Migration Letters

Volume: 21, No: 4, pp. 1250-1263

ISSN: 1741-8984 (Print) ISSN: 1741-8992 (Online)
www.migrationletters.com

Astaxanthin Prevents a Decrease of Hemopoietic Activity in Head and Neck Cancer Patients Receiving Cisplatin Chemotherapy (Randomized Controlled Trial)

Yusuf Aminullah[1], Hertanto Wahyu Subagio[2], Damai Santosa[3], Zulfikar Naftali[4]

Abstract

Decreased hemopoietic activity due to reactive oxygen species (ROS) in patients with head and neck cancer (HNC) can cause myelosuppression due to the side effects of cisplatin chemotherapy. External antioxidants, including astaxanthin, are needed to neutralize and fight ROS, preventing a decrease in hemopoietic activity. This study aims to prove and analyze the antioxidant effect of astaxanthin in preventing a decrease in hemopoietic activity through increasing levels of superoxide dismutase (SOD) and decreasing levels of malondialdehyde (MDA) in head and neck cancer patients due to cisplatin. The study design was an experimental randomized controlled trial pre-post test design with 42 research subjects randomly divided into two groups. The treatment group was given astaxanthin 2x4 mg, and the control group was given a vitamin C 1x500 mg and vitamin E 1x250 IU. Data analysis was performed by the Descriptive, Levene, Shapiro-Wilks, Wilcoxon, and Mann-Whitney tests. The results of this study stated, 42 study subjects met the inclusion criteria, most aged 41-50 years were 16 people (38.1%), male and female 2:1, Nasopharyngeal cancer 22 people (52.4%), stage IV 32 people (76.2%), Eastern Cooperative Oncological Group (ECOG) I 31 people (73.8%) and Normal Body Mass Index (BMI) 30 people (71.4%). There were significant differences in hemoglobin levels (p=0.012), erythrocyte counts (p=0.004), leukocyte counts (p=0.009), and MDA levels (p=0.014), while thrombocyte counts (p=0.178) and SOD levels (p=0.489)) was not significantly different. Supplementation of astaxanthin 2x4 mg for three weeks in head and neck cancer patients can prevent a decrease in hemopoietic activity (hemoglobin levels, erythrocytes, and leukocytes counts) by reducing malondialdehyde (MDA) levels in head and neck cancer patients receiving cisplatin.

Keywords: *astaxanthin, hemopoietic activity, head and neck cancer, cisplatin, ROS.*

INTRODUCTION

Head and neck cancer (HNC) is a variety of malignant tumors originating from the upper aerodigestive tract, including the oral cavity, nasopharynx, oropharynx, hypopharynx, and larynx, paranasal sinuses, and salivary glands.1-3

[1] Department of Doctor of Medicine and Health Science Study Program, Faculty of Medicine, Diponegoro University, Semarang, Indonesia/Kariadi Hospital Semarang, Indonesia
[2] Department of Science of Nutrition, Faculty of Medicine, Diponegoro University, Semarang, Indonesia/Kariadi Hospital Semarang, Indonesia
[3] Department of Internal Medicine, Faculty of Medicine, Diponegoro University, Semarang, Kariadi Hospital Semarang, Indonesia
[4] Departemen of Ear, Nose, Throat-Head and Neck Surgery, Faculty of Medicine, Diponegoro University, Semarang, Indonesia/Kariadi Hospital Semarang, Indonesia

HNC rank in the top six at about 4% of all cancers worldwide. According to the World Health Organization (WHO) in 2002, there were 600,000 HNC cases, with 300,000 deaths each year worldwide. HNC incidence in the United States is about 3-5% of all cancers, often occurring in men over 50 years old.4-6 According to the Indonesian Cancer Registration Agency, HNC is ranked fourth out of the top ten cancers in men and women and second out of the top ten cancers in men. During March-April 2015, the incidence of HNC at Kariadi hospital Semarang was 36 cases, and the most diagnosis was nasopharyngeal cancer, followed by sinonasal and laryngeal cancer.7,8

Squamous cell carcinoma types account for 95% of all HNC, in addition to lymphoma, sarcoma, adenocarcinoma, basal cell carcinoma, and melanoma. HNC management includes surgery, radiation, chemotherapy and combinations, depending on the type, stage of cancer and histopathology.9

Cisplatin activation is systemic and non-selective, so it has side effects. Not only cancer cells that experience apoptosis but healthy cells throughout the body are also affected due to the formation of free radical compounds. This compound is toxic in the body when the amount is excessive, so it can damage normal cells of the body, including bone marrow cells which results in a decrease in hemopoietic activity.10-12

Lee et al. examined cisplatin chemotherapy's effect on bone marrow in 274 gynecological cancer survivors and 503 breast cancer survivors. The decline in the hemopoietic system begins to occur at the beginning of the chemotherapy series and tends to increase at the end of the chemotherapy series, which is 28.8% of patients. 13 Research by Baron et al., shows a decrease in hemoglobin, leukocyte, and platelet values due to the formation of free radicals.14 A decrease in hemopoietic activity will have an impact on reducing the general state of the patient and the ability to phagocytose cancer cells, thereby worsening the prognosis. In addition, chemotherapy will be delayed so that cancer therapy cannot be carried out effectively and will extend the length of stay (LOS) of HNC patients.15,16

Based on this, it is necessary to give antioxidants from the outside in fighting free radicals to prevent a decrease in hemopoietic activity so that the quality of life of HNC patients improves and chemotherapy becomes more effective, improving the prognosis.

Antioxidants from the outside are needed because natural antioxidants in the

body cannot fight free radicals from the outside to prevent a decrease in hemopoietic activity from damage to body tissues due to free radicals caused by cisplatin chemotherapy. The way antioxidants work from the outside is to stabilize free radicals, complement the lack of electrons owned by free radicals, and inhibit the occurrence of chain reactions due to the formation of free radicals that can cause oxidative stress. Some commonly used antioxidants include green tea, vitamin C, vitamin E, lycopene, lutein, selenium, astaxanthin, and others.17-20

Astaxanthin, as an antioxidant of the alpha carotenoid group, can stop the chain reaction of free radicals and inhibit the destruction of normal cells. When given to HNC patients who get cisplatin chemotherapy, astaxanthin will bind to free radicals O2 (singlet oxygen), increase the activity of antioxidant enzymes, namely SOD (Superoxide dismutase), Catalase and Glutathione peroxidase, suppress and block lipid peroxidation through the final product, namely MDA (Malondialdehyde) as well as inhibiting and stopping the chain of free radicals that affect the bone marrow so that the side effects of decreased hemopoietic activity can be prevented.21-23

The activity and working power of astaxanthin are more potent than other antioxidants such as beta carotene, lutein, lycopene, and vitamin E because it has a carotenoid structure that is rich in electrons, not pro-oxidant, resistant to autoxidation, and has better strength in neutralizing singlet oxygen, digesting free radicals, providing protection against lipid peroxidation and oxidation damage and has minimal side effects.21-23

Research by Xue et al. (2017) proves that astaxanthin as an antioxidant has been tested in vitro. Astaxanthin can be a potential therapy to protect hemopoietic activity from bone marrow damage due to radiation in rats. 24 In this study, astaxanthin was implemented as an antioxidant in humans (HNC patients) who received cisplatin chemotherapy to prevent a decrease in hemopoietic activity.

Based on the ability and advantages of antioxidant astaxanthin in inhibiting normal cell death by neutralizing and fighting free radicals and there have been no studies that assess the influence of astaxanthin in preventing a decrease in hemopoietic activity through an increase in SOD levels and a decrease in MDA levels in HNC patients who receive cisplatin chemotherapy, a study was conducted to answer this problem.

The study aimed to prove that astaxanthin prevents decreased hemopoietic activity through increased SOD levels and decreased MDA levels in HNC patients due to cisplatin chemotherapy.

RESEARCH METHODS

Design, Time, and Place

The research design is an Experimental Randomized Controlled Clinical Trial Pre and Post Test Design carried out in the Kasuari Installation Room of Kariadi hospital Semarang from March 2022 to March 2023.

Research Subject

HNC patients undergoing cisplatin chemotherapy were taken by simple random sampling (using a random number table) and described any steps to conceal the sequence until interventions were assigned. Researcher determined the random allocation sequence, enrolled participants and assigned participants to interventions. The study was conducted in a double-blinded manner in which participants, cares providers and those assessing outcomes was blinded after assignment to interventions.

Inclusion criteria : HNC patients with cisplatin chemotherapy, stage II-IV, age >11-<80 years, ECOG I-III, and willing to follow the research stages by signing informed consent. Patients who received radiotherapy, blood transfusions, consumed other antioxidants, and the presence of gastric, liver, and kidney disorders and hematological malignancies were not included in the study. Patients are excluded from the study if the general condition worsens, drug allergies are found, do not comply with the research rules, and do not want to continue the study. The trial ended or was stopped if the patient's general condition worsened and endangered the patient's life.

The minimal sample size is calculated using based on preliminary research with the formula for calculating the estimated sample size of 2 independent groups, the error rate (α) = 5% power test = 90%. 25 The total required research subjects are 42 people. The research has been approved by the Medical Research Ethics Commission of FK UNDIP/RSUP Dr. Kariadi Semarang No. 1066/EC/KEPK-RSDK/2022.

Intervention Materials

Astaxanthin 4 mg was administered 2x daily in the treatment group. Vitamin C 500 mg and vitamin E 250 IU were administered 1x daily in the control group.

Data Collection Method

Hemoglobin levels, erythrocyte counts, leukocytes, and platelets in the study subjects were obtained from the examination results using the Hematology Analyzer method, using the Sysmex XN 1000 Hematology Analyzer tool. SOD and MDA levels were obtained from the examination results using the ELISA method using the Elx 800 Universal Microplate Reader tool.

Descriptive data includes demographic data (age, gender), HNC type, cancer stage, ECOG, and BMI.

Data Processing and Analysis

Control and treatment group data were analyzed using the Descriptive, Levene, Shapiro-Wilks, Wilcoxon, and Mann-Whitney tests with a significance value of $p<0.05$.25 Data was processed using the Statistical Package for the Social Sciences 25 (SPSS 25) computer program.

RESULT

There were 42 study subjects, divided randomly into two groups, namely the treatment and control group, to examine hemoglobin levels, erythrocyte count, leukocytes, platelets, and SOD and MDA levels, one day before cisplatin chemotherapy. In the treatment group, astaxanthin was given 2x4 mg per day. In contrast, the control group was given vitamin C 1x500 mg per day and vitamin E 1x250 IU daily after the blood draw I up to one day before the next series of cisplatin chemotherapy (administration for three weeks). In both groups, blood draw II was carried out to check hemoglobin levels, erythrocyte levels, leukocytes, platelets, and SOD and MDA levels one day before the next series of cisplatin chemotherapy.

Discussion

Characteristics of Research Subjects

Forty-two study subjects met the inclusion criteria. Most subjects belong to the ages group of 41-50 years old, consisting of 13 people (30.9%), men and women ratio 2:1, nasopharyngeal carcinoma 22 people (52.4%), stage IV 32 people (76.2%), ECOG I 31 people (73.8%) and normal BMI 30 people (71.4%) (Table 1).

Age, gender, HNC type and stage, ECOG, and BMI have been tested for homogeneity before therapy with the Levene test. There was a significant difference in the treatment and control group ($p>0.05$) in the variables of age, sex, type of HNC, ECOG, and BMI, so these variables did not affect the decrease in hemoglobin levels, erythrocytes, leukocytes, platelets, and SOD and MDA levels. The stage of cancer obtained a significant difference where $p = 0.004$ ($p<0.05$), so the stage of cancer affects the decrease in hemoglobin levels, the number of erythrocytes, leukocytes, platelets, and SOD and MDA levels. (Table 1).

These results showed that advanced HNC patients given cisplatin chemotherapy from both the treatment and control groups would experience a significant decrease in hemopoietic activity. The average age of the study sample was 48.2 years, the youngest was18 years, and the oldest was 77 years. These results are in accordance with a study at Kariadi hospital Semarang in March-April 2015, where HNC patients were the most in the age group of more than 40 years, and the incidence increased along with age. 8 The incidence of HNC in the United States often occurs in men over 50 years old. 4-6 The causes of HNC are multifactorial; exposure to carcinogenic substances and infection with the Ebstein- Barr virus can cause the accumulation of gene abnormalities that result in the transformation towards cancer cells. This process takes decades, so the frequency of HNC increases with age.26,27

The characteristics of the sample according to the sex of men were 27 (64.3%) more than women 15 (35.7%) in a ratio of 2:1. These results are in accordance with Munir's research at RSCM where the prevalence of men is greater than that of women to be exposed to HNC.3 This result is almost the same as the incident in the United States, which states that only one-third of HNC sufferers are female. 4-6 HNC incidence at RSUP Dr. Kariadi Semarang in Meret-April 2015, more found in men with a ratio of men and women 2:1.

This causes the number of male sufferers to suffer more from HNC because it is suspected to be due to habits related to carcinogenic substances (smoking, drinking alcohol) and work environments that have a high potential to be exposed to carcinogenic materials.26,27

Table 1 Description of Characteristics of Research Samples in the Treatment and Control Group

Variable	Treatment (%)n=21	Control (%)n=21	p
Age (years)			0,182
11-20	1 (4,8)	-	
21-30	2 (9,5)	1 (4,8)	
31-40	5 (23,8)	2 (9,5)	
41-50	5 (23,8)	8 (38,1)	
51-60	3 (14,3)	7 (33,3)	
61-70	5 (23.8)	2 (9,5)	
71-80	-	1 (4,8)	
Gender			0,536
Male	13 (61,9)	14 (66,7)	
Female	8 (38,1	7 (33.3)	
Head and Neck Cancer Type			0,415
Nasopharyngeal carcinoma	12 (57)	10 (47,6)	
Sinonasal carcinoma	4 (19)	4 (19)	
Laryngeal carcinoma	1 (4,8)	2 (9,5)	
Tonsil carcinoma	1 (4,8)	-	
Cavum nasi carcinoma	1 (4,8)	-	
Carcinoma of the tongue	1 (4,8)	1 (4,8)	
Parotid carcinoma	-	1 (4,8)	
Colli NHL	1 (4,8)	2 (9,5)	
Pharyngeal adenocarcinoma	-	1 (4,8)	
ECOG			0,657
I	16 (76,2)	15 (71,4)	
II	4 (19)	5 (23,8)	
III	1 (4,8)	1 (4,8)	
BMI			0,657
Underweight (<18,5)	4 (19)	3 (14,3)	
Normal (18,5- 22,9)	16 (76,2)	14 (66,7)	
Overweight (23 - 24,9)	1 (4,8)	2 (9,5)	
Obesity I (25 - 29,9)	-	2 (9,5)	
Obesity II (>30)	-	-	

*Levene test; p>0,05 (homogen)25

The distribution of HNC types shows that the most common type of malignancy is nasopharyngeal carcinoma 22 (52.4%) cases which is the most frequent malignancy in the head area of the neck. The incidence of HNC at Kariadi hospital Semarang was 448 cases, with the highest percentage being nasopharyngeal cancer (60%) followed by cancer of the nose and paranasal sinuses (18%), laryngeal cancer (16%) as well as cancer of the oral cavity, tonsils and hypopharynx with a low percentage.

The HNC stage in this study is generally advanced: many at stage IV, as many as 32 (76.2%) people, and stage III, as many as 10 (23.8%) people. Patients usually come for treatment in an advanced stage where the tumor has expanded to the surrounding tissues or lymph nodes of the neck and greatly interferes with daily activities. This is due to the lack of public knowledge about HNC, especially early symptoms, and low socioeconomic conditions, causing patients to arrive late for treatment. In the early stage, HNC has not given disturbing complaints, so patients ignore them.

ECOG in this study was generally ECOG 1, namely 31 (73.8%) people, ECOG II as many as 9 (21.4%) people, and ECOG III as many as 2 (4.8%) people. HNC patients come for treatment mostly in ECOG 1 (good) conditions. This shows that, on average, the general condition of HNC patients during the first treatment is still good regarding communication and daily activities.

The BMI of patients in this study was generally in normal circumstances (18.5-22.9), as many as 30 (71.4%) people,

underweight (<18.5), 7 (16.7%) people,

overweight (23-24.9), 3 (7.1%) people, and

obesity I (25-29.9), 2 (4.8%) people. On average, HNC patients who come for treatment have a normal BMI, where the patient has a balance between body weight and height. This is due to the increasing public knowledge about the daily nutritional intake of healthy and good foods.

Data Homogeneity and Normality Test

The results of the homogeneity test (Levene test) hemoglobin levels, erythrocyte count, leukocytes, platelets, and SOD and MDA levels before therapy in the treatment and control group obtained hemoglobin levels p=0.1000, erythrocyte count p=0.031, leukocyte count p=0.987, platelet count p=0.177, SOD level p= 0.938 and MDA level p=0.000. So, hemoglobin levels, leukocyte counts, platelets, and SOD levels are homogeneous where p>0.05, while erythrocyte counts and MDA levels are not homogeneous where p<0.05.25(Table 2).

Table 2 Homogeneity Test of Hemoglobin Levels, Number of Erythrocytes, Leukocytes, Platelets, SOD and MDA Levels Before Therapy in the Treatment and Control Group

	Treatment group (n=21)		Control group (n=21)		p
	Mean	SD	Mean	SD	
Hemoglobin(g%)	11,7	1,44	10,8	0,89	0,100
Erythrocytes (millions/mm^3)	4,1	0,76	3,7	0,37	0,031
Leukocytes (thousand/mm^3)	6,8	3,08	5,6	3,58	0,987
Thrombocytes (thousand/mm^3)	289,3	97,85	302,3	141,63	0,177
SOD (U/ml)	70,2	40,02	73,4	52,11	0,938

MDA (pg/ml)	1699	276,67	1307,4	502,45	0,000

*Levene test; p>0,05 (homogen)25

The normality test results of hemoglobin levels, the number of erythrocytes, leukocytes, and platelets, and SOD and MDA levels before therapy with the Shapiro Wilks test obtained hemoglobin levels in the treatment group is p=0.998, and the control group is p=0.792. The number of erythrocytes in the treatment group is p=0.253, and the control group is p=0.975. The number of leukocytes in the treatment group is p=0.006, and the control group is p=0.000. The number of platelets in the treatment group is p=0.011, and the control group is p= 0.313. SOD levels in the treatment group is p=0.317, and the control group is p=0.000. The MDA level in the treatment group is p=0.010, and the control group is p=0.043.25

Therefore, hemoglobin levels and erythrocyte counts before therapy in the treatment and control group, the platelet count in the control group, and SOD levels in the treatment group are normally distributed (p>0.05). In contrast, leukocyte count and MDA levels in the treatment group and control group, the platelet count in the treatment group, and SOD levels in the control group are abnormally distributed (p<0.05)25 (Table 3).

Based on the results of homogeneity and normality tests, the data were analyzed with non-parametric test.25

Table 3 Normality Test of Hemoglobin Levels, Number of Erythrocytes, Leukocytes, Platelets, SOD and MDA Levels Before Therapy in the Treatment and Control Group

	Treatment group (n=21)		Control group (n=21)		p
	Mean	SD	Mean	SD	
Hemoglobin(g%)	11,7	1,44	10,8	0,89	0,792
Erythrocytes (millions/mm^3)	4,1	0,76	3,7	0,37	0,975
Leukocytes (thousand/mm^3)	6,8	3,08	5,6	3,58	0,000
Thrombocytes (thousand/mm^3)	289,3	97,85	302,3	141,63	0,013
SOD (U/ml)	70,2	40,02	73,4	52,11	0,000
MDA (pg/ml)	1699	276,67	1307,4	502,45	0,043

*Saphiro Wilks test; p>0,05 (normal distribution)25

Average Hemoglobin Levels, Number of Erythrocytes, Leukocytes, Platelets, SOD and MDA Levels Before and After Therapy In the Treatment and Control Groups

The average hemoglobin level before therapy in the treatment group was 11.7 g%+1.44, while the average hemoglobin level after therapy in the treatment group was 11.2 g%+1.21. The average hemoglobin level before therapy in the control group was 10.8 g%+0.89, while the average hemoglobin level after therapy in the control group was 10.3 g%+1.17. The average number of erythrocytes before therapy in the treatment group was 4.1 million/mm3+0.76, while the average number of erythrocytes after therapy in the treatment group was 3.9 million/mm3 +0.54. The average number of erythrocytes before therapy in the control group was 3.7 million/mm3+0.37, while the average number of erythrocytes after therapy in the control group was 3.4 million/mm 3 + 0.37. The average number of leukocytes before therapy in the treatment group was 6.8 thousand/mm3+3.08, while the average number of leukocytes after therapy in the treatment group was 5.8 thousand/mm3 +2.77. The average number of leukocytes in the control group before therapy was 5.6 thousand/mm3+3.58, while the average number after therapy in the control gsroup was 3.8 thousand/mm3+1.59. The average platelet count before treatment

group therapy was 289.3 thousand/mm3+97.85, while the average platelet count after therapy in the treatment group was 298.7 thousand/mm3+132.2. The average platelet count before therapy in the control group was 302.3 thousand/mm3+141.63, while the average platelet count after therapy in the control group was 253.1 thousand/mm3+ 122.63. The average SOD level before therapy in the treatment group was 70.2 U/ ml+40.02, while the average SOD level after therapy in the treatment group was 91.8 U/ml +52.54. The average SOD level before therapy in the control group was 73.4 U/ml+ 52.11, while the average SOD level after therapy in the control group was 117.7 U/ml +110.73. The average MDA level before therapy in the treatment group was 1699 pg/ml+276.67, while the average MDA level after therapy in the treatment group was 1631.8 pg/ml+331.22. The average MDA level before therapy in the control group was 1307.4 pg/ml+502.45, while the average MDA level after therapy in the control group was 1249.1 pg/ml+497.14 (Table 4).

The results of the Wilcoxon test, the average hemoglobin level before and after therapy in the treatment group was p=0.085, while in the control group was p=0.014. The average number of erythrocytes before and after therapy in the treatment group is p=0.048, while in the control group, p=0.002. The average number of leukocytes before and after therapy in the treatment group is p= 0.122, while in the control group, p= 0.003. The average platelet count before and after therapy in the treatment group is p= 0.664, while in the control group, p =0.039. The average SOD levels before and after therapy in the treatment group is p=0.019, while in the control group, p= 0.000. The average MDA levels before and after treatment group therapy are p =0.028, while in the control group, p=0.027.25

There was a significant difference in the average hemoglobin levels, erythrocyte, leucocyte and platelet counts, SOD and MDA levels in the control and as well as erythrocyte levels, SOD and MDA levels in the treatment group ($p<0.05$). There was not significant difference in the average hemoglobin levels, leucocyte and platelet counts in the treatment group ($p>0.05$).

Table 4 Average Hemoglobin Levels, Number of Erythrocytes, Leukocytes, Platelets, SOD and MDA Levels Before and After Therapy In the Treatment and Control Group

	Mean Before therapy	Mean After therapy	p
Treatment Group (n=21)			
Hemoglobin (g%)	11,7 (1,44)	11,2 (1,21)	0,085
Erythrocytes (million/mm^3)	4,1 (0,76)	3,9 (0,54)	0,048
Leukocytes (thousand/mm^3)	6,8 (3,08)	5,8 (2,77)	0,122
Thrombocytes (thousand/mm^3)	289,3 (97,85)	298,7 (132,2)	0,664
SOD (U/ml)	70,2 (40,02)	91,8 (52,54)	0,019
MDA (pg/ml)	1699 (276,67)	1631,8 (331,22)	0,028
Control Group (n=21)			
Hemoglobin (g%)	10,8 (0,89)	10,3 (1,17)	0,014
Erythrocytes (million/mm^3)	3,7 (0,37)	3,4 (0,37)	0,002
Leukocytes (thousand/mm^3)	5,6 (3,58)	3,8 (1,59)	0,003
Thrombocytes (thousand/mm^3)	302,3 (141,63)	253,1 (122,63)	0,039
SOD (U/ml)	73,4 (52,11)	117,7 (110,73)	0,000
MDA (pg/ml)	1307,4 (502,45)	1249,1 (497,14)	0,027

*Wilcoxon test; $p<0,05$ (significant)25

These results show the effect of cisplatin on a decrease in hemoglobin levels, erythrocyte count, leukocytes, platelets, SOD, and MDA levels. This is because cisplatin can cause an increase in the production of reactive oxygen species (ROS) in the body. Accumulation of ROS will release cytochrome-c from the mitochondria through activation of c-Jun-N-

terminal kinase (JNK) and p38MAPK. Cytochrome-c will then activate caspase-8, -9, and -3 (intrinsic pathway apoptosis), thus causing cell apoptosis, in this case, the hemopoietic activity resulting in a decrease in hemoglobin erythrocyte count, leukocytes, platelets, SOD and MDA levels. 10,28,29

Due to cisplatin, the decrease in hemoglobin levels, erythrocyte count, leukocytes, platelets, SOD, and MDA levels is related to the dose given. The administration of cisplatin on repetition therapy for the next series will make an accumulative increase in cisplatin.10,28,29Lee et al (2005) examined the effect of cisplatin chemotherapy on bone marrow in 274 gynecological cancer patients and 503 breast cancer patients. As a result, 28.8% experienced a decrease in the hemopoietic system starting to occur in series I as much as 34.7%, in series II as much as 63.4%, and in series III as much as 75.7% p there were patients with gynecological cancer while in breast cancer patients began to decrease the hemopoietic system in series I by 26.2%, series II by 53% and series III by 72.1%. 14

Average Decrease in Hemoglobin Levels, Number of Erythrocytes, Leukocytes, Platelets, SOD, and MDA Levels After Therapy Between Treatment and Control Groups

The decrease in hemoglobin levels after therapy in the treatment group was 11.2 g%+1.21, while in the control group, it was 10.3 g%+1.17. The average decreasen the number of erythrocytes after therapy in the treatment group was 3.9 million/mm 3 +0.54, while in the control group, it was 3.4million/mm3+0.37. The average decrease in the number of leukocytes after therapy in the treatment group was 5.8 thousand/mm3+2.77, while in the control group, it was 3.8 thousand/mm3+1.59. The average decrease in platelet count after therapy in the treatment group was 298.7 thousand/mm3+132.2, while in the control group, it was 253.1 thousand/mm3+ 123.63. The average decrease in SOD levels after therapy in the treatment group was 91.8 U/ml+52.54, while in the control group, it was 117.7 U/ml+110.73. The average decrease in MDA levels after therapy in the treatment group was 1631.8 pg/ml+331.22, while in the control group, it was 1249.1 pg/ml+497.14 (Table 5).

Table 5 The Average Decrease in Hemoglobin Level, Number of Erythrocytes, Leukocytes, Platelets, SOD and MDA Levels After Therapy in the Treatment and Control Group

	Mean Treatment Group (n=21)	Control Group(n-21)	p
Hemoglobin (g%)	11,2 (1,21)	10,3 (1,17)	0,012
Erythrocytes (million/mm^3)	3,9 (0,54)	3,4 (0,37)	0,004
Leukocytes (thousands/mm^3)	5,8 (2,77)	3,8 (1,59)	0,009
Platelets (thousands/mm^3)	298,7 (132,2)	253,1 (122,63)	0,178
SOD (U/ml)	91,8 (52,54)	117,7 (110,73)	0,489
MDA (pg/ml)	1631,8 (331,22)	1249,1 (497,14)	0,014

*Mann Whitney test; p<0,05 (significant)25

Mann Whitney test results, the average decrease in hemoglobin levels between the treatment and control groups was obtained p=0.012, the number of erythrocytes obtained p=0.004, the number of leukocytes obtained p=0.009, the number of platelets obtained p=0.178, SOD levels obtained p=0.489 and MDA levels obtained p=0.014. There were significant differences in hemoglobin levels, erythrocytes, leukocytes, and MDA levels (p<0.05), while platelet counts and SOD levels did not have significant differences (p>0.05).25

The results of this study can be concluded that astaxanthin can prevent a decrease in hemopoietic activity (hemoglobin levels, erythrocyte count, and leukocytes) through a decrease in MDA levels in HNC patients who receive cisplatin chemotherapy.

This is because cisplatin works systemically non-selectively so that it can affect the body's bone marrow, as a result of which precursor cells and differentiationcells in the bone marrow and mature cells in the blood circulation will be affected. Hemopoietic activity will be damaged, mitosis decreases and apoptosis occurs so that it can result in a decrease in hemoglobin levels, the number of erythrocytes, leukocytes, and platelets through a decrease in MDA levels.30-32

Decreased hemoglobin levels, erythrocyte count, leukocytes, and platelets due to changes in hemopoietic activity, namely the death of blood embryonal cells after exposure to cisplatin chemotherapy because exposure to cisplatin causes free radicals that result in bone marrow depression (myelosuppression) through an increase in SOD levels and a decrease in MDA levels.30-32

Changes in hemopoietic activity in the form of death of blood embryonal cells within a few weeks after exposure to cisplatin chemotherapy and have different sensitivities. The most sensitive red blood cells are then followed by white blood cells and megakaryocytes. The most sensitive

leukocyte cells are lymphocyte cells, then neutrophil cells and other granulocyte cells while the less sensitive blood cells are platelet cells, besides that in cancer patients there tends to be a process of adaptation, namely reactive thrombocytosis due to the presence of cancer cells so that the number of thromboses tends to increase, therefore astaxanthin has less effect on platelet cells.

12,30-32

Due to the lack of antioxidant ability in the body to fight free radicals due to cisplatin side effects, it is necessary to use astaxanthin as an antioxidant from the outside to bind free radicals that affect the bone marrow to prevent a decrease in hemopoietic activity.

In this study, the administration of astaxanthin to prevent a decrease in hemoglobin levels, the number of erythrocytes and leukocytes due to cisplatin so that the completion of the next series of cisplatin chemotherapy is not delayed due to a decrease in the general condition of the patient and can improve the prognosis of HNC sufferers.

Astaxanthin can also increase the work activity of the SOD enzyme in fighting free radicals. In this study, there has been no increase in SOD levels in preventing a decrease in hemopoietic activity in HNC sufferers due to cisplatin. 33-37

SOD enzymes are the most critical antioxidants in the body compared to glutathione peroxidase and catalase enzymes and can improve the stressful effects of oxidative stress due to free radicals. In addition, the SOD enzyme has a three-dimensional representative structure in the form of a protein that can catalyze the change of superoxide into hydrogen peroxide and oxygen and can minimize tissue damage due to free radicals because SOD is an enzyme with extensive ramification (branching). This high SOD activity will be illustrated by the low oxidation products of lipids. 33-37

The measurement of malondialdehyde (MDA) levels was carried out in this research because direct measurement of free radicals is very difficult to do because free radicals do not stay long, their half-life is short and disappears immediately in seconds. In addition, MDA levels are used as the most frequently performed measurements among other oxidative stress biomarkers as laboratory parameters. MDA can be found in plasma, serum, and urine to assess lipid peroxidation and cellular damage. This compound was first used in the 1950s as a marker of damage to food. Currently, MDA is often used as a marker of oxidative stress, especially in various clinical conditions related to the lipid peroxidation process due to free radicals.38-41

MDA is a dialdehyde compound that is the final product of lipid peroxidation in the body through enzymatic or non- enzymatic processes. A high concentration of MDA indicates the presence of oxidation processes in the cell membranes of the human body. MDA is abundant in circulation and is the end result of lipid peroxidation due to the breaking of fatty acid chains and becoming a toxic compound for cells. This MDA is produced constantly according to the proportion of lipid peroxidation that occurs. In this study, astaxanthin was able to prevent a decrease in hemopoietic activity through a decrease in MDA levels which is the final product of lipid peroxidation. 38-41

The linear regression test of the effect of astaxanthin on decreasing hemoglobin levels, the number of erythrocytes, leukocytes, platelets and SOD and MDA levels. The correlation value (R) of hemoglobin level was 0.365, while the determinant coefficient (R square) was 0.133. The correlation value (R) of the number of erythrocytes was 0.412, while the determinant coefficient (R square) was 0.170. The correlation value (R) of leukocyte count was 0.427, while the determinant coefficient (R square) was 0.183. The correlation value (R) of platelet count is 0.214, while the determinant coefficient (R square) is 0.046. The correlation value (R) of SOD content is 0.180, while the determinant coefficient (R square) is 0.032. The correlation value (R) of the MDA level is 0.431, while the determinant coefficient (R square) is 0.186 (Table 6).

The results of the linear regression test, the effect of astaxanthin on decreasing hemoglobin levels was obtained p=0.017, decreased erythrocyte counts were obtained p=0.007, decreased leukocyte counts were obtained p=0.005, decreased platelet counts were obtained p=0.174, decreased SOD levels were obtained p=0.254 and decreased blood levels MDA obtained p=0.004. There was a significant difference in the effect of astaxanthin administration on decreasing hemoglobin levels, erythrocyte counts, leukocytes and MDA levels ($p<0.05$), while there were no significant differences in decreasing platelet counts and SOD levels ($p>0.05$) (Table 6).

Side effects of astaxanthin administration in the form of gastrointestinal disorders (nausea and heartburn) were obtained in 3 patients in the treatment group (Table 7). These side effects have been overcome by giving antacid tablets 3x1 and the patient can continue research. Based on the evidence, research results show that the benefits of giving astaxanthin are more than the disadvantages or side effects that occur.

Table 6 Linear Regression Test of The Effect of Astaxanthin on Decreasing Hemoglobin Levels, The Number of Erythrocytes, Leukocytes, Platelets and Levels of SOD and MDA

	Correlation value	Determinant (R)	p coefficient (R square)
Hemoglobin (gr%)	0,365	0,133	0,017
Erythrocytes (million/mm^3)	0,412	0,170	0,007
Leucocytes (thousands/mm^3)	0,427	0,183	0,005
Platelets (thousands/mm^3)	0,214	0,046	0,174
SOD (U/ml)	0,180	0,032	0,254
MDA (pg/ml)	0,431	0,186	0,004

*Regression test ; $p<0,05$ (significant)

Table 7 Astaxanthin Side Effects in Research Group

Complaints	Number of Patients
Nausea	2
Vomiting	-

Heartburn	1
Diarrhea	-
Total	3

The patient's adherence to taking medications and the consumption of other antioxidants was controlled by researchers by asking the rest of the drugs that had been taken and other antioxidants consumed by the patient at the time of control to the ENT clinic.

Three patients dropped out of the study, namely 2 people in the control group because the general condition worsened after cisplatin chemotherapy, while 1 person in the treatment group did not routinely take astaxanthin according to the instructions provided.

Research Limitations

The results of this study cannot explain all the problems, due to the limitations of the study, including the possibility of research subjects consuming foods and drinks containing other antioxidants, differences in the nutritional status of sufferers, differences in the ability and absorbability of food and drugs of each patient.

SUGGESTIONS AND CONCLUSIONS

Suggestions

Referring to the results of this study, it is necessary to conduct further research on the administration of other antioxidants to HNC sufferers in preventing a decrease in hemopoietic activity due to the emergence of free radicals due to cisplatin chemotherapy.

Conclusion

Supplementation of astaxanthin 2x4 mg for 3 weeks can prevent a decrease in hemopoietic activity (hemoglobin levels, erythrocyte count, and leukocytes)through a decrease in MDA levels in HNC patients who receive cisplatin chemotherapy due to the onset of free radicals.

Acknowledgment

Thanks to God, my family, Promotor & Co Promotor, supervising teachers, Kasuari Installation of RSUP Dr. Kariadi Semarang and other parties who have assisted in this research.

References

1. Mehanna H, West CML, Nutting C, Paleri V. Head and Neck Cancer. Part 2: Treatment and Prognostic Factors. J. British Medical 2010; 341: 721–5.
2. Mateos JF, et al. Epidermal Growth Factor Receptor (EGFR) Pathway Polymorphisms as Predictive Markers of Cetuximab Toxicity in Locally Advanced Head and Neck Squamous Cell Carcinoma (HNSCC) in a Spanish Population. Oral Oncology. 2016; 63: 38–43.
3. Franzmann ELS, Huang, D, Thomas, G. Head and Neck Cancer : Changing Epidemiology and Public Health Implications. Review Article Head & Neck Cancer, J. Oncology. 2010.
4. National Library of Medicine MedlinePlus. Head and Neck Cancer. [cited 16 Januari 2006]. Available from URL : www.nlm.nih.gov/medlineplus/headan dneckcancer.html.
5. WHO Global Cancer Rates Could Increase by 50 % to 15 Million by 2020. [cited 14 Januari 2006]. Available from URL : http://www.who.int/mediacentre/news/ releases/2003/pr27/en/.
6. National Cancer Institutes. Head and Neck Cancer : Types. [cited 16 Januari 2006] Available from URL : http://www.cancer.gov/cancerinfo/type s/head-and-neck.

7. Soekamto SM, Sandhika W, Fauziah D. Aspek Patologi Tumor THT-KL. Perkembangan Terkini Diagnosis dan Penatalaksanaan Tumor Ganas THT- KL. Surabaya : SMF Ilmu Penyakit THT-KL FK Unair/RSUD Dr. Soetomo. 2002; 9-37.
8. Ramdhani, Aryo. Karakteristik Pasien Keganasan Kepala dan Leher Yang Mendapatkan Kemoterapi di RSUP Dr. Kariadi Semarang periode Maret-April 2015.
9. Brockstein BE, Vokes EE. Principles of Chemotherapy in The Management of Head and Neck Cancer. In : Bailey BJ, Calhoun KH, editors. Head and Neck Surgery-Otolaryngology.4ed.Philadelphia:Lippincot- William&Wilkins. 2006; 1428-41.
10. Johnson S, O'Dwyer P. Cisplatin and Its Analogues. In : DeVita V, Hellman S, Rosenberg S, editors. Cancer Principles and Practice of Oncology. 7ed. Lippincott: 2005; 344-54.
11. Munir M. Keganasan di Bidang Telinga Hidung Tenggorok, In : Soepardi EA. Iskandar N, Bashiruddin J, Restuti RD, 6ed. Jakarta: Balai Pustaka FKUI. 2007; 162-73.
12. Vokes EE, Chong N. Chemotherapy of Head and Neck Cancer. In : Perry MC, editor. The Chemotherapy of Source Book. Philadelpia: Lippincot Williams & Wilkins. 2008; 324-38.
13. Barrett-Lee P, Bokemeyer C, Gascón P, Nortier J, Schneider M, Schrijvers D et. al. Management of Cancer-Related Anemia in Patients with Breast or Gynecologic Cancer: New Insights Based on Results from The European Cancer Anemia Survey. The Oncol. 2005; 10: 743–57.
14. Baron RE, Julve JV, Jaime SP, Santalo NB, Millan CV, Mata ML. Haemoglobin Levels and Acute Radiotherapy Induced Toxicity. Tumori: 2005; 91(1): 40–5.
15. Soebandiri. Hemopoesis. 6ed. Jakarta: InternaPublishing. 2014; 2571-74.
16. Rouli N & Pustika A. (2005). Anemia pada penyakit keganasan anak. Sari Pediatri. Vol. 6(4). Hal: 176-181.
17. Purwata M. Antioksidan. Ilmu Kedokteran Molekul. Bali: CV.Infomedika; 2016; 1-54.
18. Sayuti K, Yenrina R. Antioksidan Alami dan Sintetik.1ed. Padang. Andalas University Press. 2015; 7-73.
19. Suryohudoyo. Oksidan, antioksidan dan radikal bebas. Dalam: Kapita selekta. Ilmu Kedokteran Molekul. Jakarta: CV.Infomedika ;2000 : h 312– 47.
20. Pavlovic V, Cekic S, Rankovic G & Stoiljkovic N. Antioxidant and pro- oxidant effect of ascocbic acid. Act Med Median. 44 (1): 2005 ;65–69.
21. Suseela MR, Toppo K. Haematococcus Pluvialis A Green Alga. Richest Natural Source of Astaxanthin. Current Science. 2006; 90(12): 1602-3.
22. Nishida Y, Yamashita E, Miki W. Quenching Activities of Common Hydophilic and Lipophilic Antioxidants Againts Singlet Oxygen Using Chemiluminescence Detection System. Carotenoid Science. 2007; 11: 16-20.
23. Johanna M, Ake L, Annette P, Peter H. Oral Bioavailability of The Antioxidant Astaxanthin in Human is Enhanced by Incorporation of Lipid Based Formulations. J. European of Pharmaceutical Sciences. 2003; 19: 299-304.
24. Xue XL, Han XD, Li Y, Chu XF, Miao WM, Zhang JL, Fan SJ. Astaxanthin Attenuates Total Body Irradiation- induced Hematopoietic System Injury in Mice via Inhibition of Oxidative Stress and Apoptosis. Stem Cell Res Ther. 2017; 8: 7.
25. Sastroasmoro S, Setiabudy R, Setyawati A, Taher A, Harimurti K, Salamia IN. Uji Klinis : Landasan Teori dan Panduan Praktis. 1ed. Sagung Seto. Jakarta: 2017; 109-24
26. Franzmann E, Lilly S, Huang D, Thomas G.Oncology of head and neck tumors. In : Van De Water TR, Staecker H, editors. Otolaryngology Basic Science and Clinical Review. New York : Thieme; 2006 :159-71.
27. Hasselt CAV. Nasopharyngeal carcinoma. In: Andrew SJ, David EP editors. Disease of the Head and Neck, Nose and Throat. London: Arnold; 1998.p: 297-307.

28. Barabas K, Milner R, Lurie D, Adin C. Cisplatin: a review of toxicities and therapeutic applications. Vet & Comp Oncol. 2008;6(1):1-18.
29. Ekborn A. Cisplatin induced ototoxicity,pharmacokinetics,prediction and prevention. Stockholm: Department of Otolaryngology and Head & Neck Surgery Karolinska Hospital; 2003.p 13-16.
30. Mazza J J. Hematopoiesis and hematopoietic growth factors. In. Mazza JJ, editor. Manual of Clinical Hematology. Philadelphia: Lippincott Williams & Wilkins; 2002 : 1-17.
31. Ramnik S. Haematology for students & practitioners. 5th ed. New Delhi: Jaypee Brothers. : 2003 : 124-6.
32. Ronald AS, Richard AMcP, alih bahasa : Brahm U. Pendit dan Dewi Wulandari, editor : Huriawati Hartanto, Tinjauan klinis hasil pemeriksaan laboratorium, edisi 11. Jakarta: EGC; 2004.
33. Finkel T. Signal Transduction by Reactive Oxygen Species. J.Cell Biol. 2011; 194(1): 7-15.
34. Murray RK, Ganner DK, Rodwell VW. Biokimia Harper. (Andri Hartono). 27ed. Penerbit Buku Kedokteran EGC. Jakarta: 2009.
35. Goodsell D. Molecule of the Month: Superoxide Dismutase. [cited 27 Oktober 2012]. Available fromURL:http://www.rcsb.org/pdb/static.do?p=education_discussion/molecule_of_the_month/pdb94_1.htm.
36. Mc. Cord JM, Fridovich I. Superoxide Dismutase. An Enzymic Functionfor Erythrocuprein (Hemocuprein). J. Biol Chem. 2006; 244(22): 6049- 55.
37. Nurhayati S, Kisnanto T, Syaifudin M. Superoksida Dismutase (SOD) : Apa dan Bagaimana Peranannya Dalam Radioterapi. Iptek ilmiah Populer. 2011; 13(2): 67-74.
38. Anggraeni S, Setyaningrum T, Listiawan MY. Perbedaan Kadar Malondialdehid (MDA) sebagai Petanda Stres Oksidatif pada Berbagai Derajat Akne Vulgaris. Berkala IKK. 2017; 29(1): 1–8.
39. Gil P, et al. Malondialdehyde : A Possible Marker of Aging. Gerontology. 2002; 48(4): 209–14.
40. Situmorang N, Zulham. Malondyaldehide. J. Keperawatan & Fisioterapi. 2020; 2: 2.
41. Siswonoto S. Hubungan Kadar Malondialdehid Plasma Stroke Iskemik Akut Correlation of Plasma Malondyaldehyde. 2008: 1–116.