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Table of Contents

Chemotherapy-Induced anemia in adults and treatment.

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ABSTRACT

Chemotherapy-induced anemia is the predominant adverse event observed in individuals undergoing cancer treatment, resulting in a reduction in red blood cells and hemoglobin levels. This condition manifests through indicators such as diminished quality of life and fatigue. Effective approaches for managing recurrent chemotherapy-induced anemia encompass the use of erythropoietin stimulating agents, blood transfusions, and intravenous iron supplementation. Each of these interventions presents distinct pros and cons, with the selection of a particular treatment modality contingent upon the severity of anemia and the duration of malignancy. A comprehensive review of scholarly literature reveals a high prevalence of anemia among cancer patients receiving chemotherapy. Ongoing research endeavors are focused on the development of pharmacological agents for cancer treatment that are devoid of adverse effects, particularly concerning anemia, a common complication associated with this therapeutic approach.

Keywords: Anemia, Cancer, Chemotherapy, Erythropoietin.

Anemia, derived from the Greek word "anaimia" meaning "lack of blood," refers to a condition where the blood's capacity to transport oxygen is diminished. This condition is often associated with malignancy [1]. There is no more common and persistent hematological disorder than anemia in those with cancer [2]. Chemotherapy-induced anemia (CIA) results from bone marrow infiltration disrupting erythropoiesis, malignant metastasis to normal tissue causing lack blood and inflammation-induced functional iron deficiency [3].

One important side effect of chemotherapy is CIA, which might delay or limit therapy and increase fatigue and quality of life reduction [4]. Furthermore, the intricate landscape of CIA treatment modalities will be scrutinized, ranging from traditional approaches such as blood transfusions and erythropoiesis-stimulating agents (ESAs) to contemporary strategies informed by advancements in molecular and cellular biology [5]. A thorough review of the changing field over the past 50 years can be obtained by analyzing the literature on chemotherapy-induced anemia as shown in Figure 1. Chemotherapy-induced anemia publicationexamining publishing years can be organized to show significant advancements, patterns, and changes in the direction of research at certain points in time.

Foundation and Recognition from the 1970s-1990s Publications during this peri-

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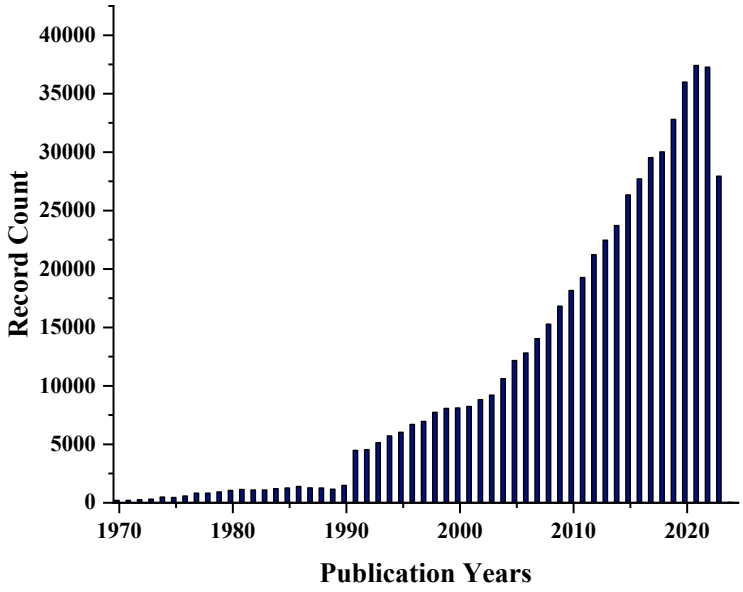

Figure 1. Chemotherapy-induced anemia publication from 1970 t0 2020
Source: Web of Sciences, Keywords: Anemia, Cancer, Chemotherapy, Erythropoietin.

od investigating the prevalence of anemia in patients with cancer, 1990 to 2020 the quality of life, and erythropoiesis-stimulating agents as a treatment this period might also have seen debates and discussions regarding the appropriate utilize of ESAs in patient and focus on produce new strategies for prevent anemia when using chemotherapy.

A five-decade timeline of publications summarized shown in Figure 2 a progression from basic statements of anemia prevalence to a more complex knowledge of its complex nature. The bar chart shows that early publications from 1970 to 1990 decreased but after that, the process of research about the widespread of anemia in cancer patients increased and reached a top in 2021.

The timeline documents turning points in the development of new treatment techniques advances in medicine, and an increasing understanding of the sig-

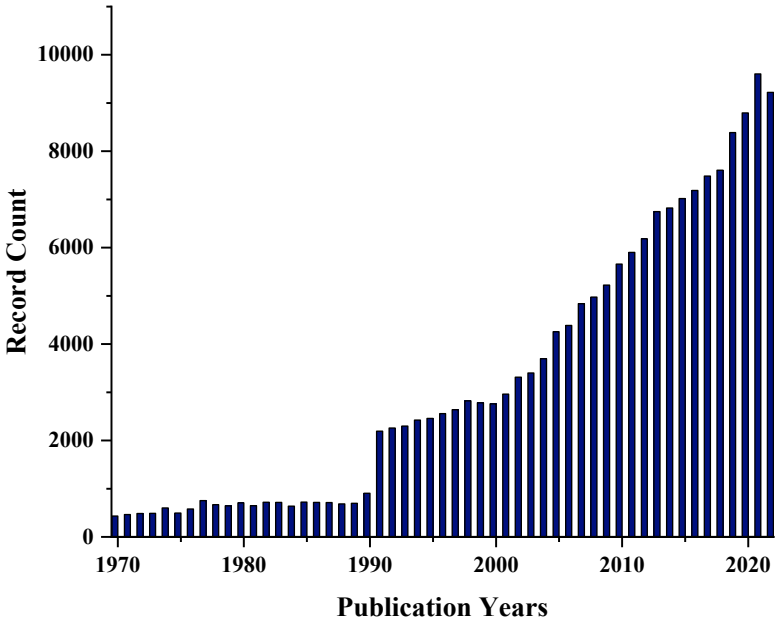

Figure 2. Anemia research landscape over time yearly publication count
Source: Web of Sciences, Keywords: Anemia, Cancer, Chemotherapy, Erythropoietin.

nificance of treating anemia as a critical component of cancer patient care. There may have been a stronger focus on patient-centered care and individualized treatment in the latter half of the study period, which runs from 2010 to 2020. Articles may provide individualized methods of treating anemia depending on a person's traits, biology, and reaction to treatment. Improvements in nutritional techniques and supporting care were probably made at this time.

1. Cancer Related Anemia

The pathogenesis of cancer-associated anemia, which includes radiation-induced anemia (RIE), chemotherapy-induced anemia , and persistent renal failure, comprises several components [6]. Anemia secondary cancer directly results from the original tumor infiltrating healthy organs and producing hemorrhage, marrow infiltration preventing the producing of red blood cells, or chronic inflammation resulting in low levels of iron [7]. Cancer-induced anemia is a typical side effect of myelosuppressive treatment such as chemotherapy, whether administered separately or in combination with radiation the pathogenesis of cancer induced anemia show in Figure 3 [7]. Most elderly cancer patients can be diagnosed with chronic kidney disease , this might result from the decline associated with aging, chemotherapy, and kidney injury from tumor penetration [8].

Anemia can be caused by underlying co-morbidities including inflammatory illnesses, hemolysis, coagulation abnormalities, genetic diseases, renal insufficiency, or nutritional deficiencies [9]. The pathophysiological origin of anemia in patients who have cancer can be divided into three main categories reduced red blood cell (RBC) production, destruction of RBC, and blood loss [10].

1.1 Reduced RBC Production

Lower erythropoiesis due to a variety of causes is the main mechanism behind of cancer-induced anemia, and also chronic renal disease and acute renal injury is one of several causes of decreased erythropoietin production [11, 12]. Inadequate intake of total iron, vitamin B 9, and vitamin B12 in the dietary regimen, or damage to the bone marrow resulting from conditions such as myelodysplasia, bone metastases, or myelosuppressive chemotherapy [13, 14]. Patients with thymomas, leukemias, pure red cell aplasia may occur in lymphomas or tumors due to cytokines related to the tumor or, in extremely rare cases, because to the development of anti-erythropoietin antibodies after the administration of external a hormone called erythropoietin [15, 16]. Furthermore, anemia is a common presentation for patients whose cancers originate from hematopoietic progenitors [7]. The excessive multiplication of blast cells in the bone marrow may contribute to this condition, since they displace the non-malignant cell population [17]. It is thought

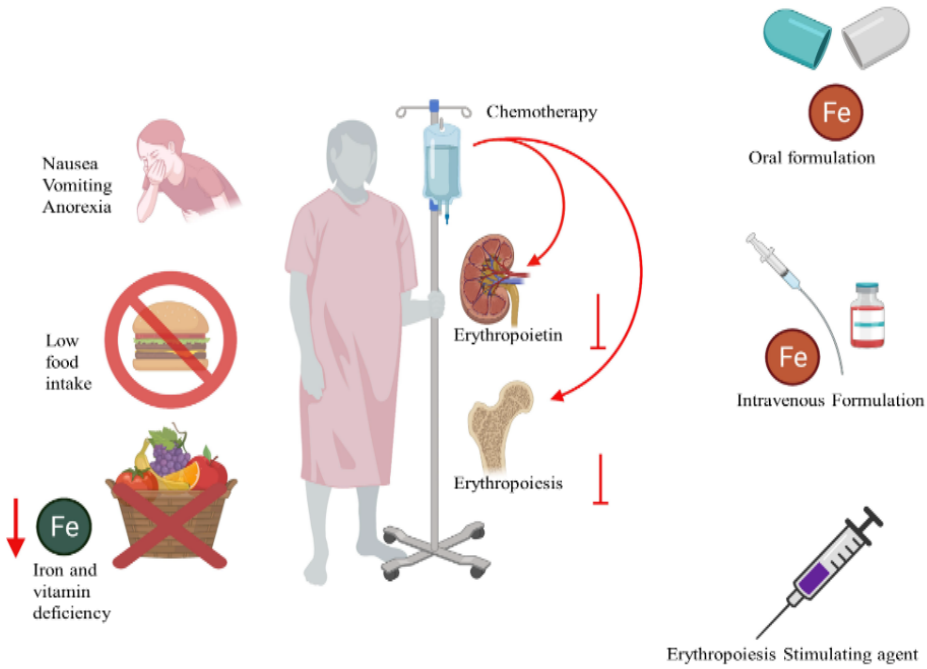

Figure 3. Pathogenesis of chemotherapy-induced anemia [42]

that normal erythroid blast-forming units and islands rely on interactions with stem cell factors and bone marrow stromal cells to sustain their differentiation, proliferation, and division [18]. However, this phenomenon hinders them from doing so [19, 20].

1.2 Destruction Red Blood Cell

Microangiopathic processes, such as erythrophagocytosis in histiocytic tumors, are among the mechanisms that causes damage the red blood cell as the result anemia will be occur [21]. Another condition that may cause the death of red blood cells is hemolytic disease anemia, which is common in persistence lymphocytic leukemia [22]. Hematopoietic cell sequestration often occurs in Hypersplenism associated with myeloproliferative neoplasms, lymphoid malignancies, cancer that invade the spleen, and tumors that induce portal hypertension [23].

1.3 Blood loss

Anemia resulting from the loss of red blood cells may be caused by treatment-related causes, such as blood loss after surgery or repeated blood drawing for laboratory testing[24]. As well as tumor-related bleeding seen in cases of uterine cancer or gastrointestinal malignancies [25]. Gynecological, genitourinary, and gastrointestinal malignancies can all result in disease-related blood loss. Both radiation and chemotherapy have the potential to affect the immune system and prevent erythropoiesis, some treatments, however, may result in anemia at a higher rate than others. Anemia of chronic illness is the term used to describe the anemia that affects a significant percentage of cancer patients who have no known etiology [26].

Unfortunately, not all medical professionals regularly measure, record, or even survey symptoms associated with anemia. Additionally, the severity and defining thresholds are not uniform. The absence of consistent, objective grading schemes for anemia and associated various expressions make quantitative assessment difficult. While some research describes anemia as a decrease in baseline hemoglobin, using recombinant human erythropoietin treatment, or requiring blood transfusions [27]. The toxicity grading system that is utilized to identify anemia is not documented by others [13]. The following anemia grading system was proposed by the National Cancer Institute Anemia [28] . An amount of hemoglobin below 12 g/dl can be regarded as suggestive of anemia, as defined by the World Health Organization and National Cancer Institute show in Table 1 severity scale for anemia [29]. The scale is made up of the following grades.

According to the European Cancer Registry's Anemia Survey a large-scale study, 39% of the 15,367 individuals who had chemotherapy were found to have anemia after being observed for a period of six months [30]. Anemia was defined as having a hemoglobin level below 10.0 g/dL, only 39% of these individuals received treatment overall, mostly with blood transfusions or erythropoietin-stimulating drugs [31]. The etiology of cancer-related anemia is complex and frequently difficult to identify, with several factors often implicated [32].

Although the exact underlying mechanisms causing this kind of anemia are unknown, it is believed that they involve the activation of cytokines such as tissue necrosis factor, interleukin-1(IL-1), and interferon-gamma γ, these cytokines may endogenous erythropoietin synthesis to be suppressed and enable iron consumption [33]. Between 30% to 90% of cancer patients are thought to have anemia at some point throughout their illness [34] .

Chemotherapy regimens that are not started or are not finished on time can have an impact on survival due to anemia, adequate oxygen levels are also necessary to prevent cytotoxicity caused by radiation therapy and certain chemotherapy regimens [35]. Because tumor hypoxia renders malignancies resistant to chemotherapy and radiation, it might perhaps contribute to the tumor's lack of response [36-38]. An imbalance between oxygen intake and delivery leads to tissue hypoxia, arises in solid tumors when the oxygen consumption rate of the neoplastic cells surpasses the ox-

Table 1. Scale of anemia in cancer patients [29, 135]

Scale	State	Range
Grade 0	Typical	Men (14-18) g/dL, Women (12-16) g/dL
Grade 1	Slight	Men (10-14) g/dL, Women (10-12) g/dL
Grade 2	Intermediate	8–10 g/dL
Grade 3	Intense	6.5–8 g/dL
Grade 4	Potentially fatal	<6.5 g/Dl

ygen supply available to them [39].

The structural and functional abnormalities of the tumor microcirculation might lead to inadequate blood flow, which can also be attributed to an increased diffusing distance, which limits the amount of oxygen available [24]. Reduced blood oxygen-carrying capacity due to anemia from the tumor or its treatment can exacerbate tumor hypoxia can modify the rate of cell division after radiation therapy or chemotherapy by influencing the cell cycle and proliferation kinetics [40]. Research has also demonstrated that persistent tumor hypoxia might, by clonal selection and genetic alterations, further promote the advancement of malignancy and perhaps heighten aggressiveness [41].

2. Immunological Alteration in Cancer Patients

Immunological changes that occur as the neoplastic disease develops have a significant influence on the patient's clinical circumstances and may even result in the patient's mortality is called cancer cachexia syndrome [42]. Apart from anemia, various symptoms related to multiple organs and processes are brought on by the immune system's alterations [43]. These include weight loss, anorexia, vomiting, fatigue, nausea elevated energy metabolism with changes in glucose, lipid, and protein metabolism, and immunosuppression, heightens susceptibility to infections [44].

It is impossible to pinpoint the exact moment these alterations occur, but it is known that the patient's immune system and cancer interact to cause these changes [45]. Different soluble factors, or cytokines, are produced by inflammatory cells, lymphocytes, and mesenchymal cells that have been activated, certain cytokines have the ability to stimulate or repress certain cell types [46].

When it comes to the cell-mediated immune response, interleukin-1(IL-1), interleukin-6(IL-6), and tumor necrosis factor alpha (TNF-a) are release these molecules also function as second messengers for the creation of interleukin-2(IL-2) which is essential for the regulation of the immune response against cancer [47].The degree of immunological dysfunction is correlated with the stage of malignancy [48]. Linked to the quantity of acute phase proteins and other inflammatory cytokines, especially IL-6 [49]. It is reasonable to suppose that compromised lymphocyte activities are a stand-in for a variety of functional alterations, the most significant of which are the immunosuppressive effects of cytokines released from macrophages and modifications to energy metabolism that can lead to an oxidative stress state. Our earlier research demonstrated that cancer patients are significantly more likely to have elevated oxidative stress [50, 51].

Notably, inflammatory cytokines have specific immune-modulatory properties, but they also vital influence in the pathophysiology of the primary metabolic abnormalities [42].

2.1 Specific Actions of Proinflammatory Cytokines

The cytokines IL-1, TNF-a, and IL-6, which are known to lead to inflammation, have been shown to have a wide range of effects on erythropoiesis, including decreased erythroid precursor proliferative response, increased macrophage destruction of erythrocytes, and decreased erythroid precursor response to EPO [52]. Furthermore, patients with advanced cancer experience several nutritional and metabolic abnormalities that are mostly brought on by the persistent action of these same cytokines , notably promote inflammatory cytokines related to the development of cancer related anemia by affecting nutritional status and energy metabolism IL-1 may reduce erythropoiesis by particularly reducing the replication and maturation of burst-forming unit-erythroid (BFU-E), and colony-forming unit-erythroid (CFU-E) cells precursors decreasing the expression of the Erythropoietin (EPO) receptor and impairing the generation of EPO and IL-1 has the ability to reduce erythropoiesis [52]. The precursors decrease the expression of the EPO receptor, hence impeding the generation of EPO [53]. Furthermore, IL-1 and other proinflammatory cytokines have been linked to the activation of macrophages for erythrophagocytosis, which causes the early erythrocyte destruction and reduced erythrocyte survival [54].

Also, IL-1 has a role in a number of modifications to nutritional status and energy metabolism, by activating its effects inside the hypothalamic nuclei central nervous system, IL-1 causes anorexia linked to decreased food intake [55].

As a result, growth hormone (GH) is inhibited, which lowers the synthesis of insulin-like growth factor-1 that causes the loss of muscle mass that is common in individuals with advanced cancer [56]. Furthermore, IL-1 prevents pancreatic beta cells from producing insulin, which results in hyperinsulinemia and insulin resistance [57]. The emergence of cancer related anemia (CRA) in individuals with advanced cancer may be associated with these IL-1-mediated actions [58]. Erythropoiesis is specifically harmed by low glucose availability and insulin resistance because glucose metabolism is the only factor that al-

lows commitment to the differentiation phases [59]. Cellular absorption of glucose and its incorporation into the tricarboxylic acid cycle are crucial for erythroid development since they provide a substantial amount of energy required for cell proliferation [60]. Additionally, there is an increased redirection of glucose towards the pentose phosphate pathway to generate carbon sugars and facilitate glutamine-dependent nucleotide synthesis [61].

Erythroid differentiation is affected by the intake of glucose and the balance among mitochondrial and non-mitochondrial glucose metabolism [62]. Furthermore, there is proof that anemia and the central nerves system (CNS) pathways regulated by IL-1 are related. Research has demonstrated a correlation between the replacement of GH and a notable increase in Hemoglobin (Hb) [63].

Moreover, results from in vitro and in vivo investigations shown that Insulin-like growth factor 1 (IGF-1) has the ability to enhance the differentiation and proliferation of both early and late erythroid progenitors [64]. clinical investigations have consistently shown that blood levels of IGF-1 are inversely correlated with hemoglobin in a variety of demographics, including adult, elderly, and dialyzed patients [65].

TNF-a has a direct impact on hemopoiesis. It may impede the production of red blood cells and their maturation, both in living organisms and in laboratory settings. Additionally, it can raise the mortality rate of immature red blood cells and reduce the quantity of mature red blood cells. Furthermore, it diminishes the responsiveness of erythroid progenitor cells to EPO [66]. Moreover, TNF-a primarily contributes to the metabolic changes in lipid metabolism that often occur in individuals with advanced cancer, particularly those experiencing cachexia [67].

Additionally, it has also been shown that regulates the development of erythroid progenitors [68]. Regarding IL-6, it affects erythropoiesis at several levels by modifying hepatic gene expression and hepcidin production, IL-6 can alter iron metabolism and limit erythroid progenitors' ability to proliferate and respond to EPO, this, in turn, causes the functional iron shortage that is characteristic of CRA [10].

Research has demonstrated that IL-6 can impact erythropoiesis by inhibiting the synthesis of hemoglobin. This effect occurs separately from the hepcidin-iron pathway and is a result of the declining mitochondrial function in maturing erythroid cells, which leads to a decrease in membrane potential or oxidative phosphorylation [69]. Numerous publications have shown the critical role that IL-6 plays in CRA determination, one of our articles gave the first evidence that IL-6, in conjunction with the stage of the illness, acted as an autonomous predictor of Hb levels in a group of ovarian cancer patients [50].

Furthermore, IL-6 significantly contributes to the pathogenesis of chronic renal failure (CRA) as well as the profound immunological and metabolic alterations that characterize advanced illness. In a previous endeavor, an animal model was used in order to simulate cancer-related situations for experimental purposes [70]. It was demonstrated that IL-6 played a critical role in causing the early start of cachexia symptoms, which were linked to the loss of muscle and adipose tissues in addition to a decrease in appetite and were independent of the rate of tumor growth. The amount of IL-6 was directly correlated with the degree of cachexia, and a notable decrease in IL-6 levels occurred after the original tumor was removed. Anti-IL-6 monoclonal antibodies consistently delayed the onset of cachexia symptoms [70].

Additionally, studies conducted on rat experimental models have shown that IL-6, similar to IL-1, directly stimulates the hypothalamus to initiate the secretion of CRF, a process that is facilitated by Prostaglandin E2 (PGE2) [71]. It may affect the pancreatic b cells' ability to produce insulin and metabolize food [72]. In more recent times, IL-6 has come to light as the primary factor influencing muscle atrophy in patients with advanced cancers [73]. Currently, there is a significant emphasis on regulating the, phosphatidylinositol-3-kinase(PI3K), protein kinase B (AKT), mammalian target of rapamycin (mTOR) pathway this system serves as the main detector of cellular energy and is responsible for stimulating muscle development. IL-6 and signal transducer and activator of transcription 3 (STAT3) have been identified as key factors in this control [74].

Patients with advanced cancer may have impaired production of red blood cells due to the activation of certain pathways controlled by IL-6, as well as the accelerated breakdown and restricted supply of amino acids associated with this condition. Increased amino acid intake stimulates mammalian target of rapamycin (mTOR) signaling, which subsequently promotes the development of red blood cells and the manufacture of hemoglobin [75]. On the other hand, decreased nutrient and amino acid pools result in decreased mTOR activity and suppressed Hb production [76].

Insulin resistance, which results in poor glucose metabolism, and anorexia, which is linked to decrease

food intake, both contribute to the mTOR pathway's suppression. Consequently, mTORC1 signaling may be inhibited by anemia, which is characterized by a less effective transport of oxygen (O2) to peripheral tissues. This is mostly due to lower adenosine triphosphate (ATP) production and poor oxidative phosphorylation, which result in mTOR suppression. Additionally, "functional iron deficiency," which is characterized by low iron levels and subsequently low heme production a key component of muscle myoglobin distinguishes CRA this implies that muscular atrophy is exacerbated by anemia [73].

3. Erythropoiesis

Several physiological processes that can also alter in response to various pathological circumstances can control the rate of new cell production [77]. Delivery of oxygen from the lungs to various body locations and carbon dioxide in the opposite direction is primarily the function of erythrocytes [78].

A stable and suitable erythrocyte mass must be maintained, but it must also be able to expand in response to tissue hypoxia, given that the body's capacity to store oxygen is 20 mL/kg and its basal oxygen used is 4 mL/kg/min [79]. In actuality, hypoxia-inducible factor (HIF)-1 stimulates erythropoietin synthesis at the molecular level during hypoxia [80]. Furthermore, vascular endothelial growth factor (VEGF) and many other growth factors are used to counterbalance the detrimental consequences of low oxygen levels [81]. As a result, the inverse relationship between the value of Hb or hematocrit and the rise in EPO levels is log-linear [52].

There is a significant correlation between energy metabolism and the availability of oxygen, since oxygen transported by hemoglobin is essential for glucose metabolism and the production of energy [82]. Conversely, protoporphyrin IX (PPIX), which is produced as a result of glucose metabolism via the Krebs cycle, is used to form the heme molecule, which serves as the cellular carrier of oxygen [83]. While hemoglobin (Hb) is essential for carrying oxygen (O2), glucose is required for the production of heme, which in turn is necessary for the synthesis of Hb. The crucial role of diet is seen in the need of iron and glucose for heme synthesis explain in Figure 4 [84].

The four primary processes that govern erythropoiesis are as follows: (1) The capacity for proliferation of the reserve of erythroid progenitors; (2) The strength of the stimuli for the creation of erythrocytes; (3) The availability of nutrients; and (4) The survival of erythrocytes, which is reduced during bleeding or due to early destruction [85].

4. Prevalence of Anemia in Cancer Patients

According to a major prospective survey from the European Cancer Anemia Survey (ECAS) with 15367 cases showed that the prevalence of anemia was 39.3% at enrollment and 67.0% during the survey [86]. Anemia prevalence at diagnosis was 18.98% in the Chinese population, according to our previous investigation of 1133 newly diagnosed cancer patients. Other factors such as age, reduced food intake, and a history of bleeding were also found to be independent risk factors for the development of anemia at the time of diagnosis in this cohort [87]. However, the incidence is significantly more common in those undergoing radiation or chemotherapy [88]. Cytotoxic chemotherapy is believed to be a primary factor contributing to anemia in cancer patients, the severity of anemia is determined by the extent of the malignancy and the dosage of treatment administered [89].

5. Sign and Symptom of Anemia and Quality of Life

The severity of symptoms can range from mild conditions like palpitations, dizziness, dyspnea, anorexia, and trouble concentrating to more serious ones like heart failure and lethargy [90].Vital symptoms that cancer patients experience is fatigue, which can hurt the patient's quality of life [91].

Depending of previous study show that the 78% of 419 cancer patients who were chosen at random to participate in a study stated they had become fatigued while undergoing treatment or dealing with their illness. Furthermore, more than cancer-related discomfort, 61% of the patients said that exhaustion hurt their lives [92]. Although anemia is thought to be a major contributing factor, cancer fatigue has diverse pathogenesis processes [93].

6. Anemia as an adverse prognostic factor

Anemia has been shown in several studies to be an autonomous risk factor for reduced survival in cancer patients, especially those undergoing both chemotherapy and radiation therapy. Furthermore, it significantly affects their quality of life [94].

There are conflicting reports, nevertheless, about increased survival after anemia correction conducted a relatively old investigation in which they examined sixty published publications describing cancer patients' survival based on their hemoglobin level

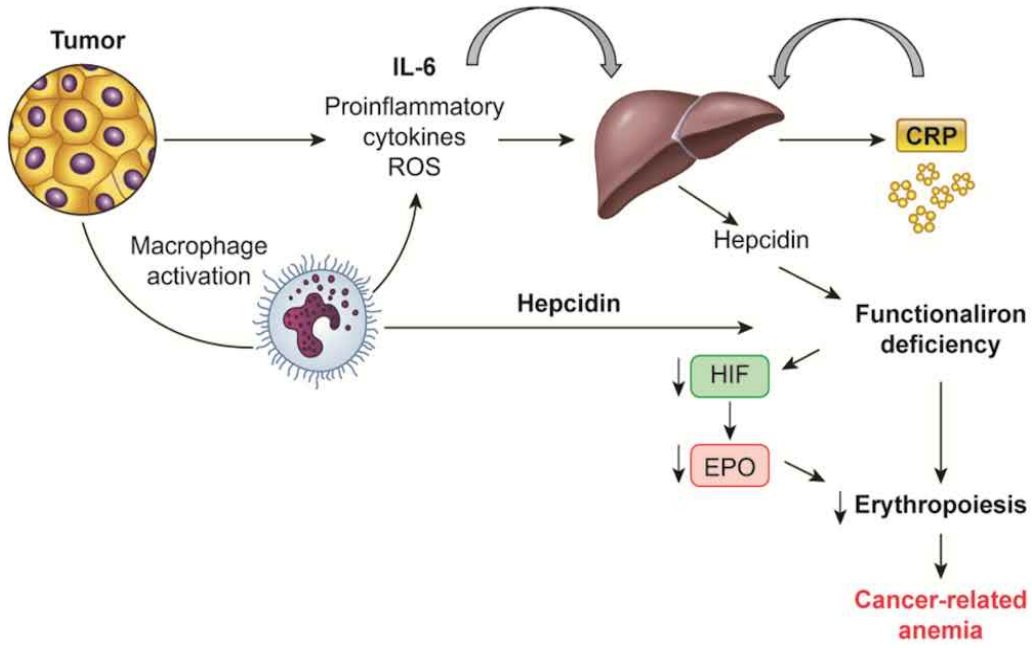

Figure 4. Pathogenesis of chemotherapy-induced anemia with erythropoiesis [135]

[95]. Anemia increased the total comparative risk of mortality in this diverse group of cancer patients by 19% in lung cancer patients, 47% in prostate cancer patients, 67% in lymphoma patients, and 75% in head and neck cancer patients [94].

7. Anemia Pathogenesis in Cancer Patients

Anemia may develop in patients with malignant illnesses for a variety of causes. The most common kind of anemia that patients with solid tumors have been known as "anemia of chronic disease" [96]. This condition has been seen in severe infections, trauma, chronic inflammatory disorders, and cancer [97]. In addition to hyperferritinemia and elevated iron storage, associated test findings include hypoferraemia and reduced transferrin saturation [98]. It is believed that the release of cytokines linked to chronic illness, IL-1, TNF and interferon beta, mediates this type of anemia [99]. These cytokines may result in decreased iron utilization, a reduction in colony-forming activity (BFU-E), and a shorter lifetime for red blood cells. An apparent low amount of EPO is commonly noted about the severity of anemia in these individuals [100-102]. These modifications result in an overall reduced RBC synthesis in the bone marrow, which is the most likely pertinent pathway for cancer-related anemia [103].

Factors contributing to the development of anemia in cancer patients

- Hemolysis
- Bleeding
- Hypersplenism, haemophagocytosis
- Nutritional deficiencies
- Chemotherapy, radiotherapy
- Anemia of chronic disorders
- Marrow damage
- Renal insufficiency)

Enumeration of distinct etiologies of therapy-induced anemia specifically associated with certain therapeutic interventions. [103]

- Radiotherapy
- Chemotherapy (depending on the drug, schedule, and dose)
- Combined radio-chemotherapy
- Surgery

List of related to underlying anemia of chronic disease[103]

- Reduced iron utilization
- Relative EPO deficiency
- Reduced RBC survival

8. Treatment of Anemia in Cancer Patient

Anemia's etiology should be determined, and treatment should focus on addressing the underlying cause. Directed therapy intervention may not work, though; pinpointing a precise causative component can occasionally be challenging. The therapeutic options represent in Table 2. Therapeutic Interventions in CIA [128]. That will be covered in the upcoming

Table 2. Therapeutic Interventions in CIA [128]

Therapy	Indications	Advantages	Disadvantages
RBC Transfusion	• Symptomatic CIA • Consider when Hb <8 g/dL • Consider comorbidities • Single-unit transfusion policy with administration of one unit at a time titrated to symptom resolution	•Rapid hemoglobin improvement •Rapid improvement in anemia symptoms • Enhance overall quality of life	• Pathogen transmission • Transfusion reaction • Alloimmunization • Increased thrombotic risk • May impact disease progression • Iron overload • Increased thrombotic risk • Seizures • Slow to improve hemoglobin
ESA administration	• Symptomatic CIA • Consider when Hb ≤10 g/dL	• Reduce RBC requirements • Diminished severity and duration of CIA • Improve quality of life	• Hypertension • Nausea, vomiting, diarrhea • Headache • Pruritus • Slow to improve hemoglobin
Intravenous iron supplementation	• Absolute iron deficiency (TSAT <20%, ferritin <30 ng/mL)7 • Functional iron deficiency (TSAT <50%, ferritin 30–500 ng/mL)7 • Consider when Hb ≤11 or has decreased by ≥2 g/dL from baseline level ≥12 g/dL in the setting of absolute iron deficiency as defined by serum ferritin <100 ng/mL29 • Consider before ESA administration	•Reduces RBC requirements • Facilitates optimal ESA response	• Hypertension • Dizziness • Dyspnea

Legend: RBC: Red Blood Cell, CIA: Cancer induced anemia, Hb: Hemoglobin, TSAT: Transferrin saturation, ESAs: Erythropoietin stimulating agents.

sections include intravenous (IV) iron therapy, erythropoietin-stimulating medications, and blood transfusions [30].

The United States Food and Drug Administration has authorized two erythropoiesis-stimulating medicines for the treatment of chemotherapy-induced anemia [40]. The Food and Drug Administration (FDA) has authorized the use of erythropoietin at a dosage of 40,000 Units (U) each week or 150 U per kg three times per week for patients with chemotherapy-induced anemia and hemoglobin levels below 10 g/dL [104]. Additionally, darbepoetin Alfa Dimensional Assessment of Repetitive Behavior (DARB) may be administered at a dosage of 500 micrograms (mcg) every three weeks or 2.25 mcg per kg weekly, empirical studies have shown that both EPO and DARB had equivalent efficacy in reducing the need for blood transfusions in individuals with chemotherapy-induced anemia [41].

8.1 Iron Supplementation

Erythropoietin deficiency and improper iron absorption into growing erythrocytes are two characteristics of anemia linked to cancer. Consequently, it has been suggested that CRA may be treated with an iron supplement and ESA combination. Depending on the severity and timing of cancer-associated anemia, the oral or IV method of iron replacement is selected. Oral and parenteral iron formulations (low-molecular-weight iron dextran, ferric gluconate, and iron sucrose) are evaluated in cancer patients. The addition of iron to ESAs versus ESAs alone for chemotherapy-induced anemia has been linked to improved Hb alterations, fewer RBC transfusions, and a higher hematological response, according to a recent systematic meta-analysis. In contrast to ESAs alone, the meta-analysis did not demonstrate any improvement reduction in time to hematological response in patients supplemented with iron. There have been no documented treatment-related fatalities [105]. There are two different ways to administer iron: intravenously or orally. Oral iron in the bivalent (ferrous) form is

Table 3. Laboratory Tests Used To Evaluate Iron Status [137]

Test	Normal Range
Serum Ferritin	Male 30-500 ng/mL Female 12-240 ng/mL
Serum Total Iron	Male 50-170 mg/dL Female 30-160 mg/dL
TIBC	240-450 mg/dL
TSAT	20%-50%
ZPP	<70 mmol/mol heme
STfR	Male 2.2-5.0 mg/L Female 1.9-4.4 mg/L
Hepcidin	<64.3 ng/Ml
Reticulocyte Count	0.5% – 2.5%
MCV	Male 83-98 fL Female 85-98 fL

Legend: TIBC: Total iron Binding Capacity, TSAT: Transferrin saturation, ZPP: Zinc protoporphyrin, STfR: Soluble transferrin receptor, MCV: Mean corpuscular volume, ng/mL: nanograms per milliliter, mg/dL: milligrams per deciliter. FL: Femtoliter.

more bioavailable than the trivalent (ferric) form. Ferrous gluconate is less dangerous than iron dextran in terms of safety [106, 107].

Parenteral iron toleration is linked to several side effects, including headache, dizziness, discomfort, hypertension, nausea, vomiting, and/or diarrhea. The majority of adverse events reported in the literature were linked to the use of high molecular weight iron dextran, which is no longer advised and is now substituted by other formulations in clinical practice [108]. Significantly, recent findings in individuals without ongoing cancer indicate that thrombocytosis associated with iron deficiency anemia may raise the risk of thrombosis by about two times when compared to individuals with IDA alone.

Oral iron is less expensive, however, there is conflicting evidence about its efficacy in treating anemia of inflammation [91, 109]. In two prospective trials, the superiority of IV iron over oral iron for improving Hb was not demonstrated in individuals with cancer-associated anemia [109, 110]. Some laboratory test need to all patient with chemotherapy induced anemia to evaluate the degree of anemia and detect amount each molecular such as iron and ferritin to see in detail in Hata! Başvuru kaynağı bulunamadı.

The primary iron regulator and frequent cause of iron homeostasis problems in cancer patients is hepcidin, an acute phase reactant generated in a setting of inflammation [111]. Patients with renal insufficiency may have even higher levels of hepcidin, impairing their ability to absorb iron through the mouth [112, 113]. Intravenous iron and are not constrained by a disturbed absorption system in an inflammatory environment, in contrast to the oral version, which is restricted by hepcidin [112, 114].

When a patient has an absolute iron shortage, as evidenced by a serum ferritin level of less than 100 ng/mL, intravenous iron is suggested in cases of CIA in patients with a hemoglobin level of less than 11 or a reduction in hemoglobin of at least 2 g/dL from a baseline level of at least 12 g/dL [115].

8.2 Erythropoietin

The hormone erythropoietin a circulatory hematopoietic glycoprotein, was first discovered in 1906 and aids in the synthesis of red blood cells [116]. EPO is expressed by the interstitial cells of the liver and kidneys, and it is increased in hypoxic environments to promote the synthesis of red blood cells in the bone marrow [116, 117]. EPO attaches to erythroid precursor cells to enhance both maturation and differentiation after being secreted by the liver and kidneys [116]. ESAs are a type of recombinant drugs that use iron stores required for healthy erythropoiesis to promote red cell growth[118].

Anemia was a common side effect of chemotherapy and cancer in the early 1980s, and red blood cell transfusion was the recommended course of treatment. Historically, doctors would sometimes transfuse patients based only on their symptoms, with the transfusion threshold being approximately 8 g/dL. In the latter part of the 1980s, Amgen successfully cloned epoetin alfa, and in the early 1990s, the CIA approved darbepoetin alfa, a molecule containing additional sugar moieties. When compared to a placebo, epoetin alfa and darbepoetin alfa has both been demonstrated

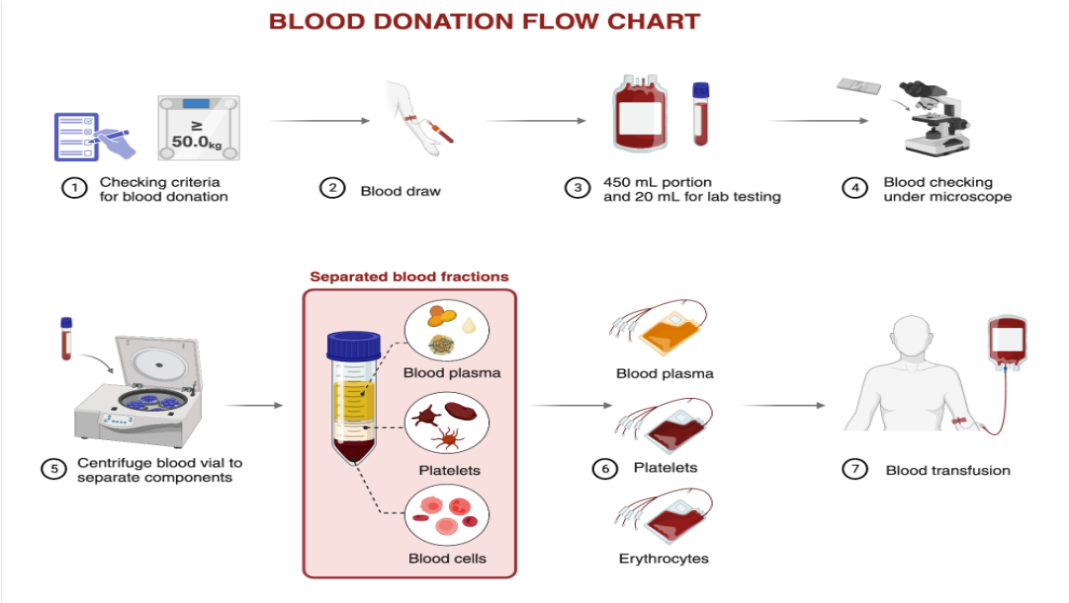

Figure 5. process of blood transfusion [136]

to improve quality of life [119, 120] . Human immunodeficiency virus(HIV), CIA, and end-stage renal disease are just a few of the illnesses that have shown that increased quality of life is correlated with higher hemoglobin levels [111, 121].

One of the main objectives of using ESAs in CIA is to reduce the requirement for RBC transfusions while simultaneously improving QOL and achieving sustained anemia correction [31]. Following the US food and drug administration's approval of darbepoetin and epoetin for use by the CIA [122]. Numerous randomized clinical trials have demonstrated that ESA improved Hb levels and decreased the need for RBC transfusions in patients with CIA [94].

8.3 Red Blood Cell Transfusion

Therefore, optimal blood transfusion shows Figure 5 in depends on the grade of anemia and hemoglobin concentration level [123, 124]. Notably, improved oxygen delivery is not achieved by transfusion of red blood cells to hemoglobin levels higher than 7 g/dL [1]. While transfusions of red blood cells can quickly increase hemoglobin, Provide a brief description of the content of the Table 4 and amount of increasing of blood after blood transfusion are 1%-3% of transfusions are associated with unfavorable outcomes [124].

A few of these side effects include hemolysis of incompatible plasma or red blood cells, allergic reaction, immunologic compromise, thrombosis, infections, acute lung injury associated with transfusions, and the development of antibodies against the human leukocyte antigen [115, 125].

Red blood cell transfusions may interact negatively with chemotherapy in addition to the concerns already discussed [126]. Blood can be kept in storage for up to 42 days after donation, but the longer it is kept, the quality of this blood can be lower, extended blood storage is linked to modifications in the metabolism, morphology, and rheology of red blood cells the loss of proteins, lipids, and carbohydrates from the membrane and changes in secretion, adhesion, and oxygen delivery [127, 128]. Acute lung injury linked with transfusion is the second most common blood transfusion complication, after delayed hemolytic response. However, donor leukocytes in the transfusion are primarily responsible for the majority of immunologic and viral issues brought on by blood transfusions. Leukocytes are the vector used by blood transfusion-transmitted viruses to infect their host, the most prevalent being hepatitis B. Hepatitis C, HIV, hepatitis A virus, cytomegalovirus (CMV), Epstein-Barr virus, human herpes virus 8, toxoplasma, parvovirus B-19, West Nile virus, spongiform encephalopathy prions, Chagas, Babesia, and malaria are among the other viruses that are less likely to be spread [129].

9. Treatment of Cancer induced anemia

It is possible to discover highly cytotoxic pharmacologic regimens and assess the effects of chemotherapy-induced myelosuppression by developing screening tests that use hematopoietic stem progenitor from peripheral blood and bone marrow [130]. Different

Table 4. Organization and recommendations for red blood cell transfusion [128]

Organization	Recommendations
AABB [128]	• Maintain hemoglobin ≥7 g/dL in hemodynamically stable hospitalized patients • Consider transfusion in patients with hemoglobin ≤8 g/dL and preexisting cardiovascular disease • Personalize all transfusions by both symptoms and hemoglobin levels
ESMO [115] (2018)	• Recommend transfusion in patients with hemoglobin level <7–8 g/dL and/or with severe anemia-related symptoms, regardless of hemoglobin level
NCCN [28] (2018)	• Recommend transfusion of patients with symptomatic anemia • Monitor and reevaluate patients with asymptomatic anemia who lack significant comorbidities • Consider transfusion in patients who are at high risk with progressive hemoglobin decline in the setting of recent chemo radiation or asymptomatic with comorbidities such as cardiopulmonary or cerebrovascular disease

Legend: (AABB) American Association of Blood Banks, (ESMO) European Society of Medical Oncology, (NCCN) National Comprehensive Cancer Network, (g/dL) Grams per Deciliter.

hormones and pharmaceutical agents have an impact on the management and prevention of CIA in addition to new screening methods. Myelosuppression of neoplastic cells may be selectively induced by tyrosine kinase inhibitors when administered after chemotherapy, but not of bone marrow progenitor cells [131]. Arginine vasopressin, transforming growth factor beta inhibitors, eryptosis inhibitors, and medications that block the HIF-1α pathway may support and maintain erythropoiesis while also shielding renal tubular and endothelial cells [132-134]. Researchers are looking into using roxadustat, a reversible HIF prolyl hydroxylase inhibitor, to lessen the need for red blood cell transfusions. Roxadustat boosts the expression of both erythropoietin and related receptors, there is hope that CIA prevention, early detection, and timely treatment will result in fewer dosage delays, fewer dose reductions, and better patient outcomes.

CONCLUSION

The prevalence of anemia in adult cancer patients undergoing chemotherapy is influenced by various factors associated with chemotherapy-induced anemia, including the cancer grade, degree of anemia, duration of malignancy presentation, and treatment intensity. The exact etiology of chemotherapy-induced anemia remains incompletely understood, although some theories suggest mechanisms that hasten the development of anemia. For instance, many commonly used chemotherapeutic agent's impact the cell cycle, leading to a slowdown in the division of normal cells. Hence, chemotherapy affects not only cancer cells but also normal cells, impeding their division. The most effective treatments for anemia in cancer patients include erythropoietin-stimulating agents, red blood cell transfusions, and iron supplementation, each with its own set of advantages and disadvantages detailed in previous literature. For future research endeavors, it is recommended that investigators focus on identifying the safest approach to managing anemia in cancer patients, one that minimizes adverse side effects.

Conflict of Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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The prognostic significance of inflammation associated blood cell markers in metastatic colorectal cancer.

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ABSTRACT

Objectives: The aim is to perform prognostic evaluation with overall survival (OS) and progression-free survival (PFS) in hematological parameter-based groups in patients with metastatic colorectal cancer (mCRC).

Methods: In a single institution, 51 patients were retrospectively analyzed mCRC diagnosed between 2019 and 2022. Pretreatment hematological parameters of patients with mCRC receiving first-line chemotherapy in a single center were examined. The receiver operating characteristic curve was used to predict the tests. Median OS was calculated by the Kaplan-Meier method and compared with the log-rank test. Multivariate analyses were performed using a Cox regression model.

Results: The median OS of the patients included in the study was 27 months (3-88 months) by statistical calculation; the median PFS was 19 months (2-84 months). The median could not be reached. Among the risk factors affecting OS, it was found effective to have a bone metastasis site and a pancreatic metastasis site (p values 0.003 and 0.027, respectively). In the analysis of the risk factors affecting PFS, bone and pancreatic metastases were found to be significant (p values 0.001 and 0.004, respectively). Patients receiving chemotherapy and anti-VEGF therapy have a significantly reduced risk of death of 0.06 times compared to those who do not receive chemotherapy, which indicates that OS is significantly longer in people receiving chemotherapy in question (p=0.020). It was observed that blood cell marker levels were not statistically significant in PFS and OS. Of the 51 patients included in the study, 30 of them were still being followed up, while 21 of them died.

Conclusions: Chemotherapy plus anti-VEGF therapy is a treatment whose effectiveness has been determined in metastatic colorectal cancer. In the future, there is a need for more prospective and large patient group studies on this topic to measure the prognostic value of hematological parameters in metastatic colorectal cancer.

Keywords: metastatic colorectal cancer, parameters, overall survival

With many studies showing the relationship between cancer and inflammatory markers, it has been seen that inflammation plays a role in carcinogenesis [1-3]. Colorectal cancer (CRC) is the third most common cancer and results in more

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than 1 million new cases and 600,000 deaths per year [4]. Various biochemical markers associated with this malignancy, prognostic and diagnostic tools are being evaluated [5-7]. In addition to the classic 'inflammatory related markers such as acute phase proteins (CRP and globulins), these are the platelet / lymphocyte ratio (PLR) and the neutrophil / lymphocyte ratio (NLR) [5, 8]. Among them, red blood cell distribution width (RDW) shows how homogeneous or heterogeneous the blood is by measuring how different the sizes of red blood cells are and is used to types of anemia [9]. Recently, the importance of RDW has increased in many chronic inflammatory and cardiovascular diseases [10-12]. Recent reports have shown that it can be used as a prognostic marker in various cancers such as lung, esophagus-gastric and breast, liver [13-18]. The prognostic role of RDW in CRC has also been studied. However, its role in the context of this malignancy remains unclear, as published reports show inconsistent results. In CRC, as in other solid tumors, carcinogenesis is caused by inflammation [19, 20].

In recent years, many studies have investigated the viability of survival and predictive immune scores associated with systemic inflammatory response in CRC. For example, some of these include the lymphocyte/monocyte ratio, PLR, and the modified Glasgow Prognostic Score, which is determined using serum CRP and serum albumin [21, 22]. Promising results have been seen in NLR risk estimation. Those with low NLR showed worse survival outcomes than patients with CRC with high NLR. This condition has been confirmed at various stages of CRC, from early localized disease to advanced stages and surgical resection [23, 24]. NLR has also been studied in CRC patients who have undergone liver metastasectomy [25]. In the mCRC, the guidelines recommend dual or triple fluoropyrimidine-based chemotherapy (CTX) regimens in first-line treatment, as well as targeted therapy in addition.

In addition, the decision on the intensity of treatment, therapeutic goals are determined usually taking into account the clinical and radiological characteristics of the patient [26]. If the goal is to transform into a resectable disease for a final surgical treatment approach, a more intensive regimen is recommended. When the goal is disease control, a less intensive CTX regimen usually controls the progression of the disease and is the first option that also protects the quality of life.

The aim of this study is to perform progostic evaluation with Overall survival (OS), Progression free survival (PFS) in hematological parameter-based groups in patients with metastatic colorectal cancer (mCRC).

METHODS

This retrospective study was conducted after the approval of the ethics committee in patients. Metastatic colorectal cancer patients followed up at Medical Oncology Unit of Bursa City Hospital between 2019 and 2022 were evaluated. Exclusion criteria from the study were: Early stage CRC, secondary malignancy, kidney and liver failure, steroid use, active uncontrollable infection. Age, gender, tm localization of patients, whether there is metastasis at diagnosis, location of metastasis, ECOG performance status (ECOG PS), First-line CTX regimen, CEA, CA 19.9, LDH, CRP, albumin, neutrophil (NEU), platelet (PLT), mean platelet volume (MPV), RDW, lymphocyte (LEU), NLR, MPV/PLT ratio, RDW/PLT ratio, NEU x PLT)/LEU, NEU x 1000/PLT ratio were recorded as laboratory data. Blood values were studied during pre-chemotherapy and at the admission. OS, PFS informations were recorded.

Peripheral blood was taken before the first CTX cycle. OS, PFS of the patients were recorded. OS was determined as the period from the diagnosis of the patient to his death or the date of the study. PFS was determined as the period from the date of diagnosis to the progression.

Statistical Analysis

Descriptive statistics of the measurements were calculated as arithmetic mean, standard deviation (SD), median and quartiles. The compliance of the numerical measurement or diagnostic markers with the normal distribution was evaluated by the Shapiro-Wilk test and deviations from the normal distribution were observed. Deceased and living patients were compared with Pearson Chi-Square test or Fisher-Freeman-Halton exact test in terms of categorical characteristics distribution. The Mann-Whitney U test was used for comparison in terms of numerical characteristics. In addition, the success of 5 numerical diagnostic markers (NLR, MPV/PLT, RDW/PLT, (NEU x PLT) / LEU and NEU x 1000/PLT) in separating the deceased was also examined with the ROC curve. In addition, the factors affecting OS and PFS durations were first considered individually and evaluated using the univariate Cox regression model, and the uncorrected effects of the factors were calculated. Then,

all the variables were taken into the multivariate Cox regression model and the final model was established by leaving the variables with significant effects on OS and PFS in the model with the help of the stepwise variable elimination method. Mean and median values of OS and PFS were calculated and Kaplan-Meier survival curves were drawn. $p<0.05$ was taken as the statistical significance level and SPSS (ver. 23) the program was used.

RESULTS

The general characteristics of the patients and the distribution of categorical characteristics are shown in Table 1. The female sex ratio was observed as 29.4%. The most common tumor localization was observed in the rectum 27.5%, metastasis at diagnosis was observed in 64.7%, and the most common metastasis was observed in the liver 88.2%. Doublet CTX was given the most frequently as chemotherapy 51.0%. Laboratory data, descriptive values of OS, PFS and numerical characteristics are shown in Table 2.

Of the total 51 patients included in the study, 30 continued their lives, while 21 had died. The shortest duration for OS was 3 months, the longest duration was 88 months; the shortest duration for PFS was 2 months and the longest duration was 84 months.Other descriptive statistics for both OS and PFS are given in Table 5. The median OS was calculated as 27 months, while the mean OS was calculated as 42 months. The median could not be reached. Median PFS is 19 months, mean PFS is 35.3 months. Survival curves of PFS and OS (survival function) were given in Figure 1. The success of NLR, MPV/ PLT, RDW/ PLT, (NEU x PLT) / LEU and NEU x 1000/PLT diagnostic markers in differentiating deceased and living patients was evaluated using the ROC curve and the results given in Figure 2 were obtained. When Figure 2 was examined, it was determined that the 5 markers in question could not distinguish between the deceased and the living successfully at a meaningful level. The cut-off value could not be given because there was no significant relationship. The ROC curves are given in Figure 2. In the analysis of the factors affecting OS with the multivariate Cox regression model, the risk factors

Table 1. Patients characteristics

		n (%)	%
Gender	Female	15 (29,4)	29.4
ECOG PS	0	11	21.6
	1	27	52.9
	2	13	25.5
Status	Ex	21	41.2
Tumor location	Rectum	14	27.5
	Descending	11	21.6
	Ascending	13	25.5
	Sigmoid	8	15.7
	Transvers	5	9.8
Progression	Yes	37	72.5
Metastasis in the diagnosis	Yes	33	64.7
Chemotherapy	No	4	7.8
	Doublet plus anti-EGFR	7	13.7
	Doublet plus anti-VEGF	8	15.7
	Doublet CTX	26	51.0
	Fluoropyrimidine	6	11.8
Location of metastasis	Liver	45	88.2
	Lung	16	31.4
	Bone	9	17.6
	Surrenal	2	3.9
	Peritoneum	6	11.8
	Kidney	1	2.0
	Pancreas	1	2.0

ECOG performance status: ECOG PS

Table 2. Descriptive values of numerical properties

	n	median(min-max)
Age	51	64.00
CEA	51	26.90
CA 19.9	51	34.10
LDH	51	231.00
CRP	51	34.40
ALBUMİN	51	39.90
NEU/ LEU	51	2.480
MPV/ PLT	51	.029
RDW/ PLT	51	.033
(NEU x PLT) / LEU	51	804.46
NEU x 1000 / PLT	51	200.00
OS	51	17.00
PFS	51	8.00

OS: Overall Survey, PFS: progression free survival, NEU: neutrophil, PLT: platelet, MPV: mean platelet volume, RDW: red blood cell distribution width, LEU: lymphocyte

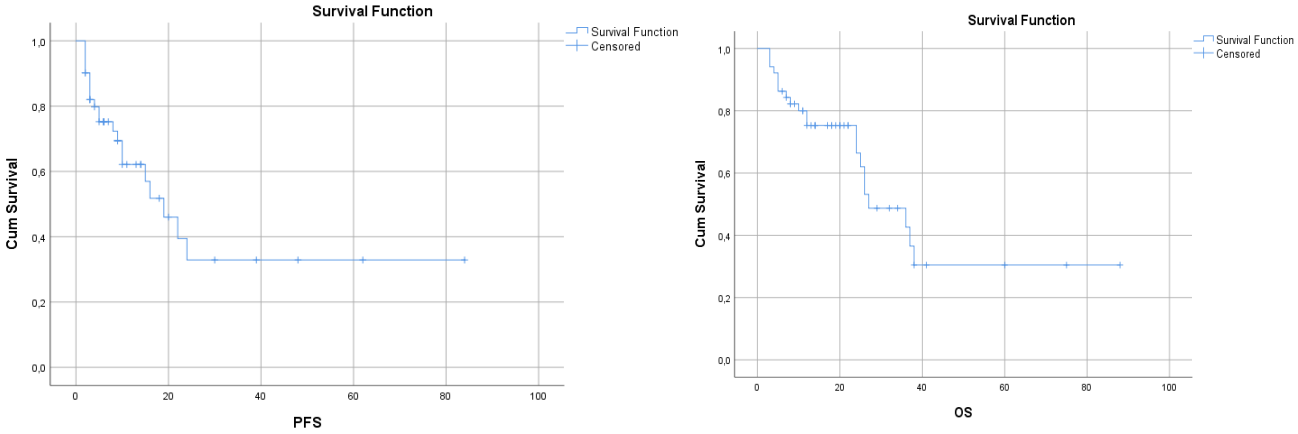

Figure 1. Survival curves of PFS and OS (survival function)

Table 3. Analysis of risk factors that have a significant impact on OS

Variable / Risk *vs* reference	HR 95.0% CI	P
Metastasis in the diagnosis		
Yes *vs* No	2.915 (0.841- 10.100)	.092
Site of metastasis		
Bone *vs* Other	6.862 (1.939 - 24.287)	.003
Pancreas *vs* Other	48.339 (1.550 -1507,25)	.027
Chemotherapy		
Doublet plus anti-EGFR *vs* no CTX	0.965 (0.169 -5.517)	.968
Doublet plus anti-VEGF *vs* no CTX	0.060 (0.006 -0.642)	.020
Doublet CTX *vs* no CTX	0.270 (0.049 -1.476)	.131
Fluoropyrimidine *vs* no CTX	0.405 (0.033 -5.036)	.483
CRP	1.008(1.003-1.013)	.003

HR: Hazard Ratio CTX: Chemotherapy, EGFR: epidermal growth factor receptor, VEGF: vascular endothelial growth factor

that have a significant effect on OS are included in Table 3. When the model results were examined, it was found that the risk of death was significantly higher by 2,915 times in those who had metastases at diagnosis, and therefore OS was significantly shorter. The risk in those with bone and pancreatic metastases has a significantly higher risk of death compared to those in other regions, so OS was found to be significantly shorter in these people. Patients who receive "Doublet plus anti-VEGF" as CTX have a significantly reduced risk of death by 0.06 times compared to those who do not receive CTX, and this indicates that the OS is significantly longer in people who receive CTX in question. Other than this, the risk of death in CTX groups did not differ significantly from those who did not receive CTX. As CRP increases by 1 unit from its own unit (>5 mg/L), the risk of death increases significantly by a factor of 1,008, and therefore the OS decreases. Examination of the factors affecting PFS with the multivariate Cox regression model Table 4 shows the risk factors that have a significant effect on PFS. The risk in those with bone and pancreatic metastases has a significantly higher risk of death compared to those in other regions, so PFS was significantly shorter in these people. As CRP increases by 1 unit from its own unit, the risk of death increases significantly by a factor of 1,005, and therefore PFS decreased. The effects of categorical factors on death are shown in Table 6. It has been observed that there is a significant effect on death with the presence of bone and peritoneal metastases. (p=0.014, 0.026)

DISCUSSION

The aim is to perform progostic evaluation with OS and PFS in groups based on hematological parameters in patients with mCRC. It is important to understand the cause-effect relationship between inflammation and cancer in terms of diagnosis and treatment of cancer. In our study, it was determined that the markers CEA, CA 19.9, LDH, CRP, Albumin, NLR, MPV/PLT, RDW/ PLT, (NEU x PLT) / LEU and NEUx1000/PLT could not distinguish between deceased and living patients successfully. Figure 2 shows that this situation is not statistically significant.

NLR and PLR are simple, easily accessible markers that indicate subclinical inflammation. Absolute neutrophil and lymphocyte counts can be affected by many factors. Many factors have proven to be useful

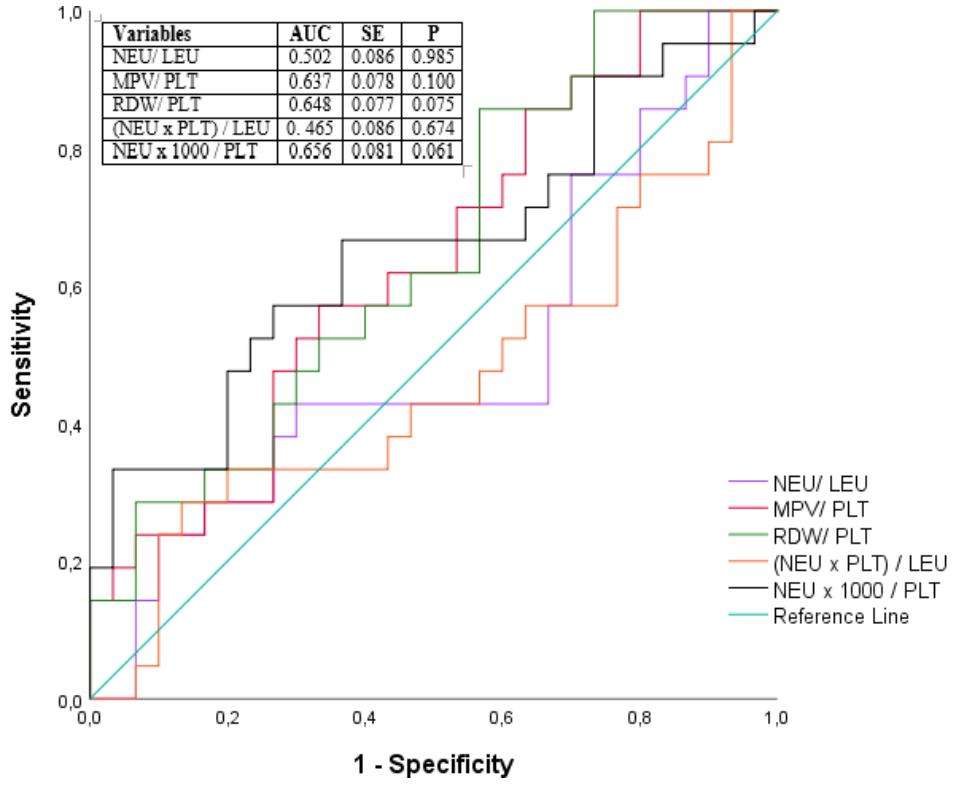

Figure 2. The power of NEU/ LEU, MPV/ PLT, RDW/ PLT, (NEU x PLT) / LEU ve NEU x 1000 / PLT to distinguish between died and alive (ROC curves)

Table 4. Analysis of risk factors that have a significant impact on PFS

Variable / Risk *vs* reference	HR 95,0% CI	P
Site of metastasis		
Bone *vs* Other	6.099 (2.125- 17.509)	.001
Pancreas *vs* Other	34.077 (3.177- 365.533)	.004
CRP	1.005 (1.001- 1.010)	.016

HR: Hazard Ratio CI: Confidence Interval, OS: Overall Survey, PFS progression free survival

Table 5. Descriptive values of OS and PFS

	Median	SE	95% Confidence Interval
OS	27.0	5.459	16.301-37.699
PFS	19.0	3.983	11.193-26.807

CI: Confidence Interval, SE: standart error, OS: Overall Survey, PFS progression free survival

Table 6. The effects of categorical factors on death

Site of metastasis	Metastasis	Ex n	Ex %	$P_{(status)}$*
Liver	No	2	33.3	.678
	Yes	19	42.2	
Lung	No	13	37.1	.387
	Yes	8	50.0	
Bone	No	14	33.3	**.014**
	Yes	7	77.8	
Surrenal	No	20	40.8	.796
	Yes	1	50.0	
Peritoneum	No	16	35.6	**.026**
	Yes	5	83.3	
Kidney	No	21	42.0	.398
	Yes	0	0.0	
Pancreas	No	20	40.0	.412
	Yes	1	100.0	

in determining the prognosis in mCRC. Patient-related (age, performance status, comorbidities), tumor-related (local growth, distant metastasis), biochemical (markers such as platelets, leukocytes, hemoglobin, CEA, LDH, alkaline phosphatase, albumin) and molecular factors (KRAS, NRAS and BRAF mutations) have all been associated with survival outcomes [27, 28].

As a result, various studies have suggested that the analysis of inflammatory factors, including the evaluation of inflammatory cells in the peripheral blood, may help in predicting survival in mCRC. Ratios between inflammatory cells such as NLR have been proposed, as other factors unrelated to cancer may also affect the systemic leukocyte count [29].

A high neutrophil count has been shown to be an independent prognostic marker for cancer recurrence and survival (including gastric cancer, metastatic melanoma, advanced non-small cell lung cancer, and metastatic renal cell carcinoma) [30, 31, 32]. Neutrophilia and lymphopenia are seen in systemic inflammation. NLR establishes the balance between antitumor functions and pre-tumor inflammatory pathways. An increase in NLR indicates that inflammatory cells affect tumor growth in the microenvironment. They also facilitate the escape of tumor cells from immunity by suppressing cell-mediated immunity [33]. High NLR is associated with tumor invasiveness, angiogenesis and metastasis [34].

The relationship between palliative CTX outcome and NLR was evaluated in 349 patients with mCRC and a significant effect of high NLR was found (p=0,002). In addition, significant improvement in PFS was observed in patients whose NLR returned to normal after one CTX cycle (p=0,012) [36].

Studies on PLT, PDW, MPV and other platelet-related indicators have appeared one after the other in recent years [37, 38]. In addition to the clotting pro-

cess, platelets also regulate the inflammatory response and cancer pathogenesis. Activating platelets can promote tumor growth, angiogenesis, and invasion [39]. Studies conducted support that the PLT counts in the CRC are based on systemic inflammation, but it is not definitive as a risk factor for prognosis and survival [40, 41]. The PLR is an index that is believed by some authors to be related to the prognosis of CRC [42, 43]. The importance of prognostic risk for PDW varied between different cancers: High PDW in breast cancer was considered a poor prognostic marker [44]; low PDW was a negative predictive factor in gastric cancers and non-small cell lung cancers [45, 46]. The role of the PDW in the CRC has been examined in a small number of publications.

In our study, the risk in those with bone and pancreatic metastases has a significantly higher risk of death compared to those in other regions, so PFS and OS are significantly shorter in these people. Duraker et al. according to the data of their study, it was found that the most common place of metastasis in CRC patients was the liver, followed by the peritoneum [47].

In our study, patients receiving "Doublet plus anti-VEGF" as CTX have a significantly reduced risk of death at a level of 0.06 times compared to those who did not receive CTX, and this indicates that OS is significantly longer in people receiving CTX in question. Other than this, the risk of death in CTX groups did not differ significantly from those who did not receive CTX. Therefore, the importance of choosing CTX in primary care should be taken into account. According to the ASCO Guideline 2023, Doublet (folinic acid, fluorouracil [FU], and oxaliplatin [FOLFOX], or folinic acid, FU, and irinotecan [FOLFIRI]) backbone chemotherapy should be offered as first-line therapy to patients with initially unresectable microsatellite stable (MSS) or proficient mismatch repair (pMMR) mCRC. Capecitabine plus oxaliplatin therapy can be used instead of FOLFOX at the clinical discretion of the treating physician and by joint decision with the patient. All patients were given the anti-vascular endothelial growth factor (VEGF) antibody bevacizumab in addition to a double or triple chemotherapy regimen (48). Dual chemotherapy has previously been shown to be superior to FU and folinic acid (49); therefore, this analysis focused on the potential for additional benefits of triple chemotherapy compared to FOLFOX or FOLFIRI (50,51).

Our study had some limitations. The fact that it is retrospective, it is single-centered, the small number of samples may cause a relatively short follow-up period in some patients. Because of these, our study resulted differently from the literature. In addition, no molecular evaluation was performed in this study, such as determining the instability of the micro-satellite. The mutation status of the patients was not evaluated.

Prospective studies are needed to further understand the prognostic value of NLR. MPV/PLT, RDW/PLT, (NEU x PLT)/LEU and NEU x 1000/PLT. There is probably a process going on in the tumor microenvironment, and we don't know the details of it.

CONCLUSION

Conflict of Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval

The protocol of the study was approved by the Medical Ethics Committee of Bursa City Hospital, Bursa, Türkiye. (Decision number: 2021-21/6, date: 17.11.2021).

Authors' Contribution

Study Conception: AHT; Study Design: AU; Literature Review: AHT; Critical Review: AHT; Data Collection and/or Processing: AHT,; Analysis and/or Data Interpretation: AHT; Manuscript preparing: AHT.

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In vitro cytotoxicity and genotoxicity/ antigenotoxicity evaluation of encapsulated black garlic extracts on A549 cells.

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ABSTRACT

Objectives: Black garlic is produced by fermenting fresh garlic under controlled temperature and humidity conditions for an extended period. Due to its sweeter taste and lack of pungent odor compared to fresh garlic, black garlic is easier to consume. Moreover, the increase in bioactive compounds such as polyphenols and flavonoids during fermentation has sparked interest in studying the health effects of black garlic. It is known that different fermentation and extraction methods can lead to variations in biological activities. Therefore, analyzing the effectiveness of black garlic processed by different methods is of critical importance. In our study, we investigated the cytotoxic, genotoxic, and antigenotoxic effects of different concentrations of encapsulated black garlic capsule extract (BGC) on lung cancer cells.

Methods: The A549 cell line was used to investigate the effects of BGC. Cells treated with BGC at different concentrations (10, 25, 50, 100, 125, 250, 500, and 1000 µg/mL) for 24 hours were subjected to MTT and NRU assays to examine the cytotoxic effects. Alkaline comet assay was performed to investigate genotoxic and antigenotoxic effects. For antigenotoxicity analysis, cells pretreated with BGC were exposed to H2O2 to explore the protective effects of BGC.

Results: According to the MTT results, cell viability remained at 90% even at concentrations higher than 125 µg/mL. However, in the NRU analysis, viability decreased to less than 70% at concentrations ranging from 50 µg/mL. Comet assay results revealed significant increases in tail length and tail intensity at different concentrations (specifically, at 250 µg/mL and above and at 50 µg/mL and 100 µg/mL, respectively). However, tail moments did not show any significant differences at any concentration. Additionally, BGC significantly reduced H_2O_2-induced DNA damage.

Conclusions: Our research demonstrated that BGC reduces the viability of lung cancer cells and can have genotoxic effects. Additionally, its protective effect against oxidative damage was shown at the DNA level. Based on these data, further research can be conducted on the use of BGC against cancer.

Keywords: Black Garlic, Antigenotoxicity, Cytotoxicity, Comet Assay

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Garlic (Allium sativum L.) is a plant widely used in global cuisine and traditional medicine. It is consumed in various forms, such as fresh, dried, pureed, and powdered. The biological functions of garlic have been investigated in numerous studies. It contains many bioactive compounds, including organic sulfides, saponins, phenolic compounds, and polysaccharides [1]. Various biological and pharmacological effects, such as antimicrobial, antioxidant, anticancer, antidiabetic, antiallergic, cardiovascular protective, anti-inflammatory, immunomodulatory, and antiobesity effects, have been demonstrated [1, 2]. However, the characteristic taste and odor of garlic may limit its consumption. Black garlic is obtained through the controlled fermentation of fresh garlic. It has a sweeter taste, is dark brown or black in color, and is odorless compared to fresh garlic. Although there is no standard method for fermentation process, fresh garlic is fermented at high temperatures (60-90 °C) and 60-90% humidity for 10-80 days during production [3]. The chemical composition of black garlic is different from that of fresh garlic after fermentation. Black garlic contains relatively high levels of antioxidants such as polyphenols, flavonoids, pyruvate, S-allyl-cysteine (SAC), S-allyl-mercapto-cysteine (SAMC), and 5-hydroxymethylfurfural (5-HMF) [3-6].

In scientific studies, black garlic has been shown to have various beneficial health effects, such as anticancer, anti-inflammatory, immunomodulatory, cardioprotective, nephroprotective, hepatoprotective, antidiabetic, and antiobesity effects [1]. In terms of hepatoprotective effects in a liver damage model, black garlic has been shown to prevent an increase in AST and ALT levels related to carbon tetrachloride-induced liver damage and to suppress hepatic damage induced by D-galactosamine. However, no changes were observed in the levels of alkaline phosphatase (ALP), a marker of hepatobiliary damage [7]. Another study revealed that treatment with black garlic extract decreased AST, ALT, ALP, and malondialdehyde (MDA) levels in a mouse model of acute hepatitis, and significant anti-inflammatory effects were identified in the mice [8]. Black garlic and yeast-fermented black garlic exhibit hepatoprotective, nephroprotective, hypolipidemic, and antiobesity effects in obese mice. However, no hypoglycemic effect was observed in the study [9]. The effects of fresh garlic and BG-ethanol extracts on immune cells obtained from the blood of 21 volunteers were examined. Garlic extract significantly influenced the proliferation, TNF-α level, and NO production of primary immune cells, with slight differences observed between garlic extract and BG. BG was determined to be a much stronger immunostimulant than raw garlic extract [10].

Studies have been conducted on the anticancer properties of BG both *in vitro* and *in vivo*. Regularly administering aged garlic extract to BALB/c mice implanted with fibrosarcoma cells increases IFN-γ and IL-4 production in splenocytes, specific cytotoxicity against fibrosarcoma cells, and strengthens the immune system, inhibiting tumor growth [11]. BG extracts from different fermentation periods have been shown to have cytotoxic effects on the HL60 leukemia cell line, albeit to a lesser extent than fresh garlic. The IC50 value for fresh garlic was determined to be 0.03 mg/mL, whereas for BG extracts, it was calculated to be 0.7 (fermented for 32 days) and 0.9 (fermented for 45 days) mg/mL [12]. SAMC found in BG reduces colorectal cancer cell viability *in vitro* in a dose- and time-dependent manner via the JNK and p38 pathways [13]. Furthermore, in colon cancer animal models, BG therapy reduces proliferative activity in adenoma and adenocarcinoma lesions [14].

Oxidative stress occurs as a result of an imbalance between reactive oxygen species (ROS) and free radicals generated during cellular metabolism and the antioxidant mechanisms. Elevated levels of ROS and free radicals can lead to DNA damage and alter various signaling pathways. Therefore, oxidative stress is known to be associated with many types of cancer. Different dietary habits, such as inadequate or excessive food intake, can trigger inflammatory and oxidative states and lead to certain pathophysiological conditions [15]. Moreover, it is known that biologically active molecules found in foods can support the prevention of many diseases. For instance, there are numerous reports on the anticancer effects of flavonoids, which possess strong anti-inflammatory and antioxidant properties [16]. Garlic, which contains a variety of phytochemicals, has emerged as an important plant with antioxidant effects. Studies have demonstrated that the antioxidant activities of garlic and its active components are influenced by garlic type and processing method. It has been shown that short-term fermented black garlic (13 days) exhibits better physicochemical qualities and greater biological activity than long-term fermented black garlic (32 and 45 days) and even white garlic [12]. The antioxidant activity of black garlic is greater than that of raw garlic according to DPPH (2,2-diphenyl-1-picrylhydrazyl), which is a method of measuring antioxidant activity, ABTS (2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfon-

ic acid)), FRAP (Ferric Reducing Antioxidant Power), H2O2 scavenging, and Fe2+ chelation analyses [17–19]. Additionally, different extraction methods also influence their biological activities. It has been reported that the DPPH radical scavenging activity of the distilled water extract of BG is higher than that of other extracts, while the Fe2+ chelating activities of the ethanol and chloroform extracts are greater than those of the distilled water extracts [17]. During a 30-day period, mice fed with BG or BG residue showed decreased levels of MDA, while SOD and GSH-Px activities significantly increased [20]. Pretreatment with black garlic significantly reduces tert-Butyl hydroperoxide (tBHP)-induced damage in rat clone-9 hepatocytes. BG facilitates the reversal of decreased antioxidant enzyme activity in hepatocytes induced by tBHP [21].

The biological activity of black garlic is influenced by various factors, such as fermentation conditions, raw material quality, and extraction methods. In our study, we aimed to investigate the effects of the encapsulated form of black garlic aqueous extract on cells and compare them with the data available in the literature. We examined the cytotoxic, genotoxic, and antigenotoxic effects of the encapsulated black garlic extract (BGC) on the human lung adenocarcinoma cell line A549.

METHODS

Preparation of the encapsulated black garlic extract

In our study, BGC produced by Cinar and colleagues in 2022, with a detailed methodology published, were utilized [22].

Sample Preparation

The BGCs were dissolved in cell culture medium at 37°C in a water bath. Serial dilutions were prepared at concentrations of 10, 25, 50, 100, 125, 250, 500, and 1000 µg/mL.

Cell Culture

The A549 cell line, which is commercially available for cytotoxicity, genotoxicity, and antigenotoxicity analyses, was used. The cells were cultured in RPMI (Roswell Park Memorial Institute) medium supplemented with 10% FBS (Fetal Bovine Serum) and penicillin-streptomycin, seeded into a 75 cm^2 culture flask with culture medium and maintained in a 37°C incubator under standard conditions with 5% CO_2. After reaching 80% confluence, they were harvested by trypsinization. The cells were seeded at a density of 10^4 cells/well in 96-well plates for the MTT (a method to measure cell viability) and NRU assays and at a concentration of 10^5 cells/well in 12-well plates for the comet assay. After 24 hours, the culture medium was removed, and the culture wells were supplemented with BG-containing media at predetermined concentrations. For the negative control, standard culture media was utilized, while for the positive control, 0.1% Triton X-100 was applied. The culture was sustained for another 24 hours in this manner, after which toxicity analyses were performed.

Cytotoxicity analysis

The potential cytotoxic effects of BGC extracts on cells were assessed using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and neutral red uptake (NRU) assays. Cells treated with BGC for 24 hours in 96-well plates were exposed to MTT solution (5 mg/mL) and incubated for 4 hours. Subsequently, the cells were washed with PBS, and the formazan crystals were dissolved in DMSO. The absorbance at 570 nm was measured for evaluation.

For the NRU assay, after the medium was discarded and the cells were washed with PBS, prewarmed NR solution (50 µg/mL NR in medium) was added to the wells. The mixture was then incubated for 3 hours at 37°C. The cells were washed with PBS three times. After removing the excess dye, a fixation solution (50% methanol, 1% acetic acid, and 49% dH_2O) was added. The plates were shaken for 20 minutes, and measurements in each well were conducted at a wavelength of 540 nm.

Genotoxicity and Antigenotoxicity Analyses

The alkali comet assay method was employed for the evaluation of genotoxic and antigenotoxic effects. Cells treated with varying concentrations (50, 100, 250, 500 and 1000 µg/mL) of BGC for 24 hours were collected by trypsinization and suspended in PBS. In the antigenotoxicity groups, the cells were treated with 50 µM/mL H_2O_2 on ice for 5 minutes. After the treatment period, the cells were centrifuged and resuspended in PBS. A negative control sample (DPBS) and a positive control (50 µM/mL H_2O_2 alone) were also included in the experiments. The cell suspensions were mixed with 0.65% LMA (low melting point agarose) and embedded in slides precoated with 1% NMA (normal melting point agarose). The slides were solid-

ified on ice, after which the coverslips were removed. Subsequently, the slides were incubated in cold fresh lysis solution (2.5 M NaCl, 100 mM EDTA, 100 mM Tris-base, 1% sodium sarcosinate, pH 10.0) supplemented with 1% Triton X-100 and 10% DMSO for 2 hours at 4°C. After incubation, the slides were transferred to cold electrophoresis buffer (1 mM sodium EDTA and 300 mM NaOH, pH 13.0) to enable DNA unwinding, after which the samples were kept at 4°C for 20 minutes. Electrophoresis was performed for 20 minutes at 21 V and 620 mA. Neutralization was achieved by washing three times in 0.4 M Tris–HCl (pH 7.5) for 5 minutes at room temperature. Next, the slides were incubated in an alcohol series (50%, 75%, and 98%) for 5 minutes each.

The dried agarose gel blocks were stained with ethidium bromide (20 µg/mL in dH2O) and covered with a coverslip. The migration of DNA was quantified using Comet Assay IV analysis software (Perceptive Instruments Ltd.), and the results are reported in terms of DNA tail length, DNA tail intensity (% tail DNA), and DNA tail moment. At least 200 cells from two replicate slides were scored at 20X for each experiment.

Statistical analysis

The statistical analysis was conducted using IBM SPSS 22 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). The study results are expressed as the mean ± standard error. Differences between the means of data were compared by the one way variance analysis (ANOVA) test and post hoc analysis of group differences by least significant difference (LSD) test. p Values of less than 0.05 were considered as statistically significant.

RESULTS

Cytotoxicity analysis

MTT and NRU assays were used to investigate the cytotoxic effects of BGC on A549 cells. MTT assay revealed that the cell viability decreased to less than 90% at concentrations higher than 125 µg/mL, although viability did not decrease to less than 70% within the applied concentration range (Figure 1).

The NRU assay results showed higher alterations in cell viability. Although cell viability did not decrease below 50% at any concentration, viability decreased to less than 70% at concentrations ranging from 50

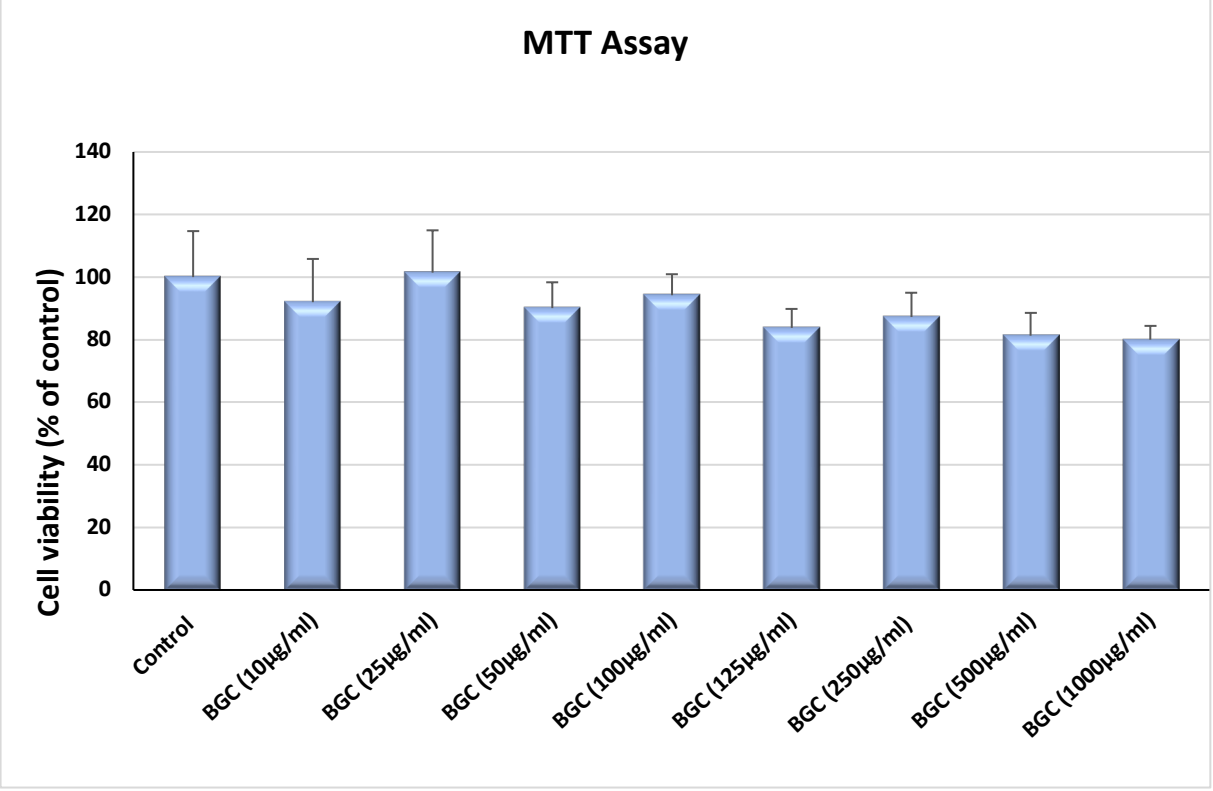

Figure 1. Cell viability rates compared to the control using the MTT assay following the treatment of A549 cells with different concentrations of BGC

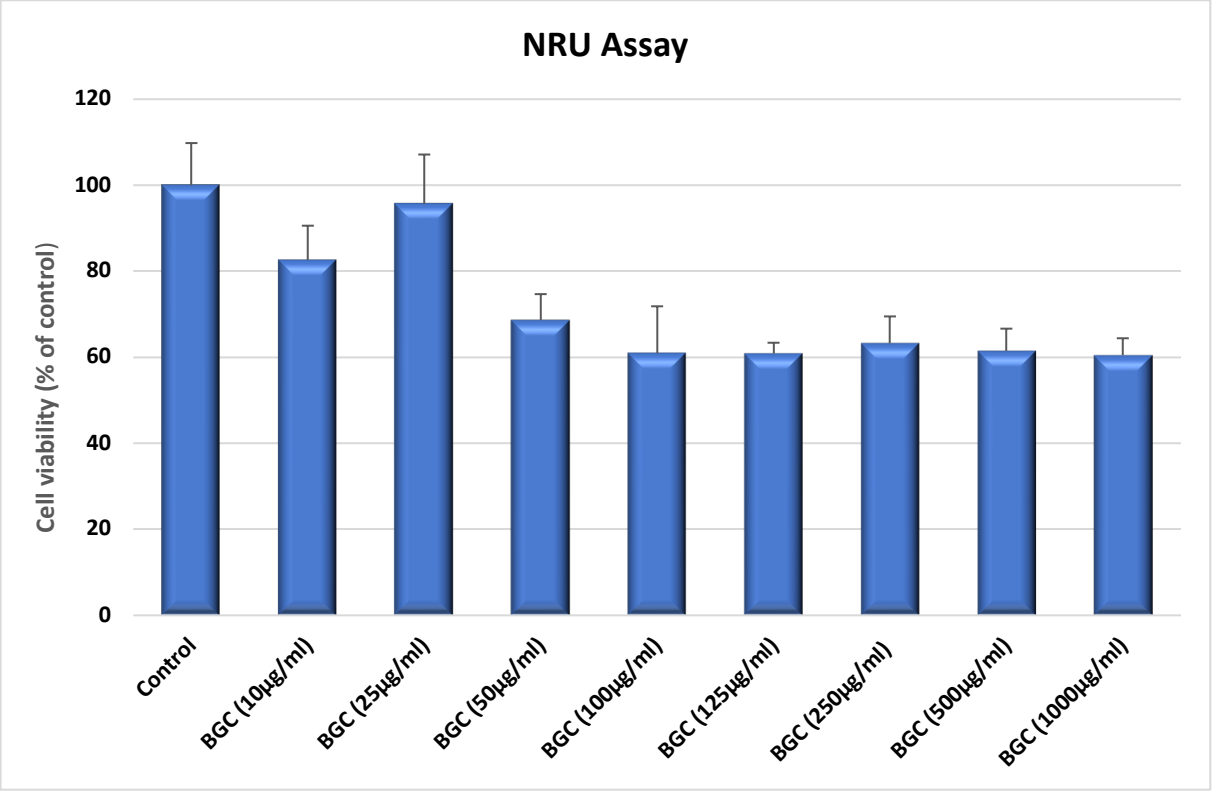

Figure 2. Cell viability rates compared to the control using the NRU assay following the treatment of A549 cells with different concentrations of BGC

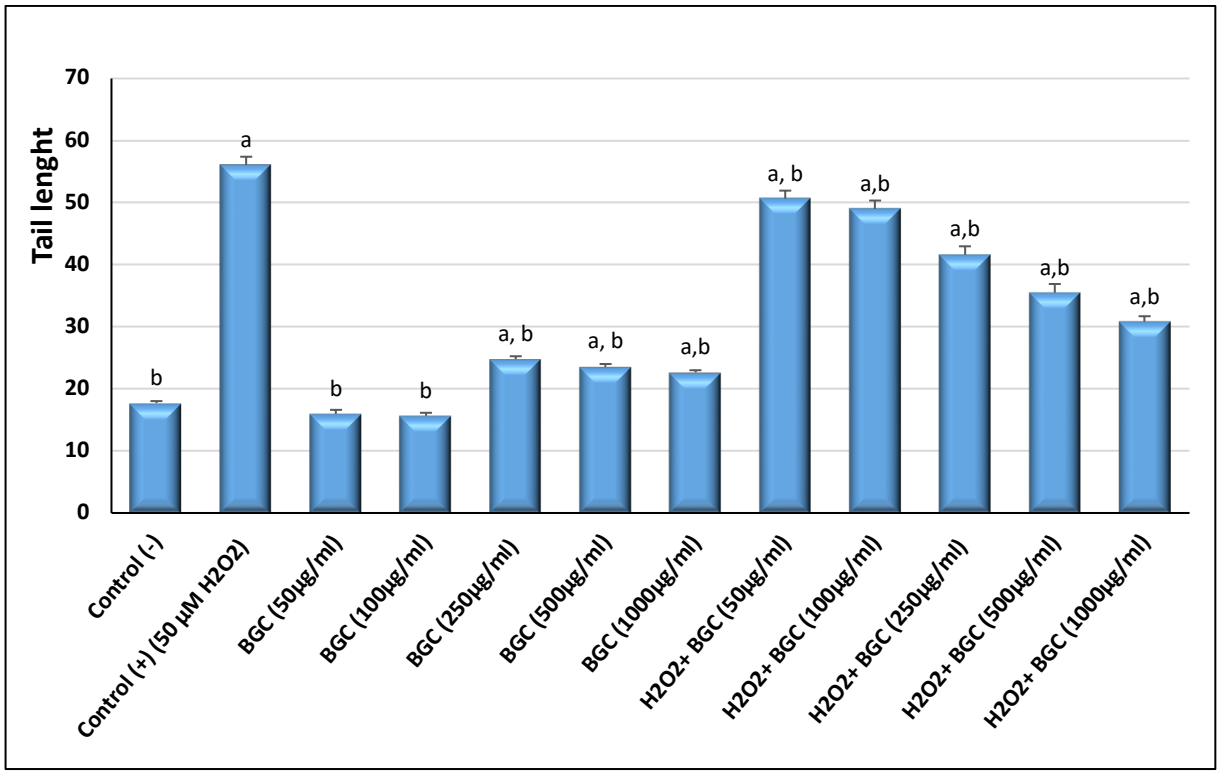

Figure 3. DNA damage, expressed as DNA tail length in the A549 cells treated with different concentrations of BGC and H_2O_2 (a: significant difference compared with Control (-), b: significant difference when compared with Control (+))

µg/mL (Figure 2).

Genotoxicity and Antigenotoxicity Analyses

The comet assay results for genotoxicity and antigenotoxicity analysis are presented in Figures 3-5, including tail length, tail intensity, and tail moment. Tail length increased significantly in the genotoxicity analysis groups when concentrations reached 250 µg/mL and above (Figure 3). Tail intensity measurements showed an increase at concentrations of 50 µg/mL and 100 µg/mL (Figure 4). When compared to the untreated control group, no differences were found in the tail moment values among the groups (Figure 5).

Pretreatment with BGC resulted in significant decreases in terms of tail length, tail intensity, and tail moment at all concentrations of H2O2-induced oxidative damage compared to the control (Figure 3-5). DNA damage was higher than in the untreated negative control but lower than in the control treated with H_2O_2.

DISCUSSION

Black garlic is a fermented type of garlic. The sensory characteristics of BG transform at the end of the aging process. The product is provided as an alternative for people who find fresh garlic to have a strong smell and irritating taste. Furthermore, after fermentation, there are significant changes in the bioactive properties of BG. There are numerous studies regarding its anti-inflammatory, antioxidant, anticancer, hepatoprotective, and hypolipidemic properties [1]. The ethanol extract of BG inhibited growth and induced apoptosis in HT29 colon cancer cells by inhibiting the PI3K/Akt pathway. Growth inhibition occurred both in a dose- and time-dependent manner. Incubation with 100 mg/mL BG at the highest dose tested inhibited 46.7±4%, 55.2±3%, and 63.9±5% of the cells at 24, 48, and 72 hours, respectively [23]. These studies suggest that black garlic may have potential health benefits beyond its mild taste and smell. Additionally, the fermentation process of black garlic enhances its nutritional profile by increasing the levels of certain beneficial compounds, such as S-allyl cysteine.

The properties of black garlic are influenced by many factors, such as fermentation conditions, processing technologies, and the quality of the raw garlic used. For instance, Bae et al. demonstrated that changing the fermentation temperature from 40 to 85°C resulted in a decrease in the amount of S-allyl cysteine

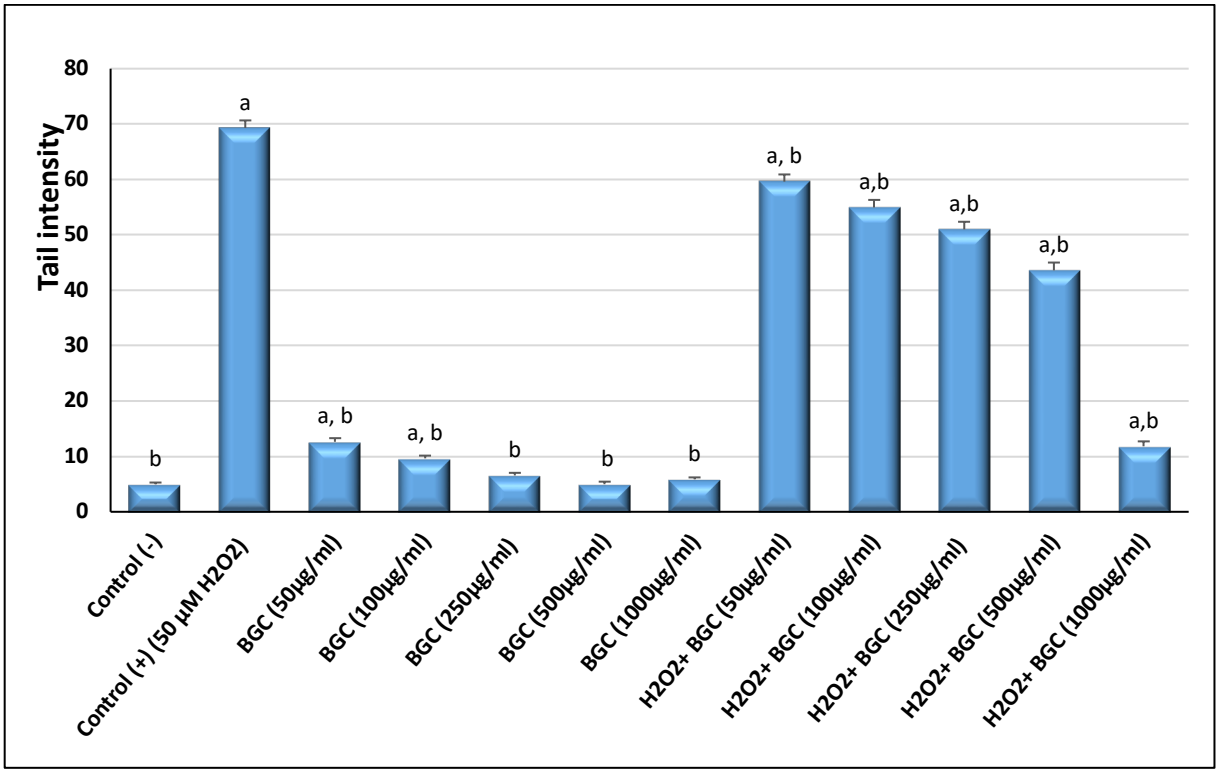

Figure 4. DNA damage, expressed as DNA tail intensity in the A549 cells treated with different concentrations of BGC and H2O2 (a: significant difference compared with Control (-), b: significant difference when compared with Control (+))

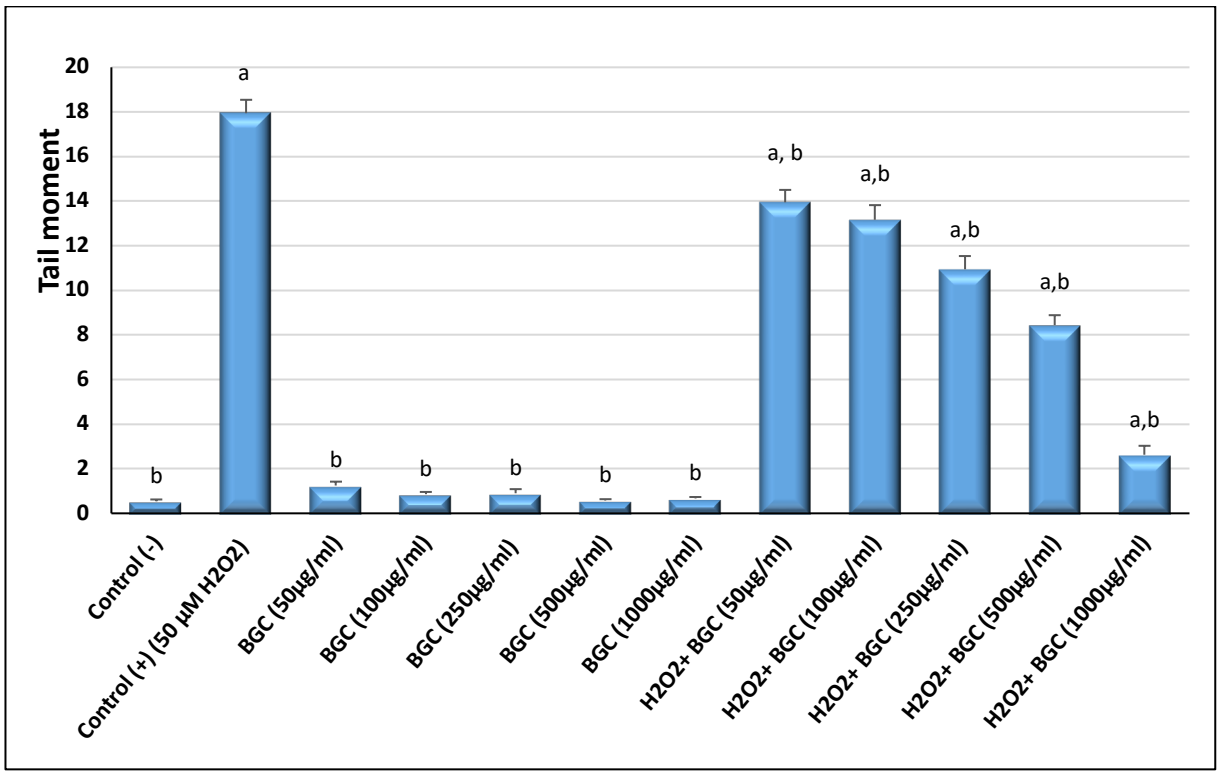

Figure 5. DNA damage, expressed as DNA tail moment in the A549 cells treated with different concentrations of BGC and H_2O_2 (a: significant difference compared with Control (-), b: significant difference when compared with Control (+))

(SAC), which is thought to be the most significant antioxidant of BG [24]. In a study investigating the 35-day aging process of black garlic, it was determined that the highest antioxidant activity was achieved on the 21st day. Along with their antioxidant activity, the quantities of polyphenols and flavonoids significantly increased on the 21st day [25]. In contrast, another study reveals that a 13-day fermentation time provides more biological activity than more prolonged fermentation periods [12].

The antiproliferative effect of BG may depend on the type of cell. The effects of the methanol extract of BG on different cancer cells (human hepatocellular carcinoma (HepG2) and human leukemia (U937)) were examined, revealing that BG dose-dependently inhibited cancer cells after 48 hours of incubation [26]. The IC50 values varied according to the cell type, with IC50 values of 0.8 mg/mL and 2.0 mg/mL observed for HepG2 and U937 cells, respectively [10]. Although the cytotoxic effect of BG on the A549 cell line has been previously investigated, this study focused on the cytotoxic, genotoxic, and antigenotoxic effects of the encapsulated form of BG on these cells.

Cytotoxic effects of BGC

The effects of BGC on cell viability were evaluated *in vitro* using the human lung cancer cell line A549. In the present study, 8 different concentrations ranging from 10 to 1000 µg/mL were used. After 24 hours of BGA treatment, the viability of the A549 cells was still greater than 80% at the concentrations used. Therefore, according to the MTT results, up to a concentration of 1000 µg/mL, BGA had no cytotoxic effect on A549 cells. These findings are consistent with those of a study conducted in 2023 involving various lung cancer cell lines, including A549 cells. Farhat et al. conducted a study comparing various forms of garlic, including BG, to assess their antioxidant and antiproliferative effects across different lung cancer cell lines. Their findings indicated that, in comparison to other forms, such as garlic powder, black garlic, water and alcohol extracts of fresh garlic, and commercial garlic supplements, BG demonstrated reduced antioxidant and antiproliferative efficacy. MTT analysis further revealed that BG had no impact on lung cancer cell lines (27). In another study investigating the anticancer effects of ethanol extracts of fresh garlic and BG on MCF-7, AGS, A549, and HepG2 cells, both extracts were found to be effective on all cell lines. However, the MCF-7 and HepG2 cells were more sensitive than the A549 and AGS cells were. The cells were treated with the extracts for 24, 48, or 72 hours,

and the highest inhibition was observed at 48 hours [10].

BG has been shown to inhibit various types of cancer cells. The cytotoxic effect of hexane extract of BG on U937 leukemia cells has been demonstrated through MTT analysis [28]. It has been determined that apoptosis is induced in cells through the caspase cascade. The hexane, chloroform, and ethyl acetate extracts of BG individually exert antiproliferative effects on breast cancer cells (T47D) [29]. The effects of BG on gastric cancer were examined, revealing that BG triggers apoptosis in SGC-7901 cells in a dose-dependent manner. Antitumor effects have also been observed in *in vivo* studies [30]. A study comparing the antioxidant properties of different garlic forms, including black garlic, reported that the greatest antiproliferative effect on ovarian cancer cell lines was achieved with fresh garlic extracts. There was no significant relationship between the potential to inhibit proliferation and the phenolic or flavonoid content, indicating that phenolics may not contribute significantly to the antiproliferative effects of garlic [31].

The results of the NRU assay conducted to investigate the effect of BGC on the proliferation of A549 cells differed from the MTT results. During the MTT assay, metabolically active cells reduce tetrazolium salts to form blue-colored formazan crystals. Neutral red is taken up by undamaged cells and shows lysosomal accumulation within the cell. Cytotoxicity analyses often yield contradictory results, as observed in this study. Putnam et al. compared four different toxicity assays (NRU, MTT, kenacid blue, and LDH) and found that during short-term incubations initiated from 3 hours onward, NRU provided more sensitive results than MTT. MTT analysis yielded results similar to those of other assays during 18- and 24-hour incubations [32]. In another study in which the viabilities of HTC (Human T-cell leukemia cell) and HepG2 cells were measured after incubation in cadmium chloride for different durations (3, 5, 8, and 24 h), the EC50 values at 24 hours were measured via NRU and MTT analyses as follows: for HTC cells, 20 µM ± 3.31 and 100 µM ± 14.47; for HepG2 cells, 8 µM ± 0.21 and 15 µM ± 5.03 (33). Furthermore, the EC50 values for HepG2 cells in the 3-, 5-, and 8-hour groups were assessed using both MTT and NRU, whereas the EC50 values for HTC cells in these groups were determined using only NRU. According to studies utilizing BG, Purev et al., 48 hours of incubation had the strongest inhibitory effect [10]. As a result, it is possible that the 24-hour incubation time we chose in our study was too short to demonstrate any effects in the MTT test. The NRU results revealed a decrease in cell viability, which was consistent with the findings of other publications suggesting that the test is more sensitive for shorter incubations.

Genotoxic and Antigenotoxic Effects of BGC

The genotoxic and antigenotoxic effects of BGC were examined using the comet assay. Single-cell gel electrophoresis is commonly used for the detection of single- and double-strand breaks in DNA. The applied alkaline comet analysis enables direct measurement of DNA damage at the cellular level. A549 cells were treated with different doses of BGC for 24 hours. Subsequently, the cells were harvested and divided into two groups. One group was subjected to comet analysis directly for genotoxicity evaluation, while the other group was treated with H_2O_2 before analysis for antigenotoxicity evaluation. According to the results, there was a significant increase in tail intensity (at 50 and 100 µg/mL) and tail length (at 250, 500, and 1000 µg/mL) in cells treated with BGC compared to those in the control group across different concentration applications. On the other hand, BGC did not significantly differ at any concentration when the tail moment was analyzed. These findings suggest that BGC may induce DNA damage in A549 cells, as evidenced by the increase in tail intensity and length. However, further investigations are needed to determine the exact mechanism of DNA damage caused by BGC and its potential implications for cellular function. The effects of black garlic extracts with different fermentation durations and fresh garlic on the genomic integrity of HL60 cells were investigated by DNA laddering, which revealed that only fresh garlic caused mild DNA fragmentation at low concentrations [12].

When examining the groups induced by oxidative damage, it was observed that H_2O_2 had genotoxic effects on the cells, while BGC had protective effects against this damage. With all administered concentrations of BGC, the outcome of oxidative damage was significantly reduced in terms of tail length, tail intensity, and tail moment. Our findings are consistent with our prior research, demonstrating that the BGC utilized in our investigation did not demonstrate any genotoxic effects on human lymphocytes at concentrations of 50, 100, or 200 µg/mL. Furthermore, BGC exhibited a protective antigenotoxic effect against the induction of micronuclei by Mitomycin C, a drug known for its ability to inhibit cell proliferation [22]. In a study conducted with the mouse macrophage line

RAW264.7, the effects of different forms of garlic on H2O2-induced oxidative damage were compared. It was determined that black garlic extract exhibited stronger antioxidant activity than fresh garlic. Upon investigating the content responsible for this effect, it was suggested that pyruvate, which is known to increase in quantity during the fermentation process, might be responsible [18]. Further analysis revealed that black garlic extract also had relatively high levels of other antioxidants, such as SAC, allicin, polyphenols and alkaloids which could contribute to its stronger antioxidant activity [3,6]. These findings suggest that the fermentation process involved in producing black garlic may enhance its antioxidant properties compared to those of fresh garlic. Studies have demonstrated the antioxidant activity of black garlic both *in vitro* and *in vivo*. Even at lower doses, black garlic has more antioxidant activity than fresh garlic [18]. The type of black garlic, processing method, and extraction method are important factors influencing its antioxidant capacity. Polyphenol content has been shown to increase with higher temperatures and decreasing humidity during the processing of black garlic. The optimal protocol for preserving antioxidant capacity involves 75°C and 85% relative humidity over a period of 8 days [34]. Water extracts of black garlic show more antioxidant activity than alcohol extracts when extraction techniques are compared [27].

CONCLUSION

In this study, we evaluated the encapsulated black garlic which we found in our previous studies that it has much higher antioxidant capacity and biological activities compared to fresh garlic and is also safe to consume due to its anti-genotoxic effect on human lymphocytes (22), for its in vitro cytotoxic, genotoxic and antigenotoxic properties on A549 cell line. According to our results, it was determined that BGC had cytotoxic and genotoxic effects depending on its concentration, on the other hand, it had protective effects against DNA damage caused by hydrogen peroxide. Considering that powerful antioxidants and antigenotoxic agents can also be anticarcinogenic, it should not be ignored that these agents can be of great benefit in cancer prevention. However, this in vitro study was planned for only a single cancer cell type. Further studies such as in different cancer cells and in comparison, with healthy cells and also in vivo studies are needed to clearly determine the potential of BGC for its use against cancer.

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Conflict of Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' Contribution

Study Conception: ÇIE, HET, GT; Study Design: ÇIE, HET, GT; Literature Review: ÇIE, GT; Critical Review: ÇIE, GT; Data Collection and/or Processing: ÇIE, HET, GT,; Analysis and/or Data Interpretation: ÇIE, GT; Manuscript preparing: ÇIE, GT.

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Evaluation of the relationship between patient blood management and anesthesia method in cesarean section: A single-center study

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ABSTRACT

Objectives: Patient blood management (PBM) aims to improve patient outcome and safety by reducing the quantity of unnecessary blood transfusions and vitalizing patient-specific anemia reserves. We aimed to determine the efficiency and practically of patient blood management in the cesarean section operating room in our hospital and the effects of the anesthesia method in cesarean section .

Methods: Between 2019 and 2021 , 6011 patients who underwent cesarean section were reviewed at Bursa City Hospital. Patients who received perioperative or postoperative blood product transfusion were divided into two groups as Group I (n=614) and patients who were not transfused into Group II (n=5397). Demographic data of the patients, preoperative hemogram laboratory values, performed anesthesia methods, transfusion rate were recorded from the database.

Results: There was no statistically significant difference between the two groups in terms of demographic data (p>0.05). The preoperative anemia rate was 35.91% (n=2159) and in these patients the blood transfusion rate was 21.86% (n=472) .Total transfusion rate was reported as 10.21% (n=614). Preoperative hemoglobin levels were statistically lower in Group I . Spinal anesthesia method was found to be statistically higher in both groups .

Conclusion: Patient blood management is very important. In line with the guidelines on this subject, it will reduce unnecessary transfusions and therefore the risks of transfusion complications. In addition, considering the difficulty of supplying blood products, blood transfusion should not be considered primarily as a treatment. Anemia treatment should be planned before surgery and hemoglobin levels should be optimized. It is recommended to prefer regional anesthesia for PBM at cesarean section. In this way, we think that we will both increase the efficiency of patient blood management and reduce the cost and complications of blood transfusion.

Keywords: anemia, anesthesia,blood transfusion, patient blood management

In obstetrics, patient blood management PBM) aims to improve clinical outcomes by avoiding unnecessary exposure to blood products. It includes three steps of optimisation of blood volume and red cell mass, minimisation of blood loss and optimisa-

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tion of the patient's tolerance of anemia [1]. Guidelines have been created to ensure the feasibility of this approach. Accompanied by guidelines, personel involved in the blood transfusion chain, especially clinicians who use blood products the most, it is aimed to help in making transfusion decisions in the light of current and evidence based information on the PBM [1, 2]. There are some publications for cases with a high risk of bleeding, such as placenta previa [3]. However, there are very few studies in the literature on the unnecessary use of blood products during cesarean section and there is a need to make recommendations for strategies to reduce the use of blood products in obstetric practices [4, 5]. The method of anesthesia is based on optimizing blood loss at PBM. Regional anesthesia is recommended in obstetric anesthesia, but general anesthesia is generally preferred in surgeries with the possibility of major bleeding such as placenta previa. Recent opinions are that regional anesthesia is preferred as much as possible because it causes hypotension [6, 7].

In our study, we aimed to examine the effectiveness and applicability of PBM and determine the anesthesia method preference and its place in the PBM approach in caesarean section at Bursa City Hospital.

METHODS

Approval was received from the Bursa City Hospital Ethics Committee (2021-18/8). Between 2019-2021, the files of 6011 adult patients aged 18 and over who underwent cesarean section at Bursa City Hospital were examined. Missing data and/or patients under 18 years of age were excluded from the study. In this retrospective study, demographic data of the patients , preoperative hemoglobin (Hb) level (gr/dL), anesthesia method performed, blood products were transfused or not, and the transfusion period data were recorded by scanning the hospital information data system. The relationship between transfusion and anemia with anesthesia management were examined.

Statistical analysis was performed with SPSS 26.0 (SPSS, Chicago, IL) program. Frequency and descriptive statistics were calculated. In descriptive statistics, continuous variables were presented as mean±SD and categorical variables were presented as percentages. Normal distribution of the data was investigated using the Kolmogorov-Smirnov test. Chi-Square test was used to compare categorical variables between groups. P value <0.05 was considered statistically significant.

RESULTS

The files of 6011 patients included in the study were examined. Patients who received blood products transfusion were divided into two groups as Group I and patients who were not transfused into Group II .While Grup I (n=614) ,10.2% received blood products transfusion, Grup II (n= 5397) , 89.8% did not receive transfusion. The transfusion rate in these patients was found to be 10.21%. There was no statistically significant difference between the two groups in terms of demographic data (p>0.05). In pregnant women, Hb level below 10.5 g/dL was considered anemia. The preoperative anemia rate was 35.91% (n=2159) and in these patients the blood transfusion rate was 21.86% (n=472) (Table 2). While Group I preoperative Hb mean values were determined as 9.64 ± 1.58 g/dL, Group II Hb mean values were determined as 11.26 ±1.38 gr/dL (Table 1).

When the period of administration of blood and blood products is examined, preoperatively 247

Table 1. Demographic data and preoperative Hemoglobin values (gr/dL)

	Group I (n=614)	*Group II (n=5397)*	*p*
Age,(mean±SD)	28,3±4,34	28,4±3,86	>0,05
Hb,gr/dL (mean±SD)	9,64±1,58	11,26±1,38	<0,001
Hb, (%,n)			
<7 gr/dL	3,2 (20)	0 (0)	<0,001
7-8 gr/dL	14,1 (87)	0,8 (46)	<0,001
8-9 gr/dL	29,3 (180)	4,5 (243)	<0,001
9-10,5 gr/dL	30,1 (185)	25,9 (1398)	<0,001
>10,5 gr/dL	23,1 (142)	68,7 (3710)	<0,001

Group I: Group blood transfusion Group II: Group not blood transfusion
Hb: Hemoglobin

Table 2. Transfusion status according to anemia status

	Group I	*Group II*
Hb< 10,5 gr/dL (n)	472 (21,9 %)	1687 (78,1 %)
Hb>10,5 gr/dL (n)	142 (3,7 %)	3710 (96,3 %)

Group I: Group blood transfusion Group II: Group not blood transfusion
Hb: Hemoglobin

(40.3%), peroperatively 53 (8.6%) and postoperatively 314 (51.1%) patients received transfusion were observed. Spinal anesthesia method was found to be statistically higher in both groups (p<0.001). The rate of spinal anesthesia was higher in Group II than in Group I (Table 3).

DISCUSSION

The patient blood management approach was first brought to the agenda in 2007 [8]. Since then, it has been aimed to minimize the problem effectively at the appropriate time, optimizing the application of the appropriate blood product to the appropriate patient with the appropriate indication [9]. Australia demonstrates very successful examples of the PBM approach. It was stated that they should make their own protocols for every hospital has blood transfusion [1, 10].

Determination and correction of anemia are included in the protocols by optimizing the erythrocyte volume. Pregnant women are one of the groups where anemia is frequently seen. For cesarean section, it is undesirable for patients to be anemic due to the risk of bleeding. Organizing anemia treatment primarily under elective conditions and providing optimal preoperative Hb values are aimed. The World Health Organization (WHO) defines anemia during pregnancy as Hb level of less than 11 g/dL, while the Centers for Disease Control and Preventions (CDC) and American College of Obstetricians and Gynecologists (ACOG) consider Hb level of less than 10.5 g/dL during the second trimester as the criterion for diagnosing anemia [11-13]. A more specific Hb range (other than statistically determined anemia) associated with the optimal gestational Hb range and perinatal outcomes has not been determined yet. In our study, we determined preoperative Hb value to be <10.5 g/dl as anemia. The rate of anemia was 35.91% detected in our study. Stevens et al while the prevalence of anemia in pregnant women worldwide was 38% in their studies, the prevalence of anemia in pregnant women in European people was reported that it was 25.8% [14]. In our study, it was determined that 472 out of 2159 patients (21.9%) in the anemic group, which had a high rate, received blood transfusion. The fact that 40.3% of blood transfusions are in the preoperative period is also a very high rate. Patterson et al. reported in their study that unnecessary blood transfusion was performed in cesarean sections and pregnant women [15]. In our study, we are thinking that unnecessary blood transfusion was performed and it can be optimized with oral and/or intravenous iron preparations before anemia treatment [16].

Optimization of total erythrocyte volume, blood loss and physiological reserve of the patient are steps of PBM [17]. The method of anesthesia is based on optimizing blood loss at PBM. It is thought that spinal anesthesia may cause less blood loss than general anesthesia due to sympathetic blockade and hypotension. General anesthesia is more preferred in patients with the risk of hemodynamic instability due to major hemorrhage such as placenta previa [18]. Additionally, since coagulation disorders caused by massive

Table 3. Transfusion status according to anesthesia method and anemia

		Group I	*Group II*	*P*
Spinal Anestesia (n,%)	Hb< 10,5 gr/dL	349 (36,8)	1406 (26,0)	<0,001
	Hb>10,5 gr/dL	82 (13,3)	2995 (55,4)	<0,001
General Anestesia (n,%)	Hb< 10,5 gr/dL	123 (20,1)	281 (5,2)	<0,001
	Hb>10,5 gr/dL	60 (9,7)	715 (13,2)	<0,001
Total		614	5397	

Group I: Group blood transfusion Group II: Group not blood transfusion
Hb: Hemoglobin

hemorrhage and transfusion may increase the risk of epidural or spinal hematoma, general anesthesia may be preferred [19]. In our study, it was determined that spinal anesthesia was performed at a very high rate in cesarean sections. As a limitation, we could not obtain sufficient data in the records regarding placental anomalies. Recently, in operations with a high risk of bleeding, such as placenta previa, it is recommended to start with regional anesthesia and return to general anesthesia when necessary [6]. We think that this result is due to the recommendation of regional anesthesia method in cesarean section and the opinion that it can reduce blood loss [7, 20].

Unnecessary blood transfusion occurs due to individual opinions and traditional approaches.

There are many minor and major complications like as allergic reactions, TRALI etc. of unnecessary blood transfusion. There have been many publications about the effects of transfusion on mortality, morbidity and the differences between clinicians' practices in recent years [21-23]. The limitation of our study is uninvestigating early and late complications of blood transfusion and anemia.

CONCLUSION

Unnecessary blood transfusion and complications can be prevented by providing early diagnosis and treatment of antenatal anemia. Correction of patients' anemia is preferred for regional anesthesia in caesarean section. To ensure that, preoperative blood transfusion is observed to be quite high. In order for PBM to be more effective, we must make our in-hospital protocols accompanied by guidelines and make them applicable. Blood products are costly and difficult to obtain. By using protocols, appropriate time, appropriate patient and appropriate product application can be achieved. In cesarean section , instead of blood transfusion to increase the preoperative Hb level, we should ensure that it is optimized with oral or intravenous iron preparations beforehand.

Conflict of Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical Approval

The protocol of the study was approved by the Medical Ethics Committee of Bursa City Hospital, Bursa, Türkiye. (Decision number: 2021-18/8, date: 06.10.2021).

Authors' Contribution

Study Conception: ST; Study Design: ST; Literature Review: ST; Critical Review: ST; Data Collection and/or Processing: ST; Analysis and/or Data Interpretation: ST; Manuscript preparing: ST.

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A rare complication of high flow nasal cannula therapy in COVID-19: Caudal septal cartilage necrosis

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ABSTRACT

A high-flow nasal cannula (HFNC) therapy has been frequently performed in adult patients with acute respiratory failure during the COVID-19 pandemic. With the increasing long-term use of HFNC, different complications have been observed. We aimed to share the nasal necrosis complication that occurred in our patient who had used HFNC for a long time.

A 65-year-old male patient with COVID PCR (+) was admitted to hospital with fever symptoms after 5 days of favipravir treatment at home and had no comorbidities. He was hospitalized due to the development of COVID pneumonia and fever symptoms. During the hospitalization, the favipravir treatment was planned to be completed to 10 days with 250 mg methylprednisolone for 3 days and 40 mg maintenance was continued. Favipravir was discontinued on the nineth day due to increased liver function tests. On the 7th day of hospitalization, he was admitted to intensive care unit with clinical deterioration such as sudden decrease in oxygen saturation, respiratory distress, and fever symptoms.

The patient's Glasgow Coma Score (GCS) was 15 at intensive care unit (ICU). HFNC therapy was started while the oxgen saturation was 72 with a reservoir oxygen mask. HFNC therapy was performed by High-flow nasal cannula. The flow was started at 80L / minute and the fraction of inspired oxygen (FiO2) was set at 50% to maintain peripheral oxygen saturation above 92%. The temperature was set between 35°C and 37°C. The patient's hemodynamics were stable without inotropic drugs and sedation was not administered. Oral feeding was continued. Diuresis, liver function tests and other laboratory parameters were monitored regularly and medical treatments were adjusted. The daily HFNC therapy settings during the follow-up of the patient are summerized in Table 1. On the 33rd day of the patient's stay at ICU, HFNC therapy was terminated and reservoir oxygen mask therapy was planned to be started. However, on the last day of HFNC therapy, epistaxis was developed. Necrosis and ulceration were noticed in our patient's nasal columella skin and upper lip filter skin due to compression. It was observed that the caudal end of the nasal septal cartilage and the medial krus of the alar cartilage were visible (Figure 1). The patient was evaluated by Otorhinolaryngology clinic. These

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Table 1.Setting parameters of High-flow nasal cannula (HFNC) and arterial blood gas parameters

Time (days)	HFNC flow (%)	HFNC Fi02 (%)	pH	Pa02 (mmHg)	PaCO2 (mmHg)	Saturation (%)
1.	50	80	7,49	78,4	32,8	96,5
2.	60	90	7,48	81,2	38,1	96,2
3.	60	90	7,52	81,4	35,7	97,3
5.	60	100	7,49	49,0	37,2	87
7.	60	90	7,49	61,8	37,0	92,9
10.	60	100	7,49	55,5	37,0	90,3
15	60	90	7,48	94,7	37,7	98,3
18.	60	80	7,47	64	42,9	93,5
21.	50	80	7,54	60,9	34,8	93,7
24.	50	65	7,61	56,2	50,9	96,7
27.	60	60	7,49	140	45,1	99,5
30.	40	50	7,54	151,2	39,6	99,8
32.	30	45	7,52	94,2	41,7	98,5

tissues of the patient were debrided, nasal columella skin and upper lip filter skin were sutured in vertical axis with primary 2/0 silk by Otorhinolaryngologist (Figure 2). Since systemic broad-spectrum antibiotic therapy was administered, only Furacin (nitrofurazone) was recommended daily. After this intervention, the patient was followed up with oxygen mask with reservoir for one week and then, when respiratory distress increases, he was entübated in clinicand mechanical ventilation was administered. But the patient died on the tenth day of intubation at ICU.

DISCUSSION

High-flow nasal cannula (HFNC) oxygen therapy, generally has been performed for acute respiratory failure in COVID-19 pandemic [1]. We used HFNC therapy in our patient who developed acute respiratory failure with COVID-19. Currently, recommendations are for HFNC usege in patients with hypoxemic respiratory failure, following extubation, in the peri-intubation period, and postoperatively for bedside clinicians [2]. Most patients tolerate oxygen therapy with HFNC. Although frequently used, complications are rare in adults. Abdominal distansion, aspiration and rarely barotrauma (eg, pneumothorax) are complications of HFNC [3-4]. In adults, as a complication of high-flow nasal cannula therapy, epistaxis has observed only in a small study sample [5].We also observed nasal necrosis complication in our patient by using HFNC for a long time in COVID-19.

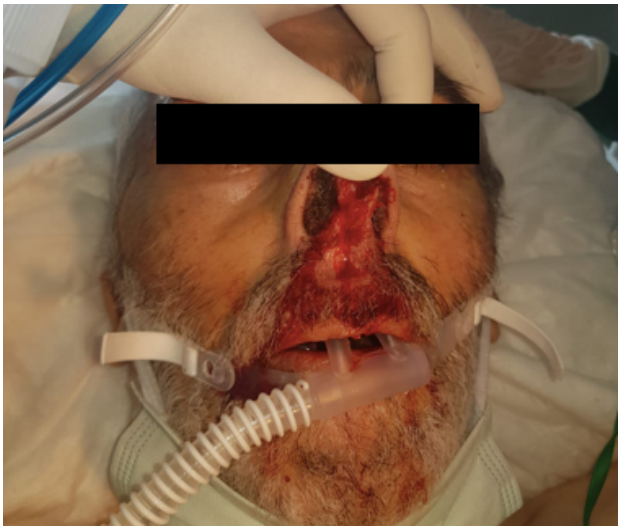

Figure 1. Caudal septal cartilage necrosis

Figure 2. Sutured nasal columella skin and upper lip filter skin

The mechanism has been developed that, reliably deliver heated and humidified oxygen at high flows via nasal cannula. High-flow nasal cannula can be administered for prolonged periods (eg, days) and patients can be switched to conventional low-flow nasal cannulae once the flow rate reaches ≤20 L/minute and FiO2 ≤50 percent. Velasco et al. attributed epistaxis to the following causes the maximum flow rate in the study by Velga et al. was 40L/min, while the high flow rate in use was 65L/min and did not differ between patients with or without epistaxis [5-6].We also used HFNC in our patient for 32 days. During this time, other invasive or non-invasive methods were not used. Since hypoxemia was very resistant to oxygen therapy, we could not reduce the flows for a long time. We used the high flow rate between 50-60L /minute for a month.

The tempature of HFNC was between 34-37°C. Warming inspired oxygen and heating it to core temperature is more effective at high flow rates (typically >40 L/minute) than low flow rates. Increased humidification results in increased water content in mucous, which can facilitate secretion removal and may also decrease the work of breathing and avoid airway desiccation and epithelial injury [7]. Mauri et al. reported that, patient comfort as significantly higher during steps at the lower temperature 31 °C in comparison to 37 °C, with the HFNC set at both 30 and 60 l/min [8].

Although proper using, epistaxis was observed in our patient on the 32th day. Necrosis and ulceration occurred in our patient's nasal columella skin and upper lip filter skin due to compression. We thought that adverse event was caused by prolonged cannula compression. Small, soft and flexible nasal prongs are recommended for patient comfort. In infants, snug fitting nasal prong systems may cause trauma to the septum as well as other mucosal complications [9]. Recommendations for children are for the cannula to occupy approximately 50 percent of the internal diameter of the nares to permit some leak and prevent excessive airway pressure [10]. Although there are recommendations about the size and diameter of the nasal cannula in adults, unfortunately there are no relevant studies. Actually ,there was only one type of nasal cannula for adults in our hospital. They were suitable for adult patients but not small enough. Temperature and humidification were in accordance with recommended standards in HFCN setting for our patient [11].

CONCLUSION

As a result, the indications for the use of HFNC in the adult patient are increasing day by day. Therefore, HFNC therapy complications are observing more common in adults. We suggest that using the lowest effective flow rates and appropriately selecting the nasal cannula size to allow adequate leakage may be protective and prevent complications. Although as a rare complication, we suggest that, the caudal septal cartilage necrosis, should be keep in mind.

Conflict of Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' Contribution

Study Conception: ST, AÇ; Study Design: ST, GÇ; Literature Review: AÇ, GT; Critical Review: ST, GÇ; Data Collection and/or Processing: ST, AÇ; Analysis and/or Data Interpretation: GÇ, GT; Manuscript preparing: ST, GÇ.

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