RESEARCH ARTICLE

Alterations of the hematologic cells in synthetic cannabinoid users

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Background: Functions, morphology, distributions, and index of the circulating cells are the most useful parameters that indicate various inflammatory and toxic conditions. The aim of this study was to investigate the clinical significance of these parameters in patients diagnosed with (synthetic) cannabis use disorder.

Methods: This study included a total of 40 patients in the study group (SG) with synthetic cannabis use; and 40 healthy individuals as the control group (CG). Participants, who had hematological disorders and other chronic diseases, were excluded from the study. All hematological parameters of SG were compared with CG. Neutrophil/lymphocyte ratio (NLR) and platelet/lymphocyte ratio (PLR) values were calculated and compared between groups.

Results: There were statistically significant differences between the groups in terms of WBC, MCH, RDW, MCV, MPV, and NEU, LYM%, MONO% parameters ($P < .05$). MPW and LYM% were significantly lower in SG compared to CG. WBC, MCH, RDW, MCV, MPV, MONO, and NEU parameters were significantly higher in SG compared to CG ($P < .05$). UIBC and TIBC levels were significantly higher in SG compared to CG ($P < .001$). Although there was statistically significant difference between groups in terms of NLR, there was no significant difference for PLR values.

Conclusion: Our data suggested that chronic use of cannabinoids can lead to deterioration of hematopoietic cells. Chronic use of cannabinoids was consistent with sub-threshold/subclinical megaloblastic anemia with iron deficiency. Inflammatory cells, especially neutrophil and monocyte counts were higher in SG compared to CG. Thus, recovery of subclinical hematological parameters should be considered in cannabis use disorder patients.

KEYWORDS

hematologic cells, inflammation, marijuana, synthetic cannabinoids

1 | INTRODUCTION

Over the last 20 years, there have been considerable researches involving the cannabinoids and its importance in regulating a variety of physiological and psychological processes such as pain, feeding behavior, lipid metabolism, pleasure sensation, and immune system.¹⁻³ Cannabinoids are usually classified as endocannabinoids, phytocannabinoids, and synthetic cannabinoids.⁴ Synthetic cannabinoids are in

the group of drugs called “new psychoactive substances” and these technically synthetic cannabinoid receptor agonists are designer drugs that mimic the psychoactive effects of cannabis.^{5,6} They were synthesized in the 1970s, and most known and common synthetic cannabinoids were coded as JWH-series (John W. Huffman), HU-series (Hebrew University), and AM-series (Alexandros Makriyannis). They were first marketed as legal cannabis alternatives in Europe in the early 2000s.⁷ Synthetic cannabinoids are synthesized in underhand

laboratories and are usually in white color like salt, mixed with acetone, and then the mixture is sprayed on dried plant materials. They are generally used like marijuana by smoking and other inhalation ways (pipes, water pipes (bongs or hookahs), cigarettes (joints or reefers), or, most recently, in the paper from hollowed out cigars (blunts)).^{8,9} The prevalence of use of synthetic cannabinoid is low in the large segment of the community (<1%), but it is higher among students, youngsters, and some special groups according to the drug tests.¹⁰ Synthetic cannabinoids are widely used in Turkey; however, there is no study for the epidemiologic data. It has both similar and different pharmacokinetic and pharmacodynamic mechanisms with the major active component of marijuana ($\Delta(9)$ -tetrahydrocannabinol-THC).^{11,12} They have hundreds of chemical types, which are usually called K2, Spice, Bonsai, Jamaica etc.¹³

DSM-V (The Diagnostic and Statistical Manual of Mental Disorders) defines cannabis use disorder as the problematic pattern of cannabis use leading to clinically significant impairment or distress, as manifested by at least two symptoms, forming within a 12-month period. Cannabis use disorder and the other cannabis-related disorders include problems that are associated with substances derived from the cannabis plant and chemically similar synthetic compounds.¹³

Effects of synthetic cannabinoids on the central nervous system are similar to other cannabinoids.^{14,15} In reference to these effects, cannabinoid ligands bind with specific G protein-coupled receptors (CB1 receptor and the CB2 receptor) and activate the endocannabinoid system. Synthetic cannabinoids have potent agonism to cannabinoid receptors differentially from THC; thus, THC has partial agonism.¹² CB1 receptors influence the central nervous system and cause thermoregulation disorders, psychotic episodes, memory disorders, antiemetic activity, appetite enhancer activity, anxiety, and stress relieving activities.^{16,17} CB2 receptors were found in immune system such as thymus, tonsils, marginal zone of the spleen, B lymphocytes, T lymphocytes, macrophages, monocytes, natural killer (NK) cells, and polymorphonuclear cells. According to the earlier studies in the literature, cannabinoids appear to influence immune function, especially through CB2 receptors^{18,19}, such as inhibition of the functions of macrophage and macrophage-like cells, suppression of B and T lymphocytes and cytolytic activity of NK cells, proliferation and maturation of cytotoxic T lymphocytes, affecting immune cells' consolidation and chemotaxis, suppressing the antibody response in humans and animals, suppressing a variety of activities of T lymphocytes. It is well known that endocannabinoid-anandamides have anti-inflammatory effects but endocannabinoid 2-Arachidonoylglycerol (2-AG) shows agonist behavior against CB1 and CB2 receptors and it is speculated that it has pro-inflammatory effects on immune function in contrast to anandamides²⁰. So there are different hypotheses about the kind of effects of endocannabinoids on immune system.

Some effects such as inflammatory disorders may impair function or synthesis processes resulting in some changes on the cell morphology. For example, hemocytometer parameters like red blood cell (RBC) count, mean red blood cell volume (MCV), and red blood cell distribution width (RDW) are frequently used for anemia diagnosis.^{21,22} Previous studies have showed that RDW is an important diagnostic

and prognostic predictor of various non-hematologic diseases such as cardiovascular and metabolic diseases,²³⁻²⁵ autoimmune disease²⁶, and cancer.²⁷ However, the exact mechanisms underlying the association between RDW and these diseases remain unknown.

Platelets (PLTs) have major effects on homeostasis cascades, and they are also related to inflammatory events. MPV is a reflection of both pro-inflammatory and prothrombotic conditions and it has been regarded as a prognostic marker of arterial and venous thrombosis.²⁸ PLTs are highly activated when inflammatory mediators are released²⁹ and the mean platelet volume (MPV) and platelet distribution width (PDW) are affected in patients with inflammatory and infectious disorders such as rheumatoid arthritis and ankylosing spondylitis.²⁹⁻³² According to literature, psychiatric disorders such as schizophrenia, unipolar depression, and bipolar disorder have abnormal (too low or too high) platelet counts and MPV parameters. For example, the mean number of platelets are lower in patients with schizophrenia and higher in patients with unipolar depression.³³ According to the results of several studies in the literature, platelet activation is associated with an increased risk of other comorbidity.^{34,35} Previous studies demonstrated endocannabinoids' effects on platelet aggregation in blood and platelet shape change, secretion, and aggregation.³⁶ Interactions between endothelial surface, platelets, and white blood cells during the inflammatory cascade may promote chronic illnesses and psychiatric disorders.^{37,38} In addition, many psychiatric disorders are shown to have a strong relationship with the immune system.

White blood cells, especially neutrophils, play a crucial role in the regulation of inflammatory response. During the inflammatory response, circulating neutrophils are increased and formed with relative lymphopenia, primarily due to increased apoptosis and secondarily due to increased stress hormones such as corticosteroids.^{39,40} The earlier studies indicated that increased leukocyte damages the endothelium surface and causes venous thrombosis.⁴¹ Neutrophil/lymphocyte ratio (NLR) and platelet/lymphocyte ratio (PLR), which are currently investigated for systemic inflammatory diseases and psychological stress, are the new predictors rising in the literature. This ratio reflects both neutrophilia, which demonstrates the acute inflammatory condition and lymphopenia, as well as physiological stress. High NLR has been reported to be a predictor for prognosis of various disorders such as cancer,⁴² cardiovascular diseases,⁴³ and autoimmune and inflammatory disorders.⁴⁴ Hemogram panels can reflect various toxic or inflammatory systemic deteriorations, although, some subclinical influences may be masked if the calculated value is in the normal range.

Synthetic cannabinoids have growing usage rate among substance users. There are many kinds of synthetic cannabinoids and it is hard to accept all of these effects of synthetic cannabinoid similar to the ones of cannabinoids or each other's. There are very limited data about the hematological effects of synthetic cannabinoids and NLR and PLR are not investigated yet to the best of our knowledge. We wondered what happens with the chronic usage of synthetic cannabinoids on hematological parameters including the new trend parameters such as NLR and PLR. The aim of our study was to investigate the clinical significance of these parameters in cannabis use disorder patients, who had normal blood hemogram test results.

2 | MATERIALS AND METHODS

2.1 | Study design

Cannabis use disorder patients, who were admitted to Sakarya University Education and Research Hospital Alcohol and Substance Treatment and Education Center inpatient clinic between October 2014 and Jun 2016, were evaluated. The control group consisted of healthy individuals, who have voluntarily participated in the study. Ethics committee approval was received from the local ethics committee.

Patients were interviewed by an experienced psychiatrist and evaluated according to the criteria of DSM-V for cannabis use disorder.⁴⁵ A total of 110 subjects, who have been synthetic cannabinoid users for more than 1 year, were subjected to pre-assessment. The ones who have polysubstance use disorder or comorbid chronic or active organic disorders were excluded from the study. All patients included in the study were active cannabinoid users according to their urine analysis. All patients were also evaluated for HIV, hepatitis, and tuberculosis and the ones with positive markers were excluded. Finally, 70 patients were excluded and the remaining 40 patients were included in the study according to the inclusion criteria. Since the majority of patients with the cannabis use disorder were cigarette smokers; the control group was similarly created with matching number of smoking individuals.

2.2 | Laboratory analysis

2.2.1 | Hematological analysis

At the first day of their hospitalization, EDTA blood samples (BD Vacutainer K₂EDTA Plus plastic tubes; Becton Dickinson, Franklin Lakes, NJ, USA) were collected from each patient at the time of admission and hemogram parameters were measured using aperture-impedance technology. Neutrophil/lymphocyte ratio (NLR) was calculated by rating neutrophil count to lymphocyte count (PLR), which was calculated by rating the count of lymphocyte to platelet count. Unsaturated iron-binding capacity (UIBC) and total iron-binding capacity (TIBC) were measured to understand iron levels for etiology of anemia diagnosis. The same procedure was applied for the control group. All hematological parameters of CG and SG groups were compared with each other.

2.2.2 | Analysis of the urine tests

Cloned Enzyme Donor Immune Assay (CEDIA) was used as the immunoassay method during the analysis of the urine tests. Detected substrates consisted of amphetamine, benzodiazepine, barbiturate, opioids, 3,4-metilenedioksi-N-metilamfetamin (MDMA), synthetic cannabinoids, THC, and buprenorphine for drugs consumption, and ethyl glucuronide for alcohol consumption.

We used the cut-off levels in workplace drug testing that are recognized values for groups of drugs to determine whether a sample is

positive or negative. Any result below the cut-off level was reported as negative, and results above the cut-off value were reported as either non-negative (for screening tests) or positive (for confirmed positive results).

2.3 | Statistical analysis

SPSS for Windows statistical package version 22 (SPSS Inc., Chicago, IL, United States) was used for all statistical analyses. The numerical data were expressed as means and standard deviations, and the categorical data were expressed as frequencies and percentages. Chi-square test was used for categorical variables and independent samples t-test was used to make comparisons between two groups to determine significant differences between groups. Linear variables of hematological parameters of the SG and CG were compared with student's t-test. In addition, neutrophil/platelet ratio and neutrophil/lymphocyte ratio were compared between two groups. A level of $P < .05$ indicated statistical significance.

3 | RESULTS

3.1 | Sociodemographic attributes and clinical features of patients with cannabis use disorders

Cannabis use disorders group (Synthetic Cannabinoid) (SG) with 40 patients (mean age: 28.55 ± 7.08 years), and 40 healthy subjects group (CG) (mean age: 30.77 ± 6.30 years) were included in the study. There was no statistically significant difference between groups in terms of age. Both groups consisted of 38 men and 2 women.

Erythrocyte series and serum iron profiles' characteristics of SG and CG groups and inflammatory series and platelets' characteristics of both groups are given in Tables 1 and 2.

3.2 | The comparison of hematological parameters between SG and CG groups

There was statistically significant difference between the groups in terms of WBC, MCH, RDW, MCV, MPV, and NEU, LYM%, MONO%, IUBC, TIBC, and NLR parameters ($P < .05$). MPV and LYM parameters were significantly lower and the others were higher in SG compared to CG ($P < .05$) (Tables 1 and 2). Comparison of SG and CG according to mean count of hematological parameters is showed in Figure 1.

4 | DISCUSSION

This study presented a relationship between hematological parameters and synthetic cannabinoid use disorder. The number of total white blood cells-neutrophils and monocytes- were higher in synthetic cannabinoid users compared to the control group. A lower level was found in the percentage of lymphocytes, but it seems as a relative decrease due to the increase in other parameters of the defence system. These results suggested an immunologic reactivity derived

Laboratory parameters	SG (n=40)	CG (n=40)	P value
Red blood cell (RBC) Count (M/ μ L)	5.05 $\pm$ 0.65	5.30 $\pm$ 0.45	>.05
Hemoglobin (HGB, g/dL)	15.29 $\pm$ 1.26	15.09 $\pm$ 1.30	>.05
Hematocrit (HCT, %)	45.43 $\pm$ 3.45	45.69 $\pm$ 3.40	>.05
Mean corpuscular hemoglobin (MCH, pg)	29.84 $\pm$ 1.65	28.94 $\pm$ 2.09	.036*
Mean corpuscular hemoglobin concentration (MCHC, g/dL)	33.63 $\pm$ 0.91	33.47 $\pm$ 0.95	>.05
Red Cell Distribution Width (RDW, %)	15.70 $\pm$ 1.29	14.94 $\pm$ 1.43	.014*
Mean corpuscular volume (MCV, fL)	88.72 $\pm$ 3.85	86.40 $\pm$ 4.89	.021*
Serum iron (SI, μ g/dL)	95.19 $\pm$ 36.71	97.46 $\pm$ 36.49	>.05
Unsaturated iron-binding capacity (UIBC, μ g/dL)	244.32 $\pm$ 57.36	185.20 $\pm$ 59.93	.000**
Total iron-binding capacity (TIBC, μ g/dL)	345.70 $\pm$ 49.76	284.52 $\pm$ 42.88	.000**

*Correlation is significant at the .05 level (two-tailed).

**Correlation is significant at the .001 level (two-tailed).

TABLE 1 Erythrocyte series and serum iron profiles' parameters of groups

Laboratory parameters	SG (n=40)	CG (n=40)	P value
Platelet (PLT, K/ μ L)	261.32 $\pm$ 57.89	240.15 $\pm$ 57.08	>.05
Mean platelet volume (MPV, fL)	7.18 $\pm$ 0.84	8.51 $\pm$ 1.64	.000**
Plateletocrit (PCT, GSD)	0.18 $\pm$ 0.04	0.20 $\pm$ 0.04	>.05
Platelet distribution width (PDW, %)	17.35 $\pm$ 0.84	16.97 $\pm$ 2.70	>.05
White blood cell count (WBC, K/ μ L)	8.75 $\pm$ 2.26	7.64 $\pm$ 1.59	.014*
Neutrophil (NEUn, K/ μ L)	5.37 $\pm$ 1.91	4.39 $\pm$ 1.25	.008*
Neutrophil (NEU%, K/ μ L)	60.37 $\pm$ 8.08	56.86 $\pm$ 7.96	.054
Lymphocyte (LYMn, K/ μ L)	2.52 $\pm$ 0.65	2.54 $\pm$ 0.65	>.05
Lymphocyte (LYM%, K/ μ L)	29.74 $\pm$ 7.23	33.70 $\pm$ 6.97	.014*
Monocytes (MONO n, K/ μ L)	0.60 $\pm$ 0.20	0.51 $\pm$ 0.13	>.05
Monocytes (MONO %, K/ μ L)	7.10 $\pm$ 2.22	6.72 $\pm$ 1.84	.026*
Eosinophils (EOSn, K/ μ L)	0.16 $\pm$ 0.12	0.22 $\pm$ 0.33	>.05
Eosinophils (EOS%, K/ μ L)	1.84 $\pm$ 1.14	2.22 $\pm$ 1.07	>.05
Basophils (BASOn, K/ μ L)	0.08 $\pm$ 0.03	0.10 $\pm$ 0.12	>.05
Basophils (BASO%, K/ μ L)	0.93 $\pm$ 0.45	0.84 $\pm$ 0.46	>.05
Platelet/lymphocyte ratio (PLR)	107.70 $\pm$ 27.08	99.29 $\pm$ 31.20	>.05
Neutrophil/lymphocyte ratio (NLR)	2.25 $\pm$ 0.99	1.81 $\pm$ 0.61	.019*

*Correlation is significant at the .05 level (two-tailed).

**Correlation is significant at the .001 level (two-tailed).

TABLE 2 Inflammatory series and platelets' parameters of both groups

FIGURE 1 Mean count of blood parameters. (*P<.05)

from synthetic cannabinoids. In addition, mean platelet volume (MPV) was affected in the study group and it was lower when compared to the control group. It is hard to decipher all of the cannabinoid effects on hematological parameters; however, a relationship was found between platelet functions and cannabinoid use.⁴⁶ The role of endocannabinoids receptors (CB1-CB2) on platelet functions still was unclear; either it had a stimulatory or an inhibitory effect.³⁶ In a previous study, it was shown that endocannabinoid receptor agonists reduce platelet activation and aggregate formation. In this study, there is a decrease in MPV, which is accepted as a reflection of both pro-inflammatory and prothrombotic conditions.²⁸ This data supports the previous one and claims for further elucidation of this novel regulatory mechanism

for platelet function, which may be beneficial to new antithrombotic therapies.⁴⁶ It is well known that so many drugs have some negative effects on the immune network and thrombocyte disorders.⁴⁷ Cannabis has been used for many years and cannabis use disorder can cause electrolyte disturbances, leukocytosis, anemia, and impair hematological cell functions.⁴⁸ Recent studies have revealed that, CB2 receptors are primarily located on immune cells and tissues; and, when they are activated, they can affect inflammatory and immune suppressive activity. Synthetic marijuana has strong activity and affinity on CB2 receptors.⁴ Our results seem to be surprising, because cannabis has been known as an immunosuppressive agent for years.¹² According to the results obtained, there is an immune reaction instead of immune suppression. We may explain these results in various ways. Firstly, our patients were users of synthetic cannabinoids at least for 1 year. Therefore, a variation in effects on the immune network in chronic use should be considered. Secondly, previous studies showed that endocannabinoid system is more complex than it is thought and some endocannabinoids may have pro-inflammatory effects.²⁰ There are various kinds of synthetic cannabinoids and each may have different effects on CB receptors that may interfere with hematological results.⁴⁹ Furthermore, different chemicals are combined during the preparation and releasing of these substances into the market; and it is nearly impossible to know the dosages and the kinds of chemicals used.¹³ The kinds of synthetic cannabinoids used in Sakarya may have a toxic chemical combination which may be responsible for unpredictable effects. Thirdly, synthetic cannabinoids are prepared with herbal compounds under unhygienic conditions and many kinds of contaminations may occur.¹³ This immune reactivity may be provoked with those contaminations for chronic users.

The other explanation for our results is a probable toxic effect of synthetic cannabinoids with their own and acute immune reaction. Patients were active users, and all of them have been using synthetic cannabinoids recently. An immune reaction to synthetic cannabinoid is considered with these data. The distribution of the white blood cells glided in favor of neutrophils and lymphocytes were relatively lower compared to the control group and this resulted as an increase in NLR. In earlier studies in the literature, NLR was found to be related to cancer, inflammatory, and autoimmune diseases. In this study, NLR was found to be higher in the study group compared to the control group. Although NLR is not investigated in synthetic cannabinoid use, no significant change was found in NLR in marijuana user patients in another study.⁵⁰ There was an increase in NLR but there was no change in PLR in our study. PLR is not informative alone, because if both parameters are reduced or increased there will be no change in the ratio. However, in our study, there is no significant change in counts of platelets and lymphocytes; so, the ratio did not change. These parameters need further investigation with higher sample size and long-term effects.

The red blood cell distribution width (RDW) is a routinely useful index to explore the etiology of anemia, and the recent studies have revealed that RDW is a diagnostic and prognostic predictor of various non-hematology diseases.⁵¹⁻⁵³ In our study, chronic use of cannabinoids is related to an increase in inflammatory cells, especially neutrophils and this immune activity may cause high RDW values that show

early signs of anemia and other comorbidity. Additionally, unsaturated iron-binding capacity (UIBC) and total iron-binding capacity (TIBC) are higher in synthetic cannabinoid users, though there is no significant difference between groups in terms of HCT and HGB. A subclinical anemia is thought to be caused by these results. It is well known that nutritional problems are frequently seen in substance use disorders and patients are not very careful about their nutrition. Patients had higher MCV compared to the control group which is generally a sign of vitamin B and folic acid deficiency.⁵⁴ These results reveal a probable nutritional problem of substance use disorder. However, due to lack of studies explaining interactions between synthetic cannabinoids and erythrocyte functions, we do not know much about if synthetic cannabinoids have a direct effect on iron metabolism, erythrocytes, and bone marrow.

As a conclusion, there is a relationship between the use of synthetic cannabinoids and hematological parameters including white blood cells, erythrocytes, and platelets. In addition, there is a significant relationship with NLR and no significant relationship was found with PLR. Understanding the nature of the relationships requires further studies.

This is a frontier study for the hematological parameters in synthetic cannabinoid users. Evaluation of NLR and PLR together is the first in the literature to the best of our knowledge. This study has a small sample size, but all of the patients had positive urine results for cannabinoid use. In addition, this study presents results from chronic use of synthetic cannabinoids with acute toxicity effects. Long-term studies are needed to see what happens after detoxification and remission periods. Smoking, which is shown to have important effects on hematological parameters, has been a serious confounding factor in previous studies.⁴⁵ We eliminated this confounding factor as the strength of the study; but we admit that there may be other confounding factors such as nutritional effects, toxic effects of combined additional chemicals, patients' life style etc. in such studies. Further detailed studies may help to eliminate other probable confounding factors.

5 | CONCLUSION

This study presents a relationship between synthetic cannabinoids and hematological parameters. A pro-inflammatory effect and change in platelet volume and a subclinical anemia with changes in iron-binding functions are found. Effects of chronic use of synthetic cannabinoids have given some contrary results with earlier studies conducted on chronic-active users. Further studies, with a larger sample size and long-term follow up, are needed to confirm and explain these results.

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AUTHORS' CONTRIBUTIONS

D.G.: Planning and writing the article. A.B.Y.: Diagnosis and treatment of the patients, providing data and writing and editing the article. E.Y.: Editing the article, statistical analysis, literature review. A.E.: Review of the article, coordination of the study team.

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