the intermittent hypoxic intervention(IHI) were given in **Table 2**. No statistical difference was observed in the hypoxic and normoxia groups before and after hypoxic intervention ($p>0.05$).

Aerobic performance values of the athletes before/after intermittent hypoxic intervention

The maximal oxygen consumption of the hypoxia group and the oxygen consumption values at the anaerobic threshold were given in **Table 3**. There was no statistical difference between VO_2 max values and VO_2 max values at anaerobic threshold before and after hypoxic intervention in both groups ($p>0.05$).

Anaerobic performance values of the cases before/after intermittent hypoxic intervention

Anaerobic power performance parameters of both groups were given in **Table 4**. In the hypoxia group in which we investigated the effect of hypoxic intervention, the statistically important difference in anaerobic parameters was not found before and after intervention ($p>0.05$).

DISCUSSION

Numerous conflicting studies have been reported on the effect of acute and chronic hypoxia on aerobic performance (**11-14**). Results of several studies for intermittent hypoxic intervention (IHI), shown that there was no increase in maximal oxygen consumption after intervention (**11, 14**). On the other hand, Bonetti et al reported that IHI increases the aerobic performance, but this was not associated with maximal oxygen consumption (**12, 13**). Gore et al. discussed that besides hematological parameters, changes in a number of cardiac, respiratory mechanisms, molecular and lactate buffering capacity might be effective in the development of aerobic performance after hypoxic training (**15**). Therefore, it is seen that all hypoxic training and intervention protocols, including IHI, are not effective only via hematological parameters at increasing maximal oxygen consumption. In a similar study on skiers, an increase of 5-10% in repeated ski sprint performance was determined after 15 sessions of IHI (**16**). The reason for this increase may be the long hypoxic interval exposure time and a performance test method known to the athletes as aerobic performance criterion.

Table 1. Physical characteristics of the athletes

	IHI (n=20) Ortalama ± SD (Min –Max)	NORMOXİ (n=20) Ortalama ± SD (Min –Max)	($P > 0.05$)
Age	24.8±2.87 (21-30)	25.46±2.09 (21-29)	
Weight	67±3.67 (58-72)	64.4±3.08 (58-70)	
BMI(kg/m^2)	23.6 ±1.8	22.9 ± 1.5	

Table 2: Baseline and post-intervention hematological parameters of athletes

	IHI		NORMOXIA	
	Baseline	After IHI	Baseline	Post Intervention
Hemoglobin (gr/dl)	15.64±0.44	15.72±0.43	15.76±0.46	15.78±0.45
Hematocrit	46.53±1.33	46.85±1.27	45.34±1.52	46.44±1.54
Eritrosit/mm^3	5.15±0.22	5.21±0.19	5.11±0.17	5.16±0.15

Table 3: Maximal oxygen and maximal oxygen consumption at anaerobic threshold test values of the groups at baseline and post-intervention

	IHI		NORMOXIA	
	Baseline	Post IHI	Baseline	Post-intervention
VO_2 max (ml/kg/dk)	66.12±1.58	67.02±1.67	67.76±1.32	67.94±1.16
VO_2 max (ml/kg/dk) (Anaerobic threshold)	57.78±1.41	58.66±1.13	59.91±2.16	60.71±0.91

IHI: Intermittant Hypoxic Intervention, **VO2 max:** Maximal oxygen consumption, **VO2 max (ml/kg/dk) (Anaerobik Threshold**): Oxygen consumption at anaerobic threshold

Table 4: Anaerobic performance values of the groups before and after the intervention

	IHI		NORMOXIA	
	Baseline	Post IHI	Baseline	Post intervention
Maximal Power watt/kg	10.57±0.81	10.68±0.79	11.47±0.87	11.44±0.80
Mean Power watt/kg	6.13±0.34	6.16±0.42	6.08±0.23	6.16±0.28
Minimum Power watt/kg	3.10±0.37	3.17±0.49	3.31±0.41	3.27±0.53

Babrock et al. also did not find a significant change as a result of 19 sessions of IHI (5 min hypoxia, 5 min normoxia) intervened by 10 recreational skiers for 3 weeks, based on lactate threshold and maximal oxygen consumption as aerobic performance criteria (17). In a study, a significant increase was found between the 3 km time-tested running tests performed on the 2nd and 17th days after 17 sessions of 90-minute IHI (5 min hypoxia, 13-10% FiO_2 5 min normoxia) to 22 non-elite athletes in different athletics branches (18). This increase may be due to lower hypoxia dose, longer application sessions and differences in the training level of the athletes. Hinckson et al. also did not detect any significant change in 5 km running performance against time after 20 sessions of 90 minutes of IHI (6 minutes hypoxia and 4 minutes normoxia; SpO_2 92 -80%) on 11 elite mountaineers, seven women and four men (19).

It is accepted that hypoxia does not have a positive effect on anaerobic performance. In our study, we did not find a significant increase in the average and maximal power of elite distance runners in the Wingate test. Tadibi et al. did not report an increase in average and maximal strength in endurance athletes after 15 sessions of IHI, evaluated with the Wingate test (11). Likewise, Bonetti et al. did not detect a significant increase in repetitive sprint times in elite cyclists and triathletes after 15 sessions of IHI (10). Bonetti et al. again did not show an increase in peak and mean strength in the elite skiers with a control group of 10, after 19 sessions of 4 weeks of IHI (13). In another study, Bonetti et al. found no difference in repetitive sprint times in 18 elite triathletes without a control group with a similar IHI protocol as in the previous study (12). Also, Hinckson et al. did not find a difference in repeated sprint performance tests against time after 14 sessions of IHI (6 min hypoxia, 4 min normoxia) in 10 rugby players (20). As an anaerobic performance test, studies were mostly evaluated with sport-specific tests such as repeated sprint tests against time that the athletes were familiar with. With the Wingate test we used, Tadibi et al. did not report an increase in mean and maximal strength after 15 sessions of IHI (6 min hypoxia 10-11% FiO_2, 4 min normoxia) at ten marathoners with the control group (10).

The purpose of hypoxia is to increase the release of Erythropoietin (EPO) hormone by accelerating the synthesis of erythrocyte precursor series in the bone marrow and; as a result to improve the oxygen carrying capacity to the tissues (21, 23). In our study, also an increase in hemoglobin, hematocrit and erythrocyte levels were not observed as a result of a study performed with 15 sessions of IHI (6 min hypoxia, 4 min normoxia; 10-11% FiO_2) to the elite athlete hypoxia group of 20 distance runners (11). After 20 sessions of IHI (5 min hypoxia, 5 min normoxia; 10-12% FiO_2) performed on 14 elite skiers with the control group, in which EPO was studied, no difference was observed in the amount of erythrocytes and hemoglobin together with the amount of EPO hormone (14). After 15 sessions of IHI (5 min hypoxia, 5 min normoxia; SpO_2 90 -76%) at elite skiers and cyclists, no significant change was observed in the amounts of reticulocyte, hemoglobin, and 2-3 diphosphoglycerate (12, 13). In another study, Hinckson et al did not report a significant change in hemoglobin, hematocrit and reticulocyte measurements after two days of 14 sessions of IHI (6 min hypoxia, 4 min normoxia; SpO_2 100-76%) in 10 elite rugby players (20). Although increased physical performance increases with IHI were observed in some studies, it was evaluated that these increases were not related to hematological parameters (24). Therefore, when people living at high altitudes in 38 different parts of the world were examined, it was found that there were significant hematological differences compared to each other (24). While the inhabitants of the Andes mountain were significantly different from those living at sea level, this ratio was found to be relatively normal for the inhabitants of Tibet (24).

We evaluated that a 4 week IHI program did not contribute to the aerobic and anaerobic performance of elite distance athletes. We did not observe the expected increases in hematological parameters, which is the most emphasized mechanism in terms of performance improvement.

CONCLUSION

Contrary to the studies in the literature with acute and chronic hypoxic intervention, we found that intermittent hypoxic intervention did not provide aerobic and anaerobic performance enhancement. There was no definite consensus on what the dose and duration of the hypoxic interval should be in previous studies. Aerobic and anaerobic performance enhancement can be achieved by increasing the dose or the duration of the hypoxic intervention. It may be another research topic that IHI can provide performance increase in sub-elite or recreational athletes instead of elite athletes applied in our study.

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The status of anatomy education in scope of the national core education program 2020: The case of Turkey

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ABSTRACT

Objective: Many branches of science are constantly changing and developing according to the innovations and the requirements of the era. Developments and medical information, especially in the field of medicine, renew itself every year. In this study, we aimed to analyze comparatively the place of anatomy education, which has an important place in undergraduate medical education, in the National Core Education Program (NCEP)-2020.

Material and Methods: In this study, the categories of basic medical practices in NCEP-2020, clinical symptoms/findings/situations, core diseases/clinical problems and the relationship between learning levels and anatomy education were evaluated one by one. Parameters were expressed in percentile using a descriptive statistical method. There are 157 Basic Medical Practices in 9 sub-categories (A-I) within the scope of NCEP-2020.

Results: It was determined that 48 of these were related to anatomy education. The number of basic medical practice was mostly in the E sub-category (n=23, 47.92%). The second sub-category with the highest number of basic medical practices was determined as B (n=16, 33.33%).

Conclusion: One of the important conclusions we have reached within the scope of the study is that NCEP-2020 has an important place in terms of scope in determining, developing and standardizing the educational curricula of medical faculties.

Keywords: Anatomy, Basic Sciences, Curriculum, Medical Education Research, Medicine

INTRODUCTION

Education has a very important role for people to inherit their culture to new generations. Although the beginning of education goes back to very old times, its treatment as a science is based on a recent history. Many branches of science are constantly changing and developing according to the innovations and the requirements of the era. Developments and medical information, especially in the field of medicine, renew itself every year. In addition, it is now a necessity to update medical education, methods and materials and to make them suitable for the conditions. One of the main conditions of being a modern society for Turkey is the modern education system as it is for every country (1,2).

It is observed that different dynamics in different time periods are effective in the development of medical education in the world. One of influential milestones worldwide is Flexner Report by Abraham Flexner (3). In this report, which is a major milestone for medical education, it states that the medical education is inadequate, education of different qualities is given and that the education model should be discussed again and comprehensively in the light of general education and training principles (3,4). Since the 1980s, due to the increase in the number of medical faculties in Turkey's medical education, different educational models and programs were implemented. Especially after the Edinburgh Declaration, which was accepted and published by World Conference on Medical Education organized by the World Federation for Medical

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Education in 1988, the studies in the medical education were more concentrated towards the end of the 1990s (5). The purpose of medical education is to train medical students who will improve the health of all people as stated in the Edinburgh Declaration (6). One of the main conditions for achieving this is the active use of up-to-date knowledge and experience in the field of education. In this regard, curricula have been developed with different committees and meetings since 2000s in order to structure the developing medical education in Turkey according to today's needs. As a result of these studies, the National Core Education Program (NCEP) was published for the first time in 2003 in order to provide an improvable medical education curriculum for medical faculties (7). NCEP-2014 has been published with its updated content in 2014. It was decided to review this program every six years due to the necessity of constantly improving it according to the needs of the day (8). However, as a result of the studies that started in 2014, NCEP-2020, which addresses a wide range of and different perspectives, was presented in April 2020. “Should there be a separate NCEP for basic medical sciences in NCEP-2020?” the question has come up. Instead, it was decided to include examples of how basic medical sciences can use NCEP in order to ensure integration in medical education (9).

NCEP contents of previous years do not include such a study on basic medical sciences. For an adequate and effective medical education, the six-year curriculum should be considered as a whole. Nevertheless, it is known that clinical information is difficult to settle without basic medical sciences. In this study, we aimed to analyze comparatively the place of anatomy education, which has an important part in undergraduate medical education, in NCEP-2020.

MATERIAL and METHODS

Ethics committee approval was obtained from Afyonkarahisar Health Sciences University Non-Invasive Clinical Research Ethics Committee for the study (Date: 04/30/2021, Decision No: 2021/310). In this study, the categories of basic medical practices in NCEP-2020, clinical symptoms/findings/situations, core diseases/clinical problems and the relationship between learning levels and anatomy education were evaluated one by one. Considering the basic medical sciences NCEP compliance table, it was analyzed according to the basic medical practices categories and learning levels related to anatomy education.

Statistical analysis

Study data were evaluated using the SPSS 24.0 program. First of all, the data were categorized. Qualitative and quantitative data were grouped. Other data were presented with descriptive statistics. Parameters were expressed in percentile using a descriptive statistical method.

RESULTS

When the NCEP-2020 sample table is examined, basic medical sciences are discussed in three categories such as Core diseases/Clinical problems, Clinical Symptoms/Conditions, and Basic Medical Practices. The distribution of all data by categories is shown in Figure 1. Symptoms, clinical problems and diseases under the categories have been determined by considering the whole of NCEP-2020.

However, it has been observed that symptoms, clinical problems and diseases that are expected to be reflected in the program in different periods during the six-year training program, which should be given more weight in each category, are particularly expressed. In this context, it has been determined that there are 68 (48.2%) symptoms, clinical problems, and diseases under the Symptoms/Conditions list and 97 (28.3%) under the list of Core diseases/Clinical problems. It was determined that these were necessary for basic medical sciences and should be included in the first three years of medical education.

It was determined that there are 157 Basic Medical Practices in 9 sub-categories (A-I) within the scope of NCEP-2020. It was determined that 48 of these were related to anatomy education. Basic medical practices and learning levels related to anatomy education were shown in Table 1.

A related practice between basic medical practice sub-categories A, G and H and anatomy education could not be detected. The number of basic medical practice was mostly in the E sub-category (n=23, 47.92%). The second sub-category with the highest number of basic medical practices was determined as B (n=16, 33.33%).

According to at the distribution of learning levels related to basic medical practices, it was found that the highest learning level was 3 (26 (54.17%)) and 4 (15 (31.25%)). The relationship between sub-categories and learning levels was presented in Table 2.

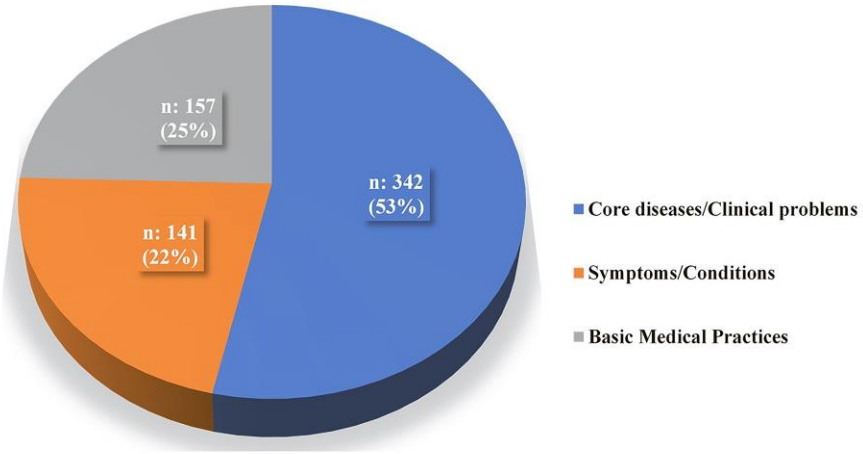

Figure 1: Basic medical sciences the National Core Education Program (NCEP) compliance categories

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Table 1: Basic medical practices related to anatomy education

Basic Medical Practices	Learning Level
A. Taking history	
B. General and problem-oriented physical examination	
B1. Forensic case examination	3
B2. Anthropometric measurements	3
B3. Abdominal examination	4
B6. Skin examination	4
B9. Evaluation of general condition and vital signs	4
B10. Eye fundus examination	2
B11. Eye exam	3
B12. Gynecological examination	3
B13. Cardiovascular system examination	4
B14. Musculoskeletal system examination	3
B15. Ear-nose-throat and head-neck examination	3
B16. Breast and axillary region examination	3
B17. Neurological examination	3
B19. Dead examination	3
B21. Respiratory system examination	4
B22. Urological examination	3
C. Record keeping, reporting and notification	
C6. To be able to prepare health reports in accordance with current legislation	3
D. Laboratory tests and other related procedures	
D1. To be able to apply the principles of working with biological material	4
D4. To be able to evaluate direct radiographs	3
D5. To be able to take and evaluate ECG	3
D19. To be able to prepare a sample of vaginal discharge	3
E. Interventional and non-invasive practices	
E9. Ability to apply bandage, tourniquet	4
E10. To be able to intervene in nosebleeds	2
E12. Evaluation of the multiple trauma patient	3
E13. Ability to establish vascular Access	3
E16. To be able to open a skin-soft tissue abscess	3
E17. Ability to take measures to stop / limit external bleeding	3
E28. Foreign body removal from the eye	2
E29. Ability to take biological samples from the patient	3
E33. To be able to provide first aid to remove the foreign body in the airway	3
E35. Intramuscular (IM), Intravenous (IV), Subcutaneous (SC), Intradermal (ID) injection	4
E36. Being able to insert a urinary catheter	3
E39. To be able to apply intraosseos	2
E40. To be able to measure blood pressure	4
E45. Enema	3
E46. To be able to perform lumbar puncture	1
E48. To be able to apply a nasogastric tube	3
E50. To be able to apply oxygen and nebul inhaler therapy	4
E51. To be able to apply oral, rectal, vaginal and topical drugs	3
E52. Autopsy	2
E59. Ability to apply a cervical collar	4
E61. To be able to evaluate respiratory function tests	3
E62. To be able to perform suprapubic bladder puncture	2
E67. Ability to take vaginal and cervical samples	3
F. Preventive medicine and community medicine practices	
F7. Teaching breast self-examination	4
G. Scientific research principles and practices	
H. Healthiness	
I. Health Screenings	
I2. Developmental hip dysplasia screening program	4
I3. Vision screening programs	4
I4. Hearing screening programs	4

Table 2: The relationship between basic medical practice subcategories and learning levels in terms of anatomy education

Learning Level	Basic Medical Practices Sub-categories						Total
	B	C	D	E	F	I	
1	-	-	-	1	-	-	1 (2.08%)
2	1	-	-	5	-	-	6 (12.5%)
3	10	1	3	12	-	-	26 (54.17%)
4	5	-	1	5	1	3	15 (31.25%)
Total	**16 (33.33%)**	**1 (2.08%)**	**4 (8.33%)**	**23 (47.92%)**	**1 (2.08%)**	**3 (6.25%)**	**48 (100%)**

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DISCUSSION

Core education program (CEP) is a concept used in all fields related to education. It emerged as a definition accompanying the development of academic sciences in the 20th century. It is used to refer to the common set of courses or curriculum that all students studying in an institution or discipline are deemed necessary to take, regardless of their choice. Basically, CEP, which was put into practice as an answer to the question of what every student should know and what he should be able to do, constitutes the common denominator of knowledge, values, sensitivities and skills related to that discipline (10).

In order to meet a common ground in medical education and to improve medical education, changes have occurred at different times depending on different dynamics in Europe, America and Turkey. Medical education has undergone many evaluations, developments and changes from the beginning of the 1900s to the present. The Flexner Report was published in 1910, examining the medical education in America and Canada, which had an important turning point in the history of medical education. In the Flexner Report, medicine was defined as an experimental discipline in which general laws of biology are valid, and it was stated that medical education should be structured on this general principle (3,11).

Another important step in medical education is the Edinburgh Declaration published in 1988. The declaration put forward by participants invited from six regions around the world has been widely accepted and applied in medical education by many countries. The declaration includes decisions such as determining the educational programs in consideration of national health needs and adding active learning methods to the curriculum to ensure lifelong learning (6).

Along with the published reports and declarations, thanks to the developing technology and today's conditions, the knowledge and practices in the field of medicine have developed greatly in recent years and a significant part of it has changed. As a result, some of the old medical skills have become unnecessary and some have changed. In addition, new skills that emerged as the product of new medical knowledge have taken their place in medical practice. Towards the end of the 20th century, the excessive theoretical load of the courses given in undergraduate medical education led to reform efforts trying to increase the quality of education programs. As a result of these efforts, concepts such as result-oriented core training program have emerged (12–14).

In Turkey, efforts to develop a core education program in this context started in the 2000s. As of 2003, NCEP-2003 was published and this process continued with updates in 2014 and 2020 (7–9). Prepared at the level of medical faculties, NCEP offers the general framework and basic principles that should be taken into account by education boards and departments during the development, implementation and improvement of all education programs in the pre-clinical and clinical period. However, a sample table for basic medical sciences was presented for the first time in NCEP-2020. Thus, it is aimed to ensure integration in pre-clinical and clinical period (9).

Anatomy is one of the departments included in basic medical sciences in the pre-clinical education process. Anatomy has a very important course in the first three years of basic medical education. In addition to enhancing basic medical performance, cadaver dissection and anatomical understanding are known to play an important role in appropriate clinical examinations, interpretation of radiographs, and execution of interventional procedures. In this context, the anatomy curriculum in medical schools at international level has been increasingly modified to expose students to multiple specialties before their clinical rotation and to accelerate their development (15–21). As stated in the literature, anatomy education should be carried out together with clinical education in today's standards. In the analysis in NCEP-2020, we determined that 48 (30.56%) of 157 basic medicine practices were related to anatomy education in accordance with the literature. In this respect, NCEP-2020 has a great place in ensuring integrity in terms of anatomy in national medical education.

Anatomy courses are one of the cornerstones of medical education, where clinicians develop their clinical skills. In this context, cadaver dissection to increase clinical skills is accepted as the gold standard in anatomy education (22,23). Also, it was predicted in the literature that anatomy education would be cadaver-free within ten years. It was believed that traditional anatomy education would be replaced by alternative training modules such as virtual dissection, medical imaging, and multimedia resources (24,25). Although today's technology and conditions have changed, cadaver dissection will always maintain its importance in anatomy education. In NCEP-2020, under the subcategories of basic medicine practices, forensic case examination, death examination and autopsy are included. Cadaver dissection training in the pre-clinical anatomy course will make a great contribution to the medical students to gain these competencies. Therefore, we are of the opinion that cadaver dissection-based anatomy course should be included in the scope of the national education program.

According to the data of the Council of Higher Education, as of 2020, there are medical faculties in 105 universities (74 state and 31 foundation) (26). According to the data of the Association for Assessment and Accreditation of Medical Education Programs, 41 of these medical faculties have accredited pre-graduation education programs. There are also 21 medical faculties that have applied for the accreditation of education programs. The purpose of medical faculties to be accredited is to contribute to the improvement of the quality of medical education in Turkey. Thus, physicians who have the knowledge, skills and attitude to respond to the health problems of the society with high quality health services by integrating the developments in science and society with medical practices, who have acquired the desire and ability to learn more than they know during their professional life, and who can contribute to scientific developments, are the level is aimed to be taken forward (27). One of the important criteria in accreditation is to evaluate medical education as a whole and to determine whether pre-graduate education programs meet the minimum standards.

From this point of view, the most important step for undergraduate medical education programs to meet the minimum standards is the NCEP. Within the scope of NCEP-

2020, symptoms, clinical problems and diseases that are expected to be reflected in the program continuously in different periods during six years of medical education are included under the titles Symptoms/Conditions and Core diseases/Clinical problems. Adding examples of basic medical sciences to the scope of NCEP-2020 will make a great contribution to the formation of the standard education program. This process may have a great contribution to ensuring the standard in medical education, maintaining integrity in the pre-clinical and clinical period, and increasing the number of accredited medical faculties.

CONCLUSION

One of the important conclusions within the scope of the study is that NCEP-2020 has an important role in terms of scope in determining, developing and standardizing the educational curricula of medical faculties. We believe that NCEP-2020, which has been implemented by all medical faculties, will be taken as an example for NCEPs to be prepared in the future by preserving its main structure. It is difficult to consolidate clinical knowledge without basic medical sciences. For this reason, the importance of basic medical sciences such as anatomy in NCEP files will ensure the standard throughout the entire education-training scope until graduation from the first year, in future. Thus, the number of accredited medical faculties will probably increase.

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Acute pancreatitis and low ascites-serum albumin gradient ascites caused by Brucellosis

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ABSTRACT

Objective: Brucellosis is a zoonotic disease seen widely around the world. Although many aspects and treatment of this disease is well known, peritoneal involvement and ascites is not well established so far.

Material and Methods: This study retrospectively enrolled 346 adult patients (aged >17 years) with acute Brucellosis attending Hepatology Clinic, Van Yuzuncu Yil University, between April 2013 and May 2016. Characteristics of those with and without ascites were analyzed using Pearson correlation coefficients and Chi-Square test in SPSS software system.

Results: Of the 346 cases, 20 (5, 7%) had ascites. Those with ascites had significantly higher transaminase, cholestatic enzyme and amylase levels compared to those without ascites.

Conclusions: We conclude that acute Brucella infection can lead to a unique low gradient ascites probably resulting from pancreatic leakage followed by peritoneal accumulation of serum proteins.

Keywords: Brucellosis, ascites, pancreatitis

INTRODUCTION

Various diseases can cause ascites, defined as accumulation of fluid within peritoneal space. Ascites is classified into two major groups depending on presence of portal hypertension. Calculation of serum ascites albumin gradient (SAAG) is the key to discriminate causes of non-portal hypertensive ascites (1). SAAG is calculated by extraction of ascites albumin from serum albumin. A value lower than 1.1 gr/dl indicates a non-portal hypertensive cause including peritoneal carcinomatosis, infectious peritonitis and other peritoneal diseases. Low gradient ascites has also higher levels of total protein levels compared to high gradient (portal hypertensive type) ascites (2). Brucellosis is a zoonotic disease caused by Brucella species B. abortus, B. melitensis, B. suis and B. canis (Table-1). Brucellosis could be viewed as an extinct disease in developed countries, but the prevalence of Brucellosis in many developing areas of the world is still high (3). Brucellae are small, gram-negative, non-motile, aerobic coccobacilli. Goat, sheep and cow are the reservoir of infection, and animal products including milk, cheese and butter can act as a bridge of transmission from animal to human. The disease is an emerging problem in Mediterranean Basin as well as elsewhere in the developing world (4). The disease has many clinical manifestations and complications including hepatitis, hepatic granulomas, peritonitis, sacroiliitis, spondylitis, meningitides, epididymoorchitis, vasculitis, bone marrow involvement (figure), pneumonia and pancreatitis. On the other hand, peritoneal involvement of the disease has rarely been reported and limited into a few case reports (5-9). Despite a well-established association of ascites with acute bacterial peritonitis, there are few data regarding the prevalence and nature of ascites in patients with acute Brucellosis. Thus, the aim of the current study is to clarify of some aspects of Brucellosis related ascites.

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MATERIAL and METHODS

The study retrospectively enrolled 346 adult patients (aged >17 years) with acute Brucellosis attending Hepatology Clinic, Van Yuzuncu Yil University, between April 2013 and May 2016. Twenty of the patients diagnosed with brucella had ascites in the abdomen. The approval of the ethical committee required to conduct the study was obtained from the Van Yuzuncu Yil University Faculty of Medicine, Clinical Research Ethics Committee(Non-invasive Clinical Research Ethics; Approved on 21.02.2020 with the 2020/02-03 number). Patients with liver cirrhosis, cardiac failure, chronic viral hepatitis, metabolic liver diseases, chronic renal failure and insufficient follow-up data were excluded from the study. Brucellosis was diagnosed by the presence of appropriate clinical signs and symptoms and at least one of these criteria: (i) positive standard tube agglutination test (STA) (a titer higher than 1/160); (ii) positive Coombs test (titer 1/160); (iii) isolation of Brucella organisms from cultures of blood, bone marrow, cerebrospinal fluid, other sterile sites, or tissue samples. Clinical and biochemical data were obtained from medical records. The diagnosis of Brucellosis-related ascites was made by both abdominal ultrasonography and computed tomography. Diagnostic paracentesis was additionally performed for every patient enrolled to obtain SAAG and other fluid parameters including complete blood count. On the other hand, 326 patients with acute Brucellosis without ascites were selected as a comparative group. All patients were treated with doxycycline plus rifampicin with respect to WHO guidelines. Patient consents were obtained.

Statistical Analysis

Descriptive statistics for continuous variables from the features mentioned; the average is expressed as standard deviation, minimum and maximum values, while categorical variables are expressed as numbers and percentages. One-way analysis of variance was performed to compare group averages for continuous variables. In determining the relationship between these variables, Pearson correlation coefficients were calculated separately in the groups. In determining the relationship between groups and categorical variables, Chi-square test was performed. The statistical significance level was taken as $p<0.05$ and the SPSS statistical software version 19.0 (SPSS Inc, Chicago, III, USA) pack has used for analyses.

RESULTS

Data of 20 patients with Brucellosis related ascites (with a male–female ratio of 1.5:1) were analyzed. In patients with ascites, the mean age was 43.6 ± 18.5 years. The mean age of control group without ascites was 56.7 ± 13.3. There was no significant difference between groups in terms of age and gender ($p>0.05$).

Serum acid albumin gradient was below 1.1 in all patients with ascites (0.6±0.3). The mean blood hemoglobin and hematocrit levels among ascites group were significantly lower compared to non-ascites group (11.1 ± 2.03 versus 12.12 ± 49.2; p=0.02; 33.56 ± 6 versus 37.350 ± 6,75; p=0.01 respectively).

The mean AST and ALT levels were found to be significantly higher in the ascites group compared to non-ascites group (630 ± 1405 versus 75 ± 336 and 454 ± 878 versus 51 ± 145 U/L.; all $p<0.001$).

Serum cholestatic enzyme levels were also analyzed as a categorical variable. Patients with ascites had a significantly higher serum alkaline phosphatase and gamma-glutamyl transferase levels compared to those without ascites (507 ± 489 versus 75 ± 336 and 183 ± 227 U/L, versus 70 ± 104 U/L.; all $p<0.001$).

In order to examine the role of Brucellosis-related pancreatitis on low gradient ascites, we also analyzed pancreatic enzyme profiles at both groups. Patients with ascites had higher levels both of amylase and lipase levels than those without ascites (1793 ± 2614 versus 75 ± 27 U/L; 1468 ± 1573 versus 57 ± 81U/L; all $p<0.001$). Additional analyses revealed that mean serum lactate dehydrogenase level was higher in ascites group (720 ± 469 versus 305 ± 108 U/L; p=0.03). In the acid fluid analysis of all patients, LDH level was found above 225 U/L (exudative).

The mean axial splenic vein diameters at both groups were measured using Doppler ultrasonography in combination with abdominal tomography. Statistical important difference was not found (134 ± 39 cm versus 122 ± 10 mm; p=0.318) (Table 2).

Inter-group comparisons were not significant for individual outcomes. No Brucellosis-related deaths were reported among study patients.

Table 1: Brucella species and Human prevalence (World Organisation for Animal Health 2006; Brucellosis in humans and animals)

Type	Reservoir	Other hosts	Prevalance in humans (%)
Brucellae mellitensis	Sheep, Goat, Camel	Cattle	70
Brucellae abortus	Cattle, Mandate, Jackal	Horse	25
Brucellae suis	Pig, Wolf, Fox	Cattle	5
Brucellae ovis	Sheep	-	No
Brucellae canis	Dog	-	Rare

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Table 2: Comparison of with acid and without acid patient parameters (AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, LDH: Lactate Dehydrogenase)

			n	Mean±Std. Dev.	Min-Max	P value
The mean of Brucella Aglutination Titer	Ascites	negative	326	541,29±447,752	160-1280	0,283
		positive	20	448,00±436,924	160-1280	
Age (years)	Ascites	negative	326	32,90±20,768	17-99	0,04
		positive	20	43,67±18,500	6-75	
Hemoglobin (gr/dl.)	Ascites	negative	326	12,125±2,0918	9,1-13,9	0,002
		positive	20	11,113±2,0336	7,7-13,6	
Hematocrit	Ascites	negative	326	37,350±6,7555	27,6-42	0,001
		positive	20	33,560±6,0085	22,8-42,1	
Platelet (/mm3)	Ascites	negative	326	259990±118617	21000-925000	0,549
		positive	20	235070±217370	33000-925000	
White Blood Cells (/mm3)	Ascites	negative	326	7,433±3,1274	1,7-25,8	0,879
		positive	20	7,080±3,3876	2,8-11,7	
ALT (U/L.)	Ascites	negative	326	51,4±145	5-2121	0,001
		positive	20	454±878	15-3044	
AST (U/L.)	Ascites	negative	326	75±336	9-5254	0,001
		positive	20	630±1405	13-5480	
Alkalyne phosphatese (U/L)	Ascites	negative	326	277±256	180-1838	0,001
		positive	20	507±489	83-1408	
Gamma glutamyl Transferase (U/L)	Ascites	negative	326	70±104.2	13-638	0,002
		positive	20	183±227	10,8-778	
Amylase (U/L)	Ascites	negative	326	75±27	13-174	0,001
		positive	20	1793±2614	55-8589	
Lipase (U/L)	Ascites	negative	326	57±81	5-536	0,00
		positive	20	1468±1573	21-4300	
Glucose(U/L)	Ascites	negative	326	97,37±31,759	56-366	0,08
		positive	20	153,83±58,854	56-254	
LDH (U/L)	Ascites	negative	326	305±108	178-411	0,002
		positive	20	720±469	327-1577	
Axial splenic diameter (mm)	Ascites	negative	326	122±10,001	100-220	0,318
		positive	20	134,42±39,330	98-220	

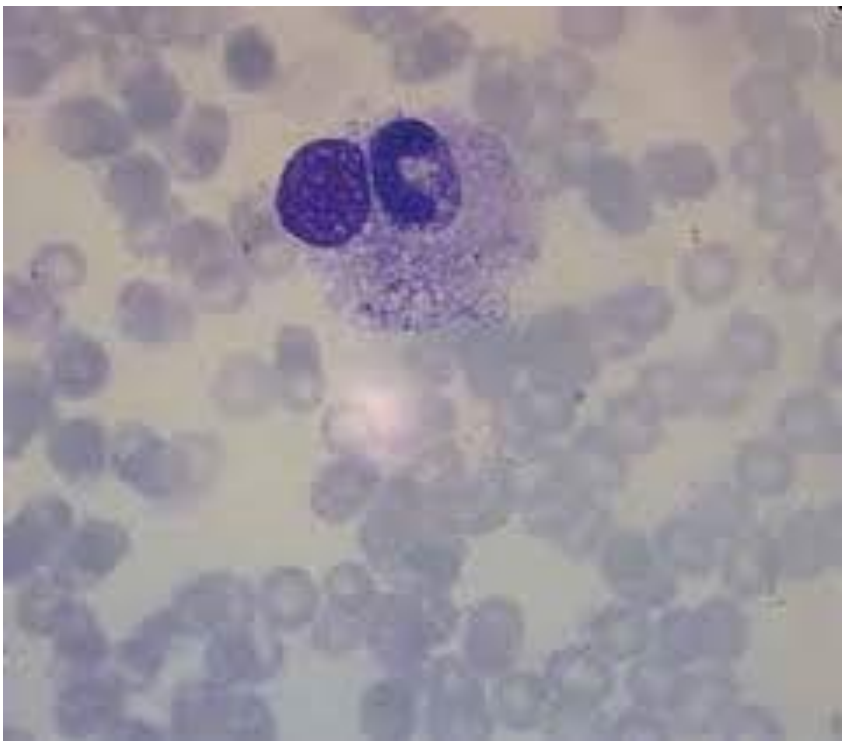

Figure: The presence of hemophagocytes in the bone marrow in a patient with Brucellosis (27).

DISCUSSION

Brucellosis is a worldwide zoonotic infection which caused by small, gram negative, oxidase and urease-positive coccobacilli from the genus Brucella, and mostly seen in Mediterranean Basin where the consumption of infected, unpasteurized animal-milk products (10). Although the Brucellosis is widely seen, it is mostly pandemic in the Mediterranean Basin, rural India and Central & South America (11). Due to extensive consumption of traditionally produced unpasteurized milk-based Turkish traditional cheese, human Brucellosis is also an endemic disease in rural areas of Turkey, where the annual incidence is 23 per 100.000 population (12).

The characteristics of ascites due to brucella have not yet been well established. According to limited case reports, patients with acute Brucellosis can develop pancreatic involvement or peritonitis, either of which could lead to ascites. Furthermore, presented cases with ascites, with a predominantly lymphocytic cell count, and may treated successfully with combination of tetracycline and rifampicin (3).

On the other hand, a case report revealed that acute Brucellosis might also cause portal hypertensive type ascites. Although the exact cause of this phenomenon was not clarified, this unique complication has been related to liver involvement of acute brucellosis infection by the authors (6).

Some authors hypothesize that the formation of ascites could be due to the primary reaction of the mononuclear–phagocytic system in the peritoneum to the infection, or to the underlying liver disease which is favored by Brucella infection (3). Our results strongly suggest that during acute phase of Brucellosis, inflammation of pancreatic tissue has a central role for developing exudative ascites via stimulating peritoneal inflammation. In the current study, the rate of Brucellosis-related ascites was 5.7%, which was higher than expected. This phenomenon might be due to more severe diseases in our patients.

Human Brucellosis may also cause spontaneous bacterial peritonitis in patients with liver cirrhosis. In the literature, there were few case reports regarding Brucellosis related peritonitis in patients with liver cirrhosis (13-17). There were also a few case reports regarding acute Brucellosis-related peritonitis in patients under peritoneal dialysis (7, 18, 19). But we did not detect spontaneous bacterial peritonitis in our cases. In those case reports; the exact cause of human Brucellosis involving cirrhotic patients mostly attributed to injured liver tissue resulting in increasing infectious mechanisms, in part through diminished opsonization and reduced anti-inflammatory signaling pathways. Those presented case reports were mostly originated from Turkey and successfully treated with six-weeks course of doxycycline plus rifampicin or doxycycline plus streptomycin regiments according to recommendations of the World Health Organization.

Lastly, two case reports involving Brucellosis-related pancreatitis were also described in patients with a ventriculoperitoneal shunt (20, 21). Brucellosis shows the involvement of thyroid gland involvement is rare (22).

In a large prospective study involving 158 patients with end-stage renal disease with Brucellosis revealed that percentage of Brucellosis-related peritonitis was as low as 0.6% (23).

In the current study we excluded patients who underwent ambulatory peritoneal dialysis. Described patients in those case reports were mostly immunocompromised patients and Brucellosis-related ascites might be related to lack of immunity against bacterial infections. Moreover, it has been shown that ascites are often present either as a temporary flare of underlying hepatic disease or as bacterial peritonitis during acute phase of Brucellosis (3). In the current study, we concluded that peritoneal and pancreatic involvement of Brucella infection causes exudative leakage from capillaries. In the current study, there was a relationship between the presence of ascites and elevated levels of cholestatic enzymes though which the mechanism was unclear. We postulated that this association might be due to presence of ascites, which was a strong finding of severe acute Brucellosis. On the liver perspective, data involving 100 patients with diagnosis of Brucellosis followed for at least one year from University Hospital of Ioannina revealed that mild hypertransaminasemia was seen in 24% of patients (5). It has been shown that cholestasis and hepatic granulomas can be present in liver-biopsy specimens in cases of both B. Melitensis and B. Abortus (24). In the current study, we found that, hypertransaminasemia was independently associated with increased risk of ascites. In a recent publication, we reported that acute Brucella infection could lead to pancreatitis. In this study, we also found that hyperglycemia, anemia, hypertransaminasemia and high cholestatic enzymes might represent new approaches for assessing disease severity in patients with Brucellosis and acute pancreatitis (25). We also identified four additional cases of acute pancreatitis secondary to Brucellosis for the literature (26).

CONCLUSION

We conclude that acute Brucella infection can lead to a unique low serum acid albumin gradient ascites probably resulting from pancreatic leakage followed by peritoneal accumulation of serum proteins.

On the other hand there were several strengths of the study. First, the findings of this study demonstrate that Brucellosis-related ascites is in low gradient nature and pancreatic type. Second, to the best of our knowledge, this is the first retrospective study comparing the Brucellosis patients with and without ascites. Further studies are needed to determine a causal link between human Brucellosis and exudative ascites.

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The effect of different doeses of aspirin application on oxidative stress in ovarian tissue

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ABSTRACT

Objective: Aspirin is a non-steroidal anti-inflammatory drug with antioxidative properties. It is recommended to use different doses and durations according to the characteristics of the patient and the type of disease. Therefore, in this study, we aimed to investigate the effect of using aspirin at different doses and for different durations on oxidative stress in ovarian tissue.

Material and Methods: Female Wistar albino rats were divided into five groups. Group 1: control group, no special treatment was applied to the rats in this group. Group 2: 1 mg/kg aspirin was administered orally to the rats in this group every day for 28 days. Group 3: 3 mg/kg aspirin was administered orally to rats in this group every three days. Ggroup 4: 5 mg/kg aspirin was administered orally to rats in this group every five days. Group 5: 7 mg/kg aspirin was administered orally to the rats in this group once a week. After fasting overnight following the last application, the rats were sacrificed, and their ovarian tissues were collected. Malondialdehyde, catalase, total thiol group, and AOPP levels were studied from ovarian tissue.

Results: Group4 and group5 ovarian tissue MDA levels were found to be significantly higher than the other groups ($p<0.05$). There was no significant difference between group1, group2 and group3 ovarian tissue MDA levels ($p>0.05$). Group1 (control group) ovarian tissue AOPP level was found to be significantly lower than all aspirin-administered groups ($p<0.05$). Group2 ovarian tissue AOPP level was found to be significantly lower than group3, group4 and group5 ($p<0.05$). TSG level was found to be significantly higher in group 5 when compared to other groups ($p0<0.05$). Group4 ovarian tissue TSG level was found to be significantly higher when compared to group1, group2 and group3 ($p<0.05$). Group3 and group4 ovarian tissue CAT activity was found to be significantly higher than group1, group2 and group5 ($p<0.05$). When group1, group2 and group5 ovarian tissue CAT activities were compared, no significant difference was found ($p>0.05$).

Conclusion: The application of aspirin at certain intervals rather than daily application may have more positive effects on the antioxidant system. especially taking aspirin at intervals of 3 or 5 days may be more effective

Key words: Aspirin, ovarian tissue, Oxidative stress, Antioxidant, Acetylsalicylic acid

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INTRODUCTION

Aspirin (acetylsalicylic acid) is a non-steroidal anti-inflammatory drug, which is widely used for relieving inflammation, fever, and pain (1). Aspirin is broadly used in the treatment of inflammatory diseases such as rheumatoid arthritis and the prevention of cardiovascular thrombosis (2). Aspirin consistently inhibits the production of prostaglandin, which leads to unexpected constriction of arterioles, resulting in tubule ischemia and cell death (3).

Aspirin administration has proven to be beneficial in the chemoprevention of various types of cancer. Unfortunately, aspirin use is still associated with serious consequences and adverse effects. Most importantly, the use of aspirin can specifically induce gastrointestinal toxicity manifested by peptide ulceration, bleeding, and dyspepsia (4).

Aspirin is prescribed for patients undergoing in vitro fertilization (IVF) or intracytoplasmic spray injection. The primary objective of acetylsalicylic acid administration to IVF candidates is to modulate excessive inflammation responses by inhibiting the production of prostaglandins synthesized by Cyxlooxiganesa-2 (5). This medication might increase blood supply to the reproductive organs during the onset of coagulation disorders, resulting in an improved rate of endometrial growth and vascularization (5). Besides, ASA is recommended in patients at high risk of developing preeclampsia as well (5).

Nowadays, aspirin is at the forefront of medical research due to its numerous adverse effects. Increasingly, once-daily medications are emerging. As members of medication therapy management in clinical practice, physicians, pharmacists, and nurses always face a common problem, that is to say, the optimal time and dose for patients to take these drugs. This critical problem reveals the dose, time, and duration of administration for optimizing medications and minimizing their adverse effects.

It has been suggested that low-dose aspirin (80 mg/day) is effective in reducing the risk of cerebral vascular attacks and myocardial infarction, and what's more, it reduces mortality by 25% in patients with cardiac risk factors; however, it has been recommended to use different doses of aspirin for different patients (6).

The treatment timing should be adapted to optimize the therapeutic outcome of the administration of medication as well as to minimize the adverse effects of treatment. Hence, it has been suggested that aspirin therapy administered at different doses and at different times may be beneficial. More frequent administration of aspirin could be an alternative to minimize adverse events (7).

Nonsteroidal anti-inflammatory medications such as aspirin are inhibitors of the cyclooxygenase enzyme and are used in the management of short-term pain and even in the treatment of chronic inflammation. COX enzyme and prostaglandins play a key role in the regulation of female reproductive function. Aspirin use can cause toxicity in many organs such as the liver and kidney. In this study, we aimed to assess the impact of aspirin administration at different doses and durations on oxidative stress in ovarian tissue.

MATERIAL and METHODS

Animals

Forty adult female albino rats (weights ranged 150-200 g, 8-10 weeks) were obtained from the Experimental Animals Unit of Van Yuzuncu Yıl University. Rats were acclimated to laboratory conditions one week before the onset of the experiment. A temperature of 25 °C and 12 hours of light/dark cycles were achieved. Animals had free access to standard pellet food and water. All animal experiments were performed following National Institutes of Health guidelines on the care and use of laboratory animals. The experiment protocol was carried out after obtaining approval from Van Yuzuncu Yil University Experimental Animals Local Ethics Committee (Date :29/04/2021 and Decision number: 2021/04-02).

Experimental Design

Rats were randomly divided into five groups, with 8 in each group.

Group 1; control group, no procedure was applied to the rats in this group.

Group 2; 1 mg/kg aspirin (Bayer AG, Leverkusen, Germany) was administered orally to the rats in this group every day for 28 days.

Group 3; 3 mg/kg aspirin was administered orally to rats in these group every three days.

Group 4; 5 mg/kg aspirin was administered orally to rats in these group every 5 days.

Group 5, 7 mg/kg aspirin were administered orally to rats in this group, for once a week.

At the end of the 28-day study, following 75 mg/kg ketamine (IP) and 5 mg/kg xylazine (IP) administration, the rats were placed on the table in the dorsoventral position and ovarian tissue was dissected for biochemical analysis.

Biochemical analysis

Ovarian tissue was homogenized with 50 mM potassium buffer (pH 7.4). The homogenate was centrifuged at 14000 RPM for 15 minutes at 4°C. The supernatant was used to identify oxidative stress and antioxidant parameters.

Total sulfhydryl content (protein and non-protein Thiols) was measured based on the method of Sedlak and Lindsay (8). In this mehod, TSG were determined with the use of 5,5’ -dithiosis (2-nitrobenzoic acid) (DTNB). DTNB, after reduction by the sulfhydryl group-containing compounds, form a yellow-colored anionic 5-thio-2-nitrobenzoic acid. The absorbance was meusered with spectrophotometer at a wavelength of 412 nm.

AOPP was determined via the method described by Witko-Sarsat (9). AOPP measurement at 340 nm by addition of acetic acid using chloramine-T in the presence of potassium iodide as standart. Ovarian tissue MDA level was measured by the method identified by Ohkava et al. (10) and MDA level was presented as mmol/gr tissue.

The principle of MDA calculation is based on pink color production as a result of the reaction between MDA and thiobarbituric acid (TBA), which is measured by a spectrophometer at 532 nm absorbance. CAT activity was spectrophotometrically analyzed at 240 nm according to the Lartillot method (11).

Statistical Analysis

All data were expressed as mean ± standard deviation. Statistical analyzes of the groups were analyzed statistically using the One-way ANOVA followed by post hoc multiple comparisons (Tukey's test) for comparative analysis between the groups.

$P < 0{\cdot}05$ was regarded as statistically significant

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RESULTS

Ovarian tissue Catalase activity and MDA, TSG and AOPP levels are presented in Table1.

Group4 and group5 ovarian tissue MDA levels were found to be significantly higher than the other groups ($p<0.05$). There was no significant difference between group1, group2 and group3 ovarian tissue MDA levels ($p>0.05$). Group 4 and group 5 ovarian tissue MDA levels were similar ($p>0.05$).

Group1 (control group) ovarian tissue AOPP level was found to be significantly lower than all aspirin-administered groups ($p<0.05$). Group2 ovarian tissue AOPP level was found to be significantly lower than group3, group4 and group5 ($p<0.05$).

TSG level was found to be significantly higher in group 5 when compared to other groups ($p0<0.05$). Group4 ovarian tissue TSG level was found to be significantly higher when compared to group1, group2 and group3 ($p<0.05$). Ovarian tissue TSG level was found to be significantly lower in group2 when compared to group1 and group3 ($p<0.05$).

Group3 and group4 ovarian tissue CAT activity was found to be significantly higher than group1, group2 and group5 ($p<0.05$). When group1, group2 and group5 ovarian tissue CAT activities were compared, no significant difference was found ($p>0.05$). There was no significant difference between group3 and group4 ovarian tissue CAT levels ($p>0.05$).

Table 1. MDA, AOPP, TSG level and CAT activity in Ovarian Tissue in study groups

	Group1	Group2	Group3	Grup4	Group5
MDA (nmol/gr tissue)	$0.39\pm0.15^{b*}$	0.41 ± 0.08^{b}	0.40 ± 0.02^{b}	0.43 ± 0.01^{a}	0.45 ± 0.014^{a}
AOPP (mmol/gr tissue	30.94 ± 1.19^{c}	32.82 ± 0.61^{b}	34.43 ± 0.67^{a}	34.41 ± 0.76^{a}	34.45 ± 0.77^{a}
TSG mmol/gr tissue)	0.59 ± 0.14^{c}	0.55 ± 0.014^{d}	0.58 ± 0.13^{c}	0.62 ± 0.11^{b}	0.66 ± 0.01^{a}
CAT (U/L)	540.38 ± 30.84^{b}	539.51 ± 57.59^{b}	737.12 ± 49.69^{a}	774.56 ± 42.42^{a}	567.81 ± 96.80^{b}

DISCUSSION

ASA is a potent anti-inflammatory medication that inhibits cyclooxygenase. It has been reported that ASA protects endothelial cells from the destructive effect of hydrogen peroxide and has free radical scavenger properties (12). Malondialdehyde (MDA) is a widely used lipid peroxidation product. This aldehyde product is used as an indicator to measure the level of oxidative stress and damage (13). It has been demonstrated that the ovarian tissue MDA level of the group administered 7.5 mg/kg aspirin was not different compared to the control group (3). It has been reported that 30 mg/kg aspirin administration increases MDA levels in the kidney, liver, brain tissues, and plasma (14). Low-dose aspirin leads to peroxidation, and therapeutic-dose aspirin administration has been shown to cause severe peroxidation in erythrocytes and increase the MDA level (15). Group4 and group5 ovarian tissue MDA levels were found to be significantly higher than the other groups ($p<0.05$). In our study, there was no significant difference between group1, group2 and group3 ovarian tissue MDA levels ($p>0.05$). Group 4 and group 5 ovarian tissue MDA levels were similar ($p>0.05$). According to these results, an increase in lipid peroxidation occurs as the aspirin administration dose increases.

Aspirin is considered to have anti-inflammatory properties as it suppresses inflammatory cytokines known to induce oxidative damage in cells. Catalase is an intracellular antioxidant enzyme. Catalase is mainly localized in cellular peroxisomes and to some extent in the cytosol. Catalase catalyzes the reduction of hydrogen peroxide to water and molecular hydrogen (3). Compared to the control group, no significant difference was found in ovarian tissue catalase activity of rats, which were administered 7.5 mg/kg/day aspirin for 15 days (3).

Administration of NSAIDs such as aspirin has been reported to reduce tissue levels of CAT, SOD, and GPx activities (16). NSAIDs are COX enzyme inhibitors. COX is the enzyme that mediates prostaglandin formation from arachidonic acid, and its level is known to increase inflammatory processes (17). It has been demonstrated that catalase activity is elevated in erythrocytes of people receiving aspirin therapy (18). In this study, Group3 and group4 ovarian tissue CAT activity was found to be significantly higher than group1, group2 and group5 ($p<0.05$). When group1, group2 and group5 ovarian tissue CAT activities were compared, no significant difference was found ($p>0.05$). There was no significant difference between group3 and group4 ovarian tissue CAT levels ($p>0.05$).

It has been revealed that anti-inflammatory agents reduce oxidative stress in experimental models involving inflammatory processes. Another remarkable issue to note here is that the suggested antitumor effects of NSAIDs may be associated with the induction of oxidative stress related to numerous signaling pathways, including apoptosis (19).

Advanced oxidation protein products (AOPP) are cross-linked proteins contain dityrosine and are safe markers used to assess oxidative modification of proteins. AOPP is a marker of oxidative stress severity and oxidative-mediated protein damage in inflammation. It is generally produced during oxidative stress or by myeloperoxidase in activated neutrophils through the interaction of hypochlorous acid or chloramines (20, 21). It has been shown that AOPP levels are higher in the serum of women with PCOS (22) and the follicular fluids of women with endometriosis (23). In this study, Group1 (control group) ovarian tissue AOPP level was found to be significantly lower than all aspirin-administered groups ($p<0.05$).

Group2 ovarian tissue AOPP level was found to be significantly lower than group3, group4 and group5 ($p<0.05$).

The balance between oxidants and antioxidants in the organism is the basis for maintaining cellular and biochemical functions. Oxidants damage lipids, proteins, and DNA in cells and even cause death. The most common and rapidly affected proteins are thiols containing sulfhydryl. Plasma thiols are robust antioxidants that remove free radicals from the physiological environment. Plasma thiols serum levels are considered among the markers that indicate antioxidant levels in the body (24). In the presented study, TSG level was found to be significantly higher in group 5 when compared to other groups ($p0<0.05$). Group4 ovarian tissue TSG level was found to be significantly higher when compared to group1, group2 and group3 ($p<0.05$). Ovarian tissue TSG level was found to be significantly lower in group2 when compared to group1 and group3 ($p<0.05$).

CONCLUSION

Aspirin is used in many oxidative stress-related diseases because of its inflammatory and antioxidant properties. Discussions continue about whether the dose of aspirin used should be given as a single dose or in divided doses. In this study, we think that the application of aspirin at intervals of 3 and 5 days may be more effective on the antioxidant system of the ovarian tissue.

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Do depression, anxiety, or stress have any effect on pain scores in patients undergoing colposcopy?

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ABSTRACT

Objective: In this study, we evaluated the effect of depression, anxiety, and stress on pain perception during colposcopy.

Material and methods: This study was performed at the gynecologic oncology department of Lutfi Kirdar Kartal Education and Research Hospital in Istanbul, Turkey between September 2017 and January 2018. After taking the informed consent form, Depression, Anxiety and Stress Scale (DASS-42) was filled out by women who attended outpatient colposcopy unit. Patients were classified into three groups according to DASS-42 (Group 1: patients without depression, anxiety, or stress; Group 2: patients with one or two of depression, anxiety, and stress; Group 3: patients with all of depression, anxiety, and stress). Patient characteristics were also recorded. The degree of pain perception was evaluated with visual analog scale (VAS) at the end of the procedure. The p values less than 0.05 were considered statistically significant.

Results: A total of 116 women were enrolled in this study. There was no statistically significant difference between the groups in terms of age, gravidity, parity, number of gynecologic examination, waiting time, BMI, VAS, having a partner, came alone to clinic, level of education, employment, the indication of procedure, number of biopsies, ECC presence ($p>0.05$). There was no difference between the subgroups in terms of VAS. There was a negative correlation between groups and VAS ($r=-0.195$, $p=0.036$).

Conclusion: According to our findings, depression, anxiety, and stress have no impact on pain perception during colposcopy, but there is a weak correlation between the absence of depression, anxiety, stress, and the pain score.

Keywords: colposcopy, depression, anxiety, stress, visual analogue scale

INTRODUCTION

Colposcopy, a procedure that allows for direct visualization of the cervix, is the gold standard procedure for detecting cervical dysplastic lesions. Although colposcopy is essentially a minimally invasive procedure, women may experience discomfort. Biopsy procedure can cause discomfort and application of acetic acid may also give rise to stinging sensation (1). Topical and oral analgesics are ineffective (2). In a Cochrane review, different forms of pain relief before, during and after colposcopy were evaluated. Although most guidelines recommend taking oral pain-relieving medicines before treatment on the cervix in the colposcopy unit, evidence from two small trials does not show that this practice reduces pain during the procedure (3).

The significance of depression, anxiety, or stress on pain perception during colposcopy is not clear. The Depression Anxiety Stress Scale (DASS) is a 42-item self-report measure of depression, anxiety, and stress that requires no special skills to administer (4). We aimed to evaluate the effect of depression, anxiety, or stress on pain perception during colposcopy procedure using DASS-42

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MATERIAL and METHODS

This study was conducted at the Department of Gynecologic Oncology, Lutfi Kirdar Kartal Education and Research Hospital, Istanbul, Turkey from September 2017 to January 2018. The study was designed according to the Helsinki declaration and approved by the Ethics Research Committee of the hospital (Protocol code: 2017.514.111.3).

A written informed consent form was also obtained from all participants. The 116 women aged between 23-55 years with HPV-16/18 positive or an abnormal pap-smear result requiring colposcopy were prospectively included in the study. Women with pregnancy, known psychiatric diseases and taking medication who had colposcopic examinations previously were excluded from the study.

Information about age, height, weight, gravidity, parity, number of gynecologic examination previously, waiting time, having a partner, coming alone to clinic, level of education, employment was collected. Psychological data were collected using the Turkish version of DASS-42 before the procedure. It is a self-report questionnaire consisting of 42 symptoms divided into three subscales of 14 items: Depression scale, anxiety scale, and stress scale.

In all patients, colposcopic examinations were performed by the same doctor (MSC). After examination with the naked eye and colposcope, 3% acetic acid solution was applied to the cervix. Suspicious areas were biopsied, and endocervical curettage was performed. All of the patients were asked to state the severity of their pain and discomfort during the procedure with VAS immediately after the procedure. Before the procedure, a detailed information about VAS was given personally to each woman. VAS was a 10-cm line scaled from 0 to 10. (0= no pain, 10= severe pain). VAS score of five and above was accepted as severe pain.

Statistical Analysis: Statistical analyses were performed using SPSS Version 20.0 for Windows software.

Numeric variables were stated as mean ± standard deviation (SD) or median (interquartile range), and categorical variables were expressed as number and percentage. Normal distribution of data was assessed using Kolmogorov-Smirnov and Shapiro Wilk tests. If numeric variables were normally distributed, the comparisons between the groups were performed with Student's t-test, comparisons between more than two groups were done with one-way ANOVA test and Tukey's HSD test. If numeric variables were not normally distributed, two group comparisons were analyzed with Mann-Whitney U test and comparisons of more than two groups were done with Kruskal Wallis test and Dunn-Bonferroni test. Differences between categorical data were evaluated using the Chi-square test. Pearson correlation analysis was used to assess the association between DASS-42 and VAS scores. The p values less than 0.05 were considered statistically significant.

RESULTS

A total of 120 patients underwent colposcopic examination within the study period. Of these, 4 women who declined to participate in the study, one woman who was pregnant, one woman who took medication for major depression and two women who had prior colposcopic evaluation were excluded from the study. One-hundred and sixteen women were included in the final analysis. The comparison of clinical and demographic characteristics of the groups are presented in Table 1. Group 1 (Patients without depression, anxiety, or stress) consisted of 40 patients, Group 2 (Patients with one or two of depression, anxiety, and stress) consisted of 36 patients, Group 3 (Patients with all of the depression, anxiety, and stress) consisted of 40 patients. There were no statistically significant differences between the groups in terms of age, gravidity, parity, number of gynecologic examination, waiting time, BMI, VAS score, having a partner, coming alone to clinic, level of education, employment, indication of procedure, number of biopsies, ECC presence (p > 0.05).

Table 1. Comparison of clinical and demographic features of patients undergoing colposcopy (n=116)

		Group 1	Group 2	Group 3	p
Age		39,4±5,9	39,2±7,9	39,2±7,3	0,988[a]
Gravidity		2±2	2±2	3±1	0,375[b]
Parity		2±2	2±2	2±2	0,189[b]
No of gyn-examination		7±16	15±15	10±15	0,127[b]
Waiting time		30±30	30±44	27,5±20	0,276[b]
BMI		25,0±5,8	25,3±7,5	24,7±4,4	0,768[b]
VAS		4,8±5,3	4,2±4,2	3±4	0,183[b]
Having a partner	**No**	9 (22,5%)	11 (30,6%)	11 (27,5%)	0,724[c]
	Yes	31 (77,5%)	25 (69,4%)	29 (72,5%)	
Came alone to clinic	**No**	11 (27,5%)	15 (41,7%)	15 (37,5%)	0,409[c]
	Yes	29 (72,5%)	21 (58,3%)	25 (62,5%)	
Level of education	**Elementary/middle school**	23 (57,5%)	19 (52,8%)	24 (60,0%)	0,179[c]
	High school	8 (20,0%)	10 (27,8%)	14 (55,0%)	
	University	9 (22,5%)	7 (19,4%)	2 (5,0%)	
Employment	**No**	25 (62,5%)	25 (69,4%)	25 (62,5%)	0,770[c]
	Employed	15 (37,5%)	11 (30,6%)	15 (37,5%)	
Indication	**HPV-16/18 +NILM**	19 (47,5%)	11 (30,6%)	14 (35,0%)	0,290[c]
	ASCUS/LSIL	18 (45,0%)	19 (52,8%)	17 (42,5%)	
	ASC-H, HGSIL, AGC	3 (7,5%)	6 (16,7%)	9 (22,5%)	
Number of biopsies	**0**	6 (15,0%)	8 (22,2%)	8 (20,0%)	0,878[c]
	1	23 (57,5%)	16 (44,4%)	18 (45,0%)	
	2	11 (27,5%)	12 (33,4%)	14 (35,0%)	
ECC presence	**No**	0 (0,0%)	0 (0,0%)	1 (2,5%)	0,384[c]
	Yes	40 (100,0%)	36 (100,0%)	39 (97,5%)	

aOneway ANOVA test (Mean±SD); bKruskal-Wallis Test (Median±Interquartil Range); cChi-Square Tests (%). BMI: body mass index, VAS: visual analog scale, NILM: negative for intraepithelial lesion or malignancy, ECC: Endocervical curettage.

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VAS score is divided into two subgroups (subgroup 1: 0-4,9; subgroup 2: 5-10) and score of five and above was accepted as severe pain. There was no statistically significant difference between groups (p=0.282, Table 2).

Table 2: Comparison of VAS subgroups between groups

			Group 1	Group 2	Group 3	Total
VAS subgroup	1	Count	21	25	26	72
		within group%	52,5%	69,4%	65,0%	62,1%
	2	Count	19	11	14	44
		within group%	47,5%	30,6%	35,0%	37,9%
Total		Count	40	36	40	116
		within group%	100,0%	100,0%	100,0%	100,0%

The comparison of VAS scores and depression, anxiety, and stress status according to the DASS-42 scale are described in Table 3. There were no statistically significant differences in terms of VAS scores between patients with and without depression, anxiety, and stress (p > 0.05).

Table 3: Comparison of the presence of depression, anxiety, stress and VAS scores.

	Group	N (%)	VAS score±IR	p
Anxiety	**No**	52 (44,8%)	4,8±5,4	0,057
	Yes	64 (55,2%)	3,5±4	
Stress	**No**	64 (55,2%)	4,6±5	0,106
	Yes	52 (44,8%)	3,3±4	
Depression	**No**	61 (52,6%)	4,4±4,5	0,369
	Yes	55 (47,4%)	3,5±4,1	

There was negative correlation between DASS-42 scores and postprocedure VAS score but it was not statistically significant (Depression r=-0.130; anxiety r=-0.103; stress r=-0.151; p > 0.05, Table 4)

Table 4: Correlations between VAS and Depression, anxiety, Stress score

		Depression score	Anxiety score	Stress score
VAS	Pearson Correlation	-.130	-.103	-.151
	Sig. (2-tailed)	.164	.269	.105
	N	116	116	116

There was statistically significant negative correlation between group 1 and VAS score (r; -0.195, p; 0.036, Figure 1).

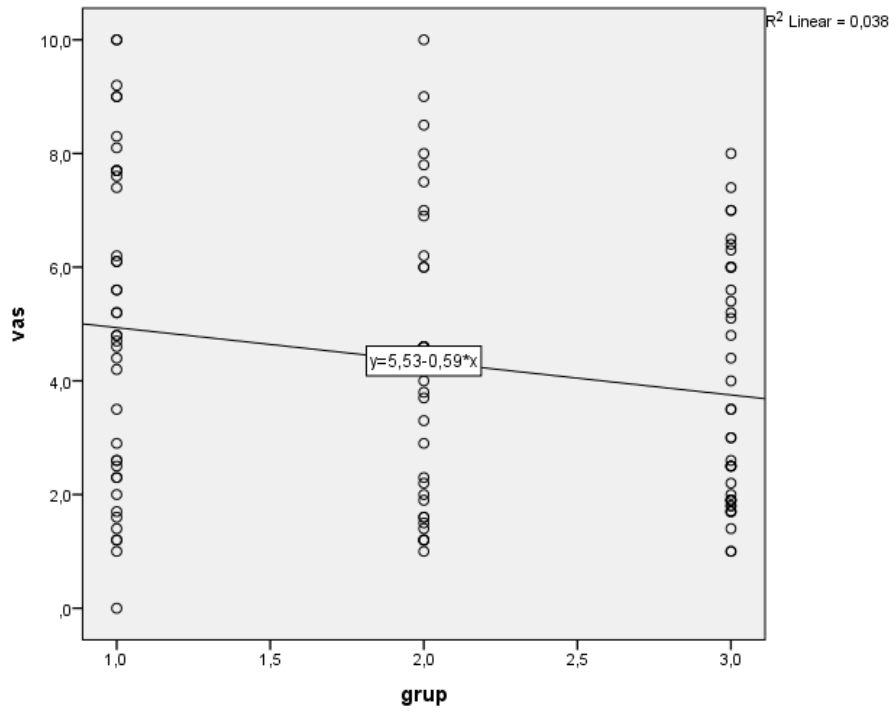

Figure 1: Correlation between groups and VAS

As a result, there is no relationship between subgroups and VAS score one by one. However, group 1 with no subgroups correlated more with pain than the others (p=0.036).

DISCUSSION

Cytological screening in the cervix prevents the progression of cervical intraepithelial neoplasia to invasive cervical cancer. Abnormal results of the smear test and a positive human papillomavirus (HPV) DNA test are usually followed by a colposcopy test that allows a detailed examination of the cervix. Colposcopy is the gold standard procedure used to detect and treat any cervical dysplastic lesions (5).

The significant level of anxiety and stress can be expected among women due to uncertainty in the status of cancer diagnosis and also in the process of colposcopy due to the pain and burning effect of the acetic acid preparation during biopsy (6). A study described in detail the entire sensory experience of undergoing colposcopy and related procedures from the perspective of the women involved and found that women can experience a range of different sensations that are quite specific to different aspects of follow-up investigations and procedures for abnormal cervical cytology. Sensory experiences of undergoing colposcopy± related procedures were pain/discomfort, stinging, shaking of the body, heart beat faster, cramping, burning smell, scraping, and cold (7).

Local anesthesia is not routinely used for biopsies of the cervix, since injection of the anesthetic is probably as painful as the biopsy. Topical and oral analgesics are ineffective (2). A systematic review suggests that music therapy has no great positive effect in reducing anxiety and pain levels and no effect in increasing satisfaction levels when compared with control groups during the colposcopy procedure (8).

Colposcopy has a potential for causing extreme anxiety, especially in single, parous, highly trait anxious patients, women who consider the information provided by the gynecologist is inadequate, women who have to wait a long time (9,10). The high levels of anxiety before and during colposcopy may have several consequences including pain, discomfort and failure to return for follow-up, impaired health related quality of life (11,12).

In similar studies, the State-Trait Anxiety Inventory (STAI) or Hospital Anxiety and Depression Scores (HADS) form were used to detect anxiety or depression. The STAI assesses anxiety on 40 items, 20 of which measure the A-state (state anxiety) intensity response scale and 20 of which measure the A-trait (trait anxiety) frequency response scale. The state scale measures anxiety at the time just prior to the colposcopy and the trait scale measures the day-to-day levels of the anxiety. The total scores of each response scale range from 20 to 80. The STAI is a self-report form, does not have a cut-off point for detecting the presence of anxiety, and only measures the level of the anxiety (13). It has been shown that colposcopy related pain and discomfort were significantly affected by pre-procedural state anxiety levels (14). The HADS consists of 14 items: 7 items that assess symptoms of anxiety and 7 items that assess symptoms of depression. The total scores for anxiety and depression range from 0 to 21. HADS is also a self-report form and has a cut-off point, but it evaluates the level of anxiety and depression; however, subgroup evaluations are not possible (15,16).

Unlike these studies, we used DASS-42 form. It is also a self-report form, has cut-off points for detecting the presence of depression, anxiety, and stress, and can evaluate their severity within subgroups (4).

One of the limitations of this study is that the DASS-42 scale is a self-report form and this type of evaluation can not take the place of a psychiatric examination performed by a psychiatrist. The other limitation is related to pain perception. Pain itself is a subjective sensation and can be related to many factors so pain evaluation is complicated.

CONCLUSION

To our knowledge, this is the first study that has evaluated depression, anxiety, and stress on pain perception during colposcopy. According to our findings, we can conclude that existing depression, anxiety, and stress before colposcopy may not be related to pain perception among the subgroups but there is a weak correlation between the absence of depression, anxiety, stress, and the pain score during the colposcopy session.

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A retrospective comparative cohort study on the routine pre-engraftment use of granulocyte colony-stimulating factor in allogeneic hematopoietic stem cell transplantation

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ABSTRACT

Objective: Myeloid growth factors have been often used in allogeneic hematopoietic stem cell transplantation settings. There are some controversies about increased graft versus host disease, relapse, and delayed platelet engraftment with those growth factors in the pre-engraftment period. In this study, we aimed to compare the transplantation outcomes of allogeneic hematopoietic stem cell transplantation recipients according to their myeloid growth factor support status.

Materials and Methods: Sixty-seven adult acute myeloid leukemia/myelodysplastic syndrome and acute lymphoblastic leukemia patients who underwent allogeneic peripheral blood stem cell transplantation from HLA-identical matched sibling donors were analyzed retrospectively. All-cause mortality at day 100, day 180, and at 1-year were the primary outcome measures. Secondary outcome measures were the engraftment kinetics, length of hospital stay, and graft-versus-host disease incidences.

Results: Growth factor supported group was younger (p=0.001), and the first complete remission status at transplantation was seen more compared to the unsupported group (p=0.04). Myeloablative conditioning was used more in growth factor supported group (p=0.004). Faster neutrophil engraftment (p=0.008) and delayed platelet engraftment (p=0.022) were seen in growth factor supported group. Graft-versus-host disease, relapse incidences, and all-cause mortality at day 100, day 180, and at 1-year were not different between groups. Steroid-resistant graft-versus-host disease was the only factor related with relapse (OR: 0.196, p=0.043).

Conclusion: This real-life study shows colony-stimulating factors are safe in HLA-identical sibling allogeneic hematopoietic stem cell transplantation. Further prospective randomized controlled studies for different stem cell sources, different donors, and different conditioning and graft-versus-host disease prophylaxis regimens are mandatory.

Keywords: Acute Myeloid Leukemia, Colony-stimulating factor, Filgrastim, Hematopoietic stem cell transplantation, Lymphoblastic Leukemia

INTRODUCTION

Myeloid growth factors have been often used in the allogeneic hematopoietic stem cell transplantation setting with the aim of fastening neutrophil engraftment. Hematologists have some concerns about increased acute graft-versus-host disease (GVHD), relapse, and delayed platelet engraftment with those growth factors in the pre-engraftment period. Conflicting results of the previous studies and the paucity of the data in recent years encouraged us to study this issue (1, 2). Herein, we present transplantation outcomes of hematopoietic stem cell transplantation recipients by their pre-engraftment granulocyte colony-stimulating factor (G-CSF) support status.

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MATERIALS and METHODS

Patient Characteristics: Adult acute myeloid leukemia/myelodysplastic syndrome (AML/MDS) and acute lymphoblastic leukemia (ALL) patients followed at a single tertiary transplantation center who underwent allogeneic peripheral blood stem cell transplantation from HLA-identical matched sibling donors between 31.01.2007-04.06.2020 were reviewed retrospectively for analysis. Patients whom data were accessible as printed or electronic records are included in the study. Patients who underwent a second transplant were excluded from the study.

Predictive models: Sorror risk index was used for hematopoietic stem cell transplantation comorbidity scoring (HCT-CI). This score analyzes 17 comorbidities as well as their degree, and is predictive for non-relapse mortality after stem cell transplantation (3). Patients were stratified as low-intermediate risk (0-2 points) or high risk (3 or more points). European Society for Blood and Marrow Transplantation (EBMT) score was employed to determine the disease risk status. This score is a validated score that predict approximately the 5-year probability of overall survival and the transplantation related mortality (4).

Conditioning regimens, graft versus host disease prophylaxis and management: Conditioning regimens were recorded as either myeloablative or non-myeloablative/reduced intensity conditioning. Cyclosporin A plus methotrexate on days +1,+3, +6, +11 (may be omitted according to toxicity) were used for GVHD prophylaxis. Anti-thymocyte globulin was not used in any of the recipients. Diagnosis and grading of acute GVHD were based on the original Glucksberg score (5). Chronic GVHD was defined according to 2014 National Institute of Health criteria (6). GVHD was treated according to institutional protocols.

Engraftment: Neutrophil engraftment was defined as the first of the three consecutive days with absolute neutrophil count of $\geqslant$ 0.5 x 10^9/l. Platelet engraftment was defined as the first of the three consecutive days with platelet count >20x10^9/l with free of transfusion requirements. Febrile neutropenia was defined as the fever $\geqslant$38 ^{0}C during neutropenia. G-CSF support protocol was as 5 µg/kg/day filgrastim beginning from day +5 until absolute neutrophil count $\geqslant$ 1.5 x 10^9/l.

Relapse: Morphological relapses were analyzed. Morphological relapse was defined as the $\geqslant$5% blasts in the bone marrow or peripheral blood (7).

Statistics: Statistical analyses were performed using the Statistical Packages for the Social Sciences software version 20. Quantitative data was defined as median (minimum and maximum value). "Student's t test" was used for normally distributed quantitative data and "Mann Whitney U" was used if the quantitative data was not normally distributed. Univariate analyses were performed via "Chi-square test" for qualitative data (or "Fisher exact test" when Chi-square assumptions do not hold due to low expected cell counts). Multivariate analyses were done by "Cox regression" analysis. A p-value of less than 0.05 was considered to show a statistical significant result.

Ethics: This study was approved by the ethics committee of our institution before the study began (E1-20-914 approval number) and the protocol conformed to the ethical guidelines of the 1975 Helsinki Declaration. Informed consent was not required as the study reports observational, retrospective data obtained from hospital records.

RESULTS

Sixty-seven patients were included in the study with median age 36 (ranging 19-67). Median follow-up time was 19 months (ranging 0.9-165 months) in the whole study group. Males (n=41), were more than the females (n=26). Cytomegalovirus statuses were all seropositive for the donors and the recipients. In the whole study group, eighty percent of the patients were AML/MDS and the remaining were ALL. Conditioning intensity was mostly myeloablative (59 of 67 patients). G-CSF was administrated to twenty-nine patients.

Patients of the growth factor supported group were younger (32 vs. 42) than the unsupported (p=0.001). Two groups were similar for sex and disease distribution, for HCT-CI grading and the median EBMT scores. First complete remission (CR1) status at transplantation was seen more in the growth factor supported group (p=0.040). Myeloablative conditioning was used more in the growth factor supported group (p=0.008). Growth factor support was mostly applied in transplantation procedures earlier than 2014 (p=0.000) and median follow-up period was longer in the growth factor supported group (51 vs. 15 months) (p=0.024). Those demographic and transplantation related clinical factors of patient groups were given in Table 1.

In the growth factor supported group, time to platelet engraftment was longer (14 vs. 12 days) (p=0.022), but time to neutrophil engraftment was shorter (14 vs. 16.5 days) (p=0.004). Febrile neutropenia incidence, days with fever, and length of hospital stay from transplantation day 0 were not different between the two groups. Those engraftment kinetics related outcomes of the patient groups were given in Table 2. In the whole study group, median time to neutrophil engraftment was faster in myeloablative conditioning versus non-myeloablative/reduced intensity conditioning (15 vs. 17 days) (p=0.027) but conditioning intensity was not related with median time to platelet engraftment (p=0.640).

Acute or chronic or both GVHD incidences were similar between the two groups. Also, growth factor support was not associated with steroid resistant GVHD. Grade I-II and grade III-IV GVHD incidences were also similar between the two groups. There was a less relapse risk tendency in the growth factor supported group without statistical significance (17.2% vs. 36.8%) (p=0.084). Mortality incidences at day 100 and day 180 in the growth factor supported group were not different from the unsupported group (Table 3).

For determining the impact of growth factor support over 1 year mortality, cases whose transplantation was in the first year of the analysis were excluded. Sixty-one cases were evaluated and there was no statistically significant 1-year mortality difference (Table 4). Kaplan-Meier analysis was performed; there was a not statistically significant overall survival difference between the growth factor supported and the unsupported group (p=0.190) which was represented in Figure 1.

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Table 1: Demographic and transplantation related clinical factors of patient groups

	No GF support (n=38)	GF support (n=29)	p
Age Mean±SD (Median)	42.05±13.06 (41)	32.21±9.44 (32)	**0.001**
Sex (Female/Male)	14/24	12/17	0.706
Disease MDS-AML/ALL	81.6% vs. 18.4%	79.3% vs. 20.7%	0.816
Conditioning regimen intensity MAC vs. RIC/NMA	78.9% vs. 21.1%	100% vs. 0%	**0.008**
Disease status at transplantation CR1 vs. >CR1	73.7% vs. 26.3%	93.1% vs. 6.9%	**0.040**
HCT-CI Low/Intermediate vs. High	94.6% vs. 5.4%	100% vs. 0%	0.502
EBMT score Mean±SD (Median)	2.54±1.12 (2)	2.04±0.922 (2)	0.060
Follow-up, mo (range)	14.6 (2.1-100.7)	50.6 (0.89-164.5)	**0.024**

AML: Acute myeloid leukemia, ALL: Acute lymphoblastic leukemia, CR1: First complete remission, EBMT: European Society for Blood and Marrow Transplantation, GF: Growth factor, HCT-CI: Hematopoietic stem cell transplantation comorbidity index, MAC: Myeloablative conditioning, MDS: Myelodysplastic syndrome, NMA/RIC: Non-myeloablative conditioning/ reduced intensity conditioning, SD: Standard deviation

Table 2: Comparison of engraftment kinetics and related outcomes between the two groups

	No GF support (n=38)	GF support (n=29)	p
Febrile neutropenia	94.7%	100%	0.502
Days with fever Mean±SD (Median)	5.21±5.63 (3.50)	4.93±2.44 (4)	0.079
Time to neutrophil engraftment Mean±SD (Median)	16.87±3.74 (16.5)	14.45±3.63 (14)	**0.004**
Time to platelet engraftment Mean±SD (Median)	13.16±4.15 (12)	15.14±4.03 (14)	**0.022**
Hospital stay Mean±SD (Median)	35.34±15.7 (32)	43.59±28.68 (37)	0.084

GF: Growth factor

Table 3: Univariate analysis for growth factor support and transplantation outcomes

	No GF support (n=38)	GF support (n=29)	OR	95% CI	p
GVHD (Yes)	55.3%	34.5%	0.420	0.157-1.155	0.094
aGVHD (Yes)	34.2%	24.1%	0.612	0.207-1.807	0.374
cGVHD (Yes)	39.5%	20.7%	0.400	0.132-1.213	0.105
srGVHD (Yes)	31.6%	27.6%	0.825	0.285-2.391	0.724
GVHD grade (I-II/III-IV)	12/9	5/5	1.333	0.294-6.043	0.709
Relapse (Yes)	36.8%	17.2%	0.357	0.111-1.148	0.084
Day 100 relapse (Yes)	15.8%	6.9%	0.395	0.074-2.120	0.279
Day 100 mortality (Yes)	7.9 %	13.8%	1.867	0.384-9.085	0.440
Day 180 mortality (Yes)	18.4%	20.7%	1.155	0.342-3.900	0.816

Table 4: Growth factor support and 1 year mortality

	No GF support (n=32)	GF support (n=29)	OR	95% CI	p
Day 365 mortality (Yes)	37.5%	27.6%	0.635	0.215-1.877	0.412

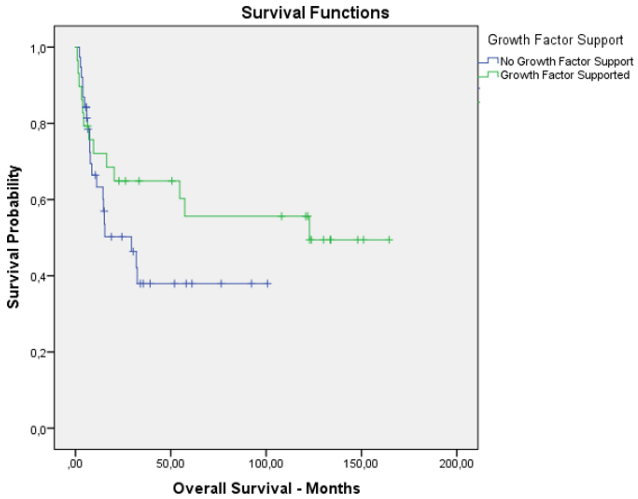

Figure 1: Kaplan-Meier analysis for overall survival according to growth factor support status

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Relapse incidence was found to be inversely related with steroid resistant GVHD (p=0.040). Other transplantation related factors in Table 5 were found to be unrelated with relapse.

Table 5: Factors affecting relapse after transplantation

	OR	95% CI	p
Age	1.006	0.964-1.050	0.778
Sex (Female vs. male)	1.123	0.375-3.364	0.836
Conditioning intensity	0.824	0.151-4.493	0.823
Leukemia type	1.156	0.309-4.325	0.830
Disease status	1.333	0.350-5.087	0.674
HCT-CI	2.937	0.173-49.74	0.455
EBMT score	1.467	0.874-2.464	0.147
GVHD (Yes)	0.791	0.270-2.310	0.667
aGVHD (Yes)	1.571	0.508-4.856	0.433
cGVHD (Yes)	0.313	0.080-1.223	0.095
srGVHD (Yes)	0.196	0.040-0.949	**0.043**
Transplantation period (Earlier)	1.625	0.556-4.753	0.375

aGVHD: Acute graft versus host disease, cGVHD: Chronic graft versus host disease, CI: Confidence interval, EBMT: European Society for Blood and Marrow Transplantation, GVHD: Graft versus host disease HCT-CI: Hematopoietic stem cell transplantation comorbidity index, OR: Odds ratio, srGVHD: Steroid resistant graft versus host disease

DISCUSSION

Granulocyte colony-stimulating factors are cytokines enhancing neutrophil production with safe usage in acute leukemia and myelodysplastic syndrome (8). Expectation from myeloid growth factors in allogeneic hematopoietic stem cell transplantation setting is faster neutrophil engraftment (9) but it should be noted that the engraftment kinetics is influenced by several factors such as the stem cell source, graft composition, the underlying disease, the conditioning regimen, and the type of GVHD prophylaxis (10). Limited up-to-date data exist in the literature regarding the efficacy and safety of myeloid growth factors after allogeneic stem cell transplantation. The increasing numbers of patients older than 60 years undergoing stem cell transplantation and increased reduced intensity conditioning encourage the researchers to study pre-engraftment myeloid growth factors and its effects in the era of improved supportive care relevant to infectious complications (11). Since 2014 our institutional guideline was changed regarding the empiric use of filgrastim after allogeneic stem cell transplantation based on concurrent guidelines and studies demonstrating increased GVHD and mortality (1, 12-14). This change in practice was the reason for the transplantation timeline difference between groups.

There have been studies evaluating the effects of myeloid growth factors on engraftment kinetics. Bishop et al. found faster neutrophil engraftment (11 vs. 15 days for placebo, p=0.008) with filgrastim (10 µg/kg/day) versus placebo in a double blind placebo-controlled study in 2000. In this study with fifty-four recipients filgrastim was started at day of transplantation. There were no significant differences for red blood cell transfusion and time to platelet engraftment (15). In a randomized trial of Prepizioka et al. with forty-two adult recipients of allogeneic blood stem cells from human leukocyte antigen-matched related donors, 10 µg/kg/day filgrastim subcutaneously from day 1 through neutrophil recovery was compared with no growth factor support after transplantation. The group receiving filgrastim had a shorter time to neutrophil engraftment (12 vs. 15 days, P =0.002) and a trend for earlier discharge (16 vs. 20 days, p=0.05). There were no significant differences for the number of transfusions, time to platelet engraftment, and infections (16). In 2001, in a retrospective comparative trial of Ozcan et al., fifty-six allogeneic stem cell transplantation recipients with different hematological neoplasms were involved. Both the neutrophil and platelet engraftments were faster with myeloid growth factor administration. Besides, less febrile episodes and less mucositis were seen (17). In a retrospective trial of Remberger et al., in matched related donors with various hematological neoplasms, neutrophil engraftment was fastened without an effect on platelet engraftment, red blood cell transfusion burden, and infections (12). Ringden et al., retrospectively analyzed myeloid growth factors effects in matched related (14) or matched unrelated (13) bone marrow and peripheral blood stem cell transplantation recipients and showed faster neutrophil but delayed platelet engraftment. In the meta-analysis by Dekker et al., the studies were heterogeneous regarding transplantation setting (allogeneic/autologous), stem cell sources (bone marrow/peripheral blood), age group (adult/pediatric), and cytokines G-CSF or granulocyte and monocyte colony-stimulating factor (GM-CSF). Fewer infections and parenteral antibiotic use accompanied fastened neutrophil engraftment with both G-CSF and GM-CSF, but infection-related mortality risk was comparable between the groups (18). Following those studies, Khoury et al. showed faster neutrophil engraftment (16 vs. 20 days, p<0.001) with myeloid growth factors administration. In this retrospective trial, the stem cell source was unrelated donor bone marrow, related donor bone marrow, or peripheral blood (19). A phase III trial with filgrastim after HLA-matched related bone marrow transplantation with various hematological neoplasms showed faster neutrophil engraftment, although days with neutropenic fever and antibiotic usage durations were not affected (20).

Authors conducted a prospective randomized controlled trial of filgrastim 5 µg/kg/day from day +7 until neutrophil recovery in HLA-identical allogeneic bone marrow transplantation in various hematological neoplasms and showed accelerated neutrophil recovery (16 vs. 23 days for placebo, p<0.001), reduced intravenous antibiotic therapy (18 vs. 26 days, p=0.001) and reduced hospitalization (27 vs. 34 days, p=0.017) but platelet recovery rate was not affected (21). One of the most recent largest studies demonstrated shorter hospitalization with myeloid growth factors administration for HLA-matched unrelated donor stem cell transplantation in acute leukemia and myelodysplastic syndrome (11), and the most recent retrospective study demonstrated earlier neutrophil engraftment and shorter post-transplant hospital stay in peripheral blood stem cell transplantation for various hematological neoplasms including lymphomas (22).

G-CSF has effects on the immune system, mainly exerting Th2 polarization and anti-inflammatory profile and might alter the risk of GVHD. Some authors recommend using of the myeloid growth factors in allograft recipients with leukopenia persisting for 3 weeks after transplantation (23). The first meta-analysis in the field, searching for GVHD, was done by Ho et al. at 2003. A total of 1198 allogeneic hematopoietic stem cell transplantation recipients were analyzed. In this meta-analysis, the studies were heterogeneous regarding stem cell sources (bone marrow/peripheral blood), age group (adult/pediatric), cytokines (G-CSF/GM-CSF), and trial design (randomized controlled/retrospective cohort). There was not a significant difference in the risk of grade 2-4 acute GVHD, grade 3-4 acute GVHD, and chronic GVHD when hematopoietic growth factors were used (24). A double-blind placebo-controlled study by Bishop et al., demonstrated comparable incidences of acute GVHD and 100-day mortality with filgrastim (15). In the retrospective comparative trial of Ozcan et al., pre-engraftment filgrastim support was not associated with increased acute GVHD, relapse, disease free survival, and overall survival (17). There were trials resulting with unfavorable results. One of them, was conducted in 2003, was a retrospective analysis of various hematological neoplasms with matched related donors and with bone marrow or the peripheral blood as the stem cell source. In this trial, there was an increase in the risk of grade 2-4 acute GVHD (34% vs. 9%, P<0.001) without a detrimental effect on chronic GVHD, relapse, and survival (12). In the retrospective analysis of Ringden et al., there was a 1.33 times more grade 2-4 acute GVHD, 1.29 times more chronic GVHD, increased transplantation related mortality, and decreased survival with G-CSF in bone marrow transplant recipients. These detrimental effects were not seen when peripheral blood stem cells were used (14). In 2010, Ringden et al. demonstrated 1.52 times more grade 2-4 acute GVHD and 1.51 times more chronic GVHD with G-CSF; however equal non-relapse mortality, relapse, and survival were seen with G-CSF. The interesting result of this study was that the G-CSF increases the risk of acute GVHD, especially with peripheral blood stem cell transplantation and chronic GVHD with bone marrow transplantation (13). In the aforementioned meta-analysis by Dekker et al., colony-stimulating factors were not found to be detrimental on grade 2-4 acute GVHD and transplantation related mortality (18). Contemporaneous with those studies, American Society of Clinical Oncology 2006 guidelines recommended the use of G-CSF after autologous, but not after allogeneic HSCT (2). In Khoury et al. cohort of acute myeloid leukemia and chronic myeloid leukemia, G-CSF use was not a determinative factor for day 30 and day 100 mortality, acute GVHD, chronic GVHD, and survival in the multivariate analysis (19). Randomized placebo-controlled trial of Ernst et al., showed comparable GVHD, mortality, and relapse rate with 2 years follow-up (20). A randomized clinical trial with long-term follow up, demonstrated less non-relapse mortality and comparable GVHD and relapse incidences. In this trial, the age range was 16 to 49 (younger than our cohort), myeloablative conditioning was used, and the stem cell source was bone marrow (21). American Society of Clinical Oncology revised recommendations on the use of G-CSFs in 2016, as they may be administered after allogeneic stem-cell transplantation to reduce the duration of severe neutropenia with low quality of evidence and weak strength of recommendation (1).

G-CSF administration was not related with overall survival neither in matched sibling donor nor matched unrelated donor peripheral blood stem cell transplantation in a recent and large retrospective multicenter cohort transplantation of acute leukemia and myelodysplastic syndrome patients (11). Unlike the aforementioned studies, a recent study with peripheral blood stem cell transplantation for various hematological neoplasms showed G-CSF use was associated with higher rate of extensive chronic GVHD (22). This study was also similar to our study by more myeloablative conditioning usage in the G-CSF supported recipients.

Filgrastim dosage can have an impact on transplantation outcomes. In a study with mycophenolate mofetil for GVHD prophylaxis, higher doses of G-CSF was/were associated with higher acute GVHD incidence with cord blood and bone marrow as the stem cell sources and decreased progression free survival with cord blood stem cell transplantation (9). One of the recent interests is the difference between biosimilar versus originator filgrastim and a study showed similar transplantation outcomes (25).

Our study clearly showed faster neutrophil recovery with growth factor administration but delayed platelet engraftment. Faster neutrophil recovery did not provide less neutropenic fever incidence or days with fever. Likewise, faster neutrophil recovery did not translate into shorter length of hospital stay after transplantation. However, mucositis or severe mucositis incidence, invasive fungal infections were not evaluated in our study. In this study, we clearly demonstrated the safety of pre-engraftment growth factor in terms of GVHD. Our study shows a trend through less early (day 100) relapse and cumulative relapses with growth factor administration, although statistically insignificant. Only the steroid resistant GVHD was a better factor for relapse in the univariate analysis. This can be related to the graft versus leukemia effect. We found the growth factor support was safe in terms of day 100, day 180 and 1-year mortality. Mortality incidences have a tendency to be higher at day 100 and day 180 in growth factor supported group, but mortality at 1-year showed a tendency for decreased mortality favoring G-CSF use.

There are limitations of this study. First of all due to the institutional protocol switch by 2014, growth factor supported recipients had a shorter follow-up duration. Median age, disease status at transplantation and conditioning regimen intensities were not comparable between groups as this is a real-life data. In our study, molecular relapses, measurable residual disease determination and loss of chimerism are not considered as relapse due to lack of patient records transplanted in the earlier times.

CONCLUSION

This study shows colony-stimulating factors are safe in HLA identical allogeneic hematopoietic stem cell transplantation. Heterogeneity between randomized trails and lack of up-to-date meta-analyses mandate further prospective randomized controlled studies to be performed in order to make routine suggestions regarding pre-engraftment growth factor administration for different stem cell sources, different donors, and different conditioning and GVHD prophylaxis regimens.

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Ethical approval: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Local Ethical Committee

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One Disease, Two Approaches. Acute Post-Streptococcal Glomerulonephritis – A Case Report of Two Young Patients

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ABSTRACT

Objective: Acute post-streptococcal glomerulonephritis (APSGN) is the most studied immune-mediated glomerulonephritis, being caused by streptococcal infections such as pharyngotonsillitis or skin infections (impetigo, erysipelas). Globally it is the main form of glomerular suffering among pediatric patients, especially between the ages of 3 and 15, but in the developed countries, the incidence of this condition has decreased significantly in the last decades. The majority of the medical literature indicates that the treatment in such a disease is symptomatic (the major goal being controlling of the edema and hypertension) associated with antibiotics for streptococcal infection. Due to some situations where antibiotic treatment cannot be administered, this case report hypothesized that homeopathy is a possible alternative treatment method for conventional therapy. We presented two cases diagnosed with APSGN: a nine-year-old boy treated homeopathically at home and a three-year-old girl treated conventionally during hospitalization. Analyzing the two cases, we observed that the patients were cured regardless of the therapeutic method approached. This aspect has an advantage in situations where there are limitations in the administration of allopathic treatment. According to homeopathic understanding, the success rate is higher when there are clear homeopathic symptoms for choosing the right remedy. Many more cases and much more research is needed to conclude that classical homeopathy can be a treatment option for this pathology. In conclusion, it is important to find a personalized therapy (allopathy or homeopathy) for each patient that will bring the maximum benefit, depending on the particularity of the case

Keywords: Acute Post-Streptococcal Glomerulonephritis, ASPGN, Allopathy, Homeopathy

INTRODUCTION

Hippocrates, considered to be the father of medicine, has taught us since the 4th century BC that the nature of the human body can only be understood if it is viewed as a whole (1).

Classical, conventional medicine, also known as allopathic medicine, is the medicine that defines health as the absence of the disease. Allopathy is the procedure of treating a disease using resources contrary to its symptoms. In allopathic medicine, the main cause of diseases is considered to be bacteria and viruses or biochemical disorders that produce illness in specific organs. In the diagnosis of diseases, scientific tests are used, and drugs and surgery are the key tools in treating health problems (2). Sometimes, conventional treatment is limited due to external factors as multiple allergies, special conditions as pregnancies when some allopathic medicines are contraindicated, the reluctance of some patients as a result of religious beliefs, etc. (3, 4). According to Samuel Hahnemann, homeopathic medicine assumes that the disease is seen as a manifestation of a dysfunction of the whole being and not as an isolated event (5). Homeopathy is the process of treating a disease using resources similar to its symptoms.

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The defense mechanism starts to produce symptoms whenever it's necessary to protect vital organs. Its principle is based on the fact that diseases can be cured by strengthening the body's defense mechanism with substances selected for their energy-giving properties. This principle is the law of similarity (Similia Similibus Curantur) (5). Homeopathy uses remedies, selected from herbs, minerals, or chemicals, which would produce, in a healthy body, the same symptoms found in a sick person suffering from the specific disease. However, this original substance is diluted and purified beyond the point of harm to its quintessential state of energy (6). It is stated that matching the patient's symptoms to those of the remedy is vital in choosing the correct remedy (6). Homeopathic treatment assumes to be effective in conditions such as infectious diseases (9, 10), children-related diseases (11-13), apparently in certain types of glomerulonephritis (14). However, homeopathic medicine requires much more research and evidence-based medicine methods to be considered a reliable therapeutic option throughout the world (16, 17).

Acute post-streptococcal glomerulonephritis (APSGN) is immune glomerulonephritis, secondary to streptococcal infection. From the entrance gate, the extracellular antigens of streptococcus enter the circulation and trigger the formation of anti-streptococcal antibodies which react with the antigens and begin the formation of circulating immune complexes that cause characteristic glomerular lesions. These are responsible for the loss of proteins and elements shown in the urine. The main consequence of the histological lesions is the decrease of the glomerular filtrate, without the parallel decrease of the renal blood flow, which causes oliguria and arterial hypertension (15, 18).

The clinical presentation of APSGN varies from a benign asymptomatic or oligosymptomatic condition with an acute nephritic syndrome that shows glomerular impairment (edema, hypertension, oliguria, hematuria, minimal proteinuria, nitrogen retention) to rapidly progressive glomerulonephritis requiring dialysis (19). The treatment is mainly supportive, as there is no specific therapy for renal injury. Symptomatic therapy is considered regarding the severity of the illness. For edema and hypertension, diuretics are commonly used (20). Antibacterial treatment is usually given where there is evidence of a still active (pharyngeal) infection, and also for controlling local symptoms and limiting dissemination to contacts (to sterilize any outbreaks) (20). The antibacterial treatment is recommended for ten days with Penicillin or another antibiotic with anti-streptococcal action such as Erythromycin, Amoxicillin with Clavulanic Acid, Clarithromycin, Cefuroxime, Cephalexin (20). Corticosteroid treatment is indicated only in rapidly progressive forms (20, 23). Homeopathic treatment may be considered as an alternative treatment method in the specific situations mentioned above when conventional treatment cannot be administered.

Considering the principles of conventional and homeopathic therapy and the recommended treatment for APSGN I hypothesized that homeopathy is a possible treatment method for conventional therapy.

To demonstrate the hypothesis, we will examine the degree of the disturbance of the general state (fully favorable/ unfavorable condition both physically, mentally, and socially) and the presence or the absence of the symptoms belonging to the nephritic syndrome (edema, oliguria, cola-colored urine, and the values of hypertension) (22). Edema represents the swelling caused by excess fluid stuck in the tissues of the body (32). Oliguria means the quantity of urine output that is less than 0.5 mL/kg/h in children and less than 400 mL daily (33). Hematuria, also known as cola-colored urine, refers to the presence of blood in the urine, either visible (macroscopic) or non-visible (microscopic) (30). Hypertension is a medical condition in which the blood pressure in the arteries is elevated, the normal range for children until 16 years old being under 90 percentiles for height, weight, and sex (29).

Additionally, I will present the laboratory investigations that are the most useful in APSGN assessment. They include tests to provide evidence of preceding streptococcal infection, renal function tests, serologic studies, and urine analyzes (21).

Evidence of a preceding streptococcal infection is determined by measuring antistreptolysin titer (ASO), antibodies that are usually elevated after both pharyngitis and skin infections. The antibody titers may elevate at one week, following a possible rising to a peak at one month, and decrease toward pre-infection levels after several months (21). Kidney function tests include blood urea nitrogen (BUN) and serum creatinine values that are typically elevated during the acute phase reflecting the decrease in the glomerular filtration rate. The elevations are usually transient (22). Serology studies find low serum complement (C3) levels indicating an antigen-antibody interaction. C3 is consumed in the inflammatory reaction. Its values return to normal in 6-8 weeks. Occasionally, low complement levels persist for three months (22). Urine analysis shows both macroscopic and/or microscopic hematuria and mild proteinuria. Proteinuria in APSGN represents the presence of an increased level of protein in the urine, the values being lower than three g/L/24 hours in the nephritic syndrome. Hematuria usually disappears within 3-6 months but may persist as long as 18 months. Proteinuria may persist until six months. However, there can be found an increase in the urinary protein excretion even after three years from the debut of the disease in 15% of the cases and also ten years after the debut in 2% of the cases (21, 31).

MATERIAL and METHODS

I performed a prospective clinical study carried out while shadowing a physician in Romania.

The study included two cases of children, one male, another female who were clinically diagnosed with edema, oliguria, cola-colored urine, and hypertension to whom the final diagnosis of APSGN was established. The boy was treated at home and the girl was treated at the hospital.

I searched the variables that could describe the evolution of the disease in those two cases.

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studied: the degree of the disturbance of the general state (fully favorable/unfavorable condition both physically, mentally, and socially), the presence or the absence of the symptoms belonging to the nephritic syndrome (edema, oliguria, cola-colored urine, and the values of hypertension), the laboratory tests (complete urine examination, blood urea, ASO titers, serum complement), and the time interval between the initiation of the therapy until the normalization of clinical signs and laboratory parameters. A detailed history enlisting the presenting complaints, history of present illness, personal history, and treatment history were studied.

RESULTS

Here are two cases diagnosed with APSGN: a nine-year-old boy and a three-year-old girl. The girl was treated conventionally during hospitalization, and the boy was treated homeopathically at home. These cases were studied while shadowing a physician, and they belong to the caseload of the clinic.

First case:

The 9-year-old male patient presented with a generalized altered condition, headache, high blood pressure, dyspnea, and low back pain. Besides, he presented non-medical characteristic symptoms, useful for the homeopathic diagnosis: the patient was not thirsty at all, patient was warm-blooded, during his sleep patient uncovered his feet, constantly asked for ice cream, patient sought the attention of his mother and liked to be caressed and cried quickly. In the past, he frequently developed respiratory infections, including otitis and bronchitis. After treating those infections conventionally, he developed a severe form of atopic dermatitis for five years, which was also treated conventionally. Moreover, he was allergic to almost all antibiotics (penicillin, macrolides, and cephalosporins), dust mites, mold, pollen, and particularly to chlorinated water. At the physical exam, the patient presented significant periorbital and facial edema, pale skin, high blood pressure (170/120 mmHg), oliguria (200 ml/day) with macroscopic hematuria.

Laboratory tests confirmed hematuria, minimal proteinuria, elevated ASO, low level of C3, and elevated level of BUM. The APSGN diagnosis was established based on clinical and laboratory data (Table 1).

Because the patient had multiple allergies and due to a clear picture for a homeopathic remedy, his parents consented that he would receive homeopathic treatment. He was administered Pulsatilla 200 ch two times per day, for five days. Thirty-six hours after the medication was given, the patient was sweating, blood pressure decreased to 120/70 and lumbar pain diminished. On the third day, blood pressure normalized and after five days, macroscopic hematuria was improved, the headache and lumbar pain disappeared, and the edema decreased significantly along with the decrease of proteinuria from 200 mg/dl to 30 mg/dl and which was negative at fifteen days after onset. The urine test revealed only microscopic hematuria after two weeks which also disappeared until the sixth week after onset and remained negative at fourth-month control. Also, the C3 consumption normalized six weeks after the onset (**Table 1**)

Second case:

The 3-year-old female patient was brought by her parents for macroscopic hematuria and minimal palpebral edema. She had a good general condition. She did not present oliguria, high blood pressure, or any non-medical symptoms which could be part of a homeopathic diagnosis. Patient did not have any relevant family history and her personal medical history, by then, consisted of two episodes of gastroenteritis. Physical exam pointed out minimal palpebral edema. Laboratory tests found hematuria, elevated ASO, and C3 consumption (**Table 2**).

After the diagnosis of APSGN was established she was administered Penicillin throughout the hospitalization, which lasted ten days. Macroscopic hematuria normalized after two weeks, and microscopic hematuria disappeared after four months after the onset of the disease. The minimal proteinuria disappeared at six weeks. Also, C3 consumption normalized after six weeks (**Table 2**).

Table 1. Laboratory tests of the first patient, presented at the onset and in the evolution of the disease.

Lab Test	Normal Range	Values Onset	Values 2 weeks after Onset	Values 6 weeks after Onset	Values 16 weeks after Onset
ASO (UI/ml)	0-200	1483	1447	300	110
C3 (mg/dl)	90-180	42	80	100	110
Hematuria (no./ul)	0	250	50	0	0
Proteinuria (no./ul)	0	200	10	0	0

Table 2. Laboratory tests of the second patient presented at the onset and in the evolution of the disease.

Lab Test	Normal Range	Values Onset	Values 2 weeks after Onset	Values 6 weeks after Onset	Values 16 weeks after Onset
ASO (UI/ml)	0-200	607	590	185	70
C3 (mg/dl)	90-180	28	65	85	110
Hematuria (no./ul)	0	300	100	10	0
Proteinuria (no./ul)	0	30	30	0	0

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DISCUSSION

Through these two cases, we wanted to show that personalized therapy should be considered when treating a patient. The cases reported had favorable evolution as long as the treatment modality was judiciously chosen, depending on the particularity of the case. Our results indicate that the APSGN could be treated not only allopathically but also homeopathically, the healing occurring in both situations.

In the two cases presented, the evolution of the C3 complement and proteinuria values during each chosen treatment followed the same pattern of gradual reduction (Fig. 1, 2).

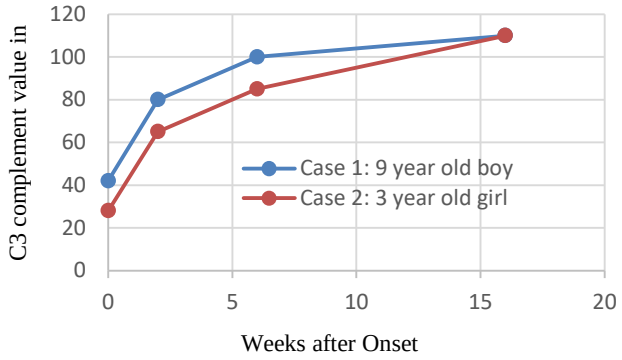

Figure 1: Evolution of C3 complement in the two cases presented throughout the disease

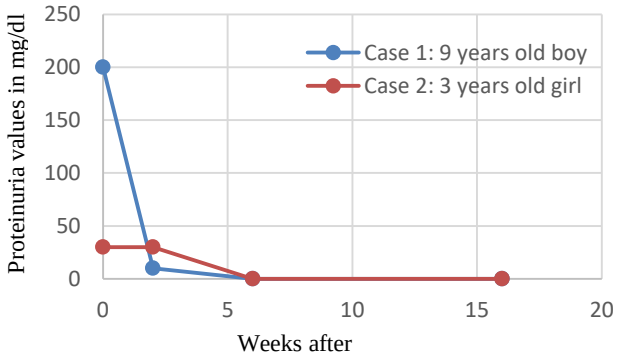

Figure 2: Proteinuria evolution in the two cases presented throughout the disease

Regarding the hypocomplementemia, the data obtained from this study resonates with the literature as the C3 complement returns to normal after 6 weeks (21). Although the curves for proteinuria decreased similarly in both cases during the therapy, I would remark the evolution in the male patient's case where the decline took place from a higher level, close to the superior limit of the nephritic syndrome from 2g/L/day to 0.3 g/L/day in the third day. The difference between the evolution of proteinuria in the first 6 weeks after the onset and the medical literature, which states that the proteinuria may persist until 6 months cannot be taken into consideration because of the poor number of considered subjects in my study (21).

The distinction between the curves of hematuria in each case could be explained through the different presentations for each patient and the particular treatment used (fig. 3).

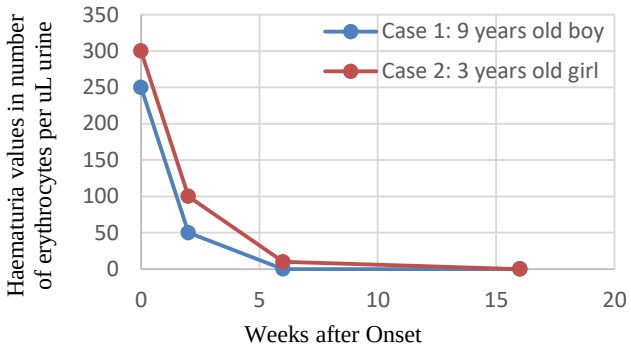

Figure 3: Evolution of Hematuria in the two cases presented throughout the disease

Hematuria is a sensitive indicator of renal injury. The presence of an oligosymptomatic presentation in the female patient's case confirms the minor histological renal lesion. The presence of oliguria and arterial hypertension along with important edema and proteinuria in the male patient's case certify more significant histopathological destructions (28). Accordingly, the remission of hematuria in the second case (male patient) where the kidney was more affected should have occurred later than in the girl's case where the renal damages were minor, but in the studied cases, it happened the contrary. We could assume that the particular homeopathic treatment for a 9-year boy was efficient. A peculiarity of this case report is choosing an unconventional homeopathic therapy for treating APSGN.

During the homeopathic interview, good observation of both the physician and the patient is one of the keys to solving the case (7). When the patient or the parent observes and knows very well his particular symptoms, general symptoms, or preferences, it is in his favor to be diagnosed correctly, according to that information. The result would be an accurate treatment and therefore a positive change in evolution. That was the case of the nine years old boy. As a result of the personal history of allergies, it was decided an alternative therapy with the parent's consent. The physician observed a gentle, timid boy who was crying easily and sought his mother's consolation. Starting with the observation, she asked for particular supplemental homeopathic symptoms: the little boy was not thirsty at all, he was warm-blooded, in sleep he uncovered his feet, he constantly asked for ice cream. Pulsatilla seemed to be the perfect remedy for the boy because it covered the totality of the symptoms (15). Pulsatilla nigricans is a homeopathic remedy derived from the windflower. The mother tincture is prepared from the inflorescent whole plant. Higher potencies are prepared from the mother tincture (24). Its active principles are potassium sulfate, anamonic acid, oil of anemone, anemone camphor, isoanemonic acid, and saponin (26). According to the homeopathic levels of health, the boy belonged to the superior hierarchy (as the picture of the remedy appears so clear), and according to this, healing was established quicker (7, 8). This observation was sustained by the shorter period required for normalizing the urine test including microscopic hematuria that clarified six weeks after onset. Another

peculiar aspect essential to be mentioned was that despite the more severe acute nephritic syndrome (significant edema, high blood pressure, dyspnea, severe oliguria with hematuria), the boy recovered very well using homeopathic treatment. That means that his defense mechanism strengthened by the homeopathic treatment was strong enough to bring back the homeostasis, recovering the favorable general state, normalizing the blood pressure, and ceasing the edema five days after onset and urinary symptoms in the first month. (25).

On the contrary, the efficacy of the treatment may not be directly proportional to the chosen therapeutic method. There are certain studies that conclude the placebo effect in regard to homeopathic treatment (27). Thereby, the necessity of inclusion of homeopathy in evidence-based medicine will decide if this is a viable therapeutic treatment.

Analyzing the two cases, we observed that the patients were cured regardless of the therapeutic method approached. Therefore, alternative treatments, as the homeopathic ones, could be considered for diseases, especially in situations where allopathic treatment is limited or unsuccessful (e.g.: multiple allergies, special conditions as pregnancy when some allopathic medicines are contraindicated, or the reluctance of some patients to take particular drugs) (3, 4).

CONCLUSION

In conclusion, it is important to find a personalized therapy (allopathic or homeopathic) for each patient that will bring the maximum benefit, depending on the particularity of the case. Although we observed the healing of one patient using homeopathic therapy in APSGN, there are not many pieces of evidence of APSGN treated homeopathically, so many more cases and much more research are needed to conclude that classical homeopathy can be a treatment option for this pathology.

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