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Negative Impact of Cigarette Smoking on Haematological Parameters in Healthy Libyans

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Abstract: Smoking causes about 6 million deaths annually, increases the risk for many diseases and alters hematological parameters. Thus, the aim of this study was to evaluate the negative impact of cigarette smoking on the hematological parameters of healthy smokers in Libya. A total of 145 smokers and 145 non-smokers, with age range 39-45 years, were recruited. Blood samples were collected from each participant and were analysed for complete blood count. The obtained results were statically analysed using descriptive statistics and ANOVA. The mean for all study variables for smokers (except for MCHC) was greater than that for non-smokers. The coefficient of variation (CV%) showed that the smokers group was more homogeneous for most variables than the non-smokers group, except for BMI and LYMP. The results of ANOVA test showed that only in the case of MCHC the non-smokers had a significantly higher mean ($p < 0.05$). For other variables, the smokers had significantly higher means ($p < 0.05$), except for the RBC, where the mean was non-significantly higher compared to the non-smokers. There was no statistically significant effect for the age variable or for the interaction between smoking and age on all variables. In conclusion, the study revealed that cigarette smoking has a significant impact on most haematological parameters; confirming that smoking has severe adverse effects on most haematological parameters.

Keywords: cigarette smoking, haematological parameters, ANOVA, descriptive statistics, negative impact.

1. Introduction:

Smoking is one of the leading causes of death, as it causes approximately 6 million deaths per year throughout the world [1], which represents 9% of all deaths worldwide [2]. Nicotine, the chemical compound responsible for tobacco addiction, is used by around 22% of the world's population, according to the WHO in 2020 [3], nearly 1.3 billion individuals [4].

In addition to the negative impact of tobacco addiction on smokers, smoking affects many body systems by increasing the risk of

cardiovascular diseases [1,5], respiratory ailments [2,6], various diseases of the digestive system [7], and disorders of the immune and reproductive systems [8]. It is also associated with cancer of different human body organs including lung, head, neck, pancreas, bladder, kidney, ovaries, and myeloid leukemia [7].

Smoking has been found to alter the levels of several biochemical and hematological parameters in serum. A previous study [9] revealed significant increases in total cholesterol, triglycerides, low-density lipoprotein cholesterol, and a decrease in high-density lipoprotein cholesterol in smokers compared to non-smokers. Additionally, smoking is linked to higher levels of free thyroxine and triiodothyronine, lower levels of thyroid stimulating hormone [10] reduced circulating vitamin D levels [11], and elevated levels of urea, creatinine [12], heavy metals [13], and volatile organic compounds [14].

Regarding the hematological parameters, several comparative studies have indicated that white blood cells (WBC), hemoglobin (Hg), and mean corpuscular volume (MCV), are significantly higher in the smokers [15-17]. However, results for other hematological parameters are controversial. For instance, some studies have found that red blood cells (RBC) counts are significantly higher in smokers [18, 19], while others report no significant difference [20, 21]. Thus, the aim of this study was to assess the negative impact of cigarette smoking on the hematological parameters of healthy smokers by comparing their hematological levels with those of non-smokers.

2. Materials and Methods:

This study wasconducted to investigate the effects of smoking on haematological parameters in a group of clinically healthy volunteers including both smokers and non-smokers. Oral consent was obtained from each participant. Approval to conduct the study was obtained from the Libyan Centre for Biotechnology Research in Tripoli, with ethical authorization under reference number NBC: 001. H. 24. 15.

2.1 Subjects:

A total of 290 subjects participated in the study, consisting of 145 smokers and 145 non-smokers, aged between 39 and 45 years. Subjects were recruited from the Medical Analysis Reference Laboratory and Ghadames General Hospital in western Libya during the period from March 2022 to December 2023. Smokers had a history of smoking at least 15 to 25 cigarettes per day for at least 5 years. Subjects included in this study had no evidence of active hepatic or renal disease, chronic pancreatitis, gastrointestinal disease, intestinal inflammation, ischemic heart disease,

history of diastolic blood pressure issues, endocrine disorders, infections, or hormonal therapy.

2.2 Anthropometric measurements:

Anthropometric data (height, weight, and waist circumference) were measured for all subjects. Waist circumference is measured midway between the lowest rib and the iliac crest. BMI is calculated as weight in kilograms divided by height in meters squared.

2.3 Complete blood count:

Blood samples were collected after an overnight fast. Each test was conducted between 8 am and 12 pm.

All samples underwent all of the following tests: haemoglobin (Hb), red blood cells (RBCs), haematocrit (HCT), mean corpuscular Volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBCs), lymphocyte test (LYMP), monocyte and granulocyte.

These tests were carried out using a fully automated Sysmex XN-330 haematology analyser (Sysmex Corporation, Shisumekkusu Kabushiki-gaisha, Kobe, Japan).

2.4 Statistical analysis:

Statistical analysis was performed using Minitab 17 (Minitab Inc., State College, Pennsylvania, USA) and SPSS 26 (SPSS Inc., Chicago, IL, USA). The following tests were conducted: descriptive statistics, including maximum and minimum values, mean, standard deviation (St Dev), and coefficient of variation (CV%) to measure relative dispersion; Two-Way Analysis of Variance (ANOVA), with significance determined at $p < 0.05$; and the effect size of smoking on the study variables, evaluated using eta squared (η^2) and Cohen's d. The interpretations of eta squared were as follows: $\eta^2 < 0.2$ indicates a very small effect size, $0.2 \leq \eta^2 < 0.5$ indicates a small effect size, $0.5 \leq \eta^2 < 0.8$ indicates a medium effect size, and $\eta^2 > 0.8$ indicates a large effect size.

3. Results:

Table (1) shows the descriptive statistics for the study variables in the smokers and non-smokers groups. The mean values for all study variables in smokers were greater than those in the non-smoking sample. The coefficient of variation (CV%) indicated that the smokers' group was more homogeneous (less dispersed) for most variables than the non-smoking group, except for BMI and LYMP, where the coefficient of variation indicated that the smokers' group was less homogeneous (more dispersed) compared to the non-smokers group.

In general, the waist circumference variable for the non-smokers group exhibited the least homogeneity (highest coefficient of variation) compared to all study variables, while the HCT variable for the smokers group showed the most homogeneity (lowest coefficient of variation) among all study variables.

Table (1) Descriptive statistics for study variables for smokers group and non-smokers group.

Variable	Group	Min	Max	Mean	S.Dev	CV %
BMI	Smokers	22.49	28.47	26.03	1.09	4.18
	Non-smokers	22.17	27.11	25.24	0.83	3.28
Waist circumference	Smokers	0.82	0.93	0.87	0.02	2.29
	Non-smokers	0.63	0.97	0.79	0.07	8.86
WBC	Smokers	6.16	8.05	6.85	0.29	4.23
	Non-smokers	4.99	7.10	5.80	0.29	5
LYMP	Smokers	29.99	36.54	34.45	1.08	3.13
	Non-smokers	24.65	30.38	28.65	0.66	2.30
Monocyte	Smokers	4.05	5.19	4.63	0.16	3.46
	Non-smokers	4.04	5.12	4.57	0.19	4.16
Granulocyte	Smokers	50.34	59.27	56.19	1.04	1.85
	Non-smokers	48.72	58.79	53.74	1.74	3.24
RBC	Smokers	4.32	5.01	4.70	0.13	2.77
	Non-smokers	4.23	5.13	4.68	0.17	3.63
HB	Smokers	12.40	15.70	14.06	0.58	4.13
	Non-smokers	11.00	16.00	13.67	1.21	8.85
HCT	Smokers	41.12	42.01	41.61	0.17	0.41
	Non-smokers	33.34	47.41	40.95	3.18	7.77
MCV(fl)	Smokers	85.34	91.00	88.48	0.69	0.78
	Non-smokers	79.70	88.80	83.36	1.49	1.79
MCH(pg)	Smokers	28.49	30.04	29.61	0.28	0.95

	Non-smokers	25.60	31.10	28.43	0.48	1.69
MCHC(g/l)	Smokers	329.72	338.81	334.85	1.45	0.43
	Non-smokers	329.01	350.40	336.11	2.81	0.84

The results of the ANOVA test showed that only in the case of MCHC the non-smokers group had a significantly higher mean ($p < 0.05$). For the other variables, the smokers group had a higher mean, and with the exception of RBC, these means were significantly higher ($p < 0.05$) compared to the non-smokers group. Additionally, there was no statistically significant effect of the age variable or for the interaction between smoking and age on any of the variables.

Table (2) Statistical significance of ANOVA test with effect size

Variable	P	Effect size	
		η_P^2	Cohen's d
WBC	0.000*	0.766	Medium
LYMP	0.000*	0.914	Large
Monocyte	0.005*	0.033	Very Small
Granulocyte	0.000*	0.423	Small
Hb	0.000*	0.400	Small
HCT	0.004*	0.021	Very Small
MCV	0.000*	0.830	Large
MCH	0.000*	0.692	Medium
MCHC	0.000*	0.074	Very Small

*Significant at $p < 0.05$

As shown in table (2), the maximum effect size (highest values for η_P^2) for smoking was on LYMP, while the minimum effect size (lowest value for η_P^2) for smoking was on HCT. The effect size was also large on MCV. The effect size of smoking was medium on both of WBC and MCH, and the effect was higher on WBC compared to the effect on MCH. In addition, the effect of smoking was small on granulocyte and Hb, a slightly higher on granulocyte. Furthermore, smoking had a very small effect on Monocyte and HCT and the order of the effect on these variables was Monocyte > HCT. Finally, for MCHC, the effect was very small, indicating that the mean for non-smokers was higher than that for smokers.

4. Discussion:

During the past three decades, smoking has caused approximately 175 million deaths worldwide. Cigarette smoking remains a major risk factor for

avoidable morbidity and mortality globally, despite a significant decline in smoking prevalence in many nations. In 2021, smoking was responsible for around 142 million years of lost life and accounted for more than one in ten deaths globally [22-24].

Although the various impacts of tobacco cigarette smoking are well established, some connections, including its relationship with hematological indices, are still under discussion. Therefore, this study is important in contributing to the growing pool of literature. Our findings indicated that cigarette smoking significantly increases BMI and waist circumference, along with significantly raising levels of most hematological parameters (i.e., WBCs, LYMP, monocytes, granulocytes, Hb, HCT, MCV, MCH) in healthy smokers compared to healthy non-smokers. Although controversial, routine and heavy cigarette smoking is often associated with higher waist circumference than in non-smokers [25, 26]. Many studies suggest that the haematological parameters are significantly elevated in smokers compared to never-smokers: Hb [16-18, 20, 27-32], HCT [20, 28-31], WBCs [16-18, 20, 28, 30-35], LYMP [16, 25, 30, 32, 33, 35], monocytes [16, 31, 35], granulocyte [16, 35], RBCs [18, 28, 30, 32], MCV [16, 17, 31, 32, 34], MCH [17, 18, 31, 32].

One proposed cause of the high hemoglobin concentration among smokers is carbon monoxide released from cigarette smoke, which binds to hemoglobin to form carboxyhemoglobin (a functionally inactive Hb form), reducing the oxygen-carrying capacity of RBCs and leading to tissue hypoxia. This, in turn, stimulates increased erythropoietin production and secretion, resulting in heightened erythropoiesis [17, 18, 34]. Smokers often develop erythrocytosis or polycythemia (increased RBC count, HCT level, and blood viscosity) due to increased capillary permeability and decreased plasma volume, both caused by carbon monoxide inhalation from cigarettes [17, 18, 28, 30, 36].

Three main RBC parameters assist in measuring the average size and hemoglobin composition of RBCs are MCV, MCH, and MCHC. Our study exhibited significantly higher MCV and MCH levels among smokers compared to non-smokers, while significantly higher MCHC levels were observed among non-smokers compared to smokers. These findings were either consistent with or different from existing literature. One study [17] was consistent with our results, except for insignificant increases in lymphocyte, monocyte, and granulocyte levels among smokers, and insignificant higher MCHC among non-smokers. Another study [35] had significantly higher WBC, granulocyte, monocyte and lymphocyte levels among smokers compared to non-smokers, which were consistent with our results.MCHC findings remain controversial; in our study, MCHC levelswere significantly lower in smokers, which was similar to other studies [37-40], while others reported non-significant lower MCHC values in smokers [17, 33, 34]. Conversely**,** other studies suggest that MCHC levels are higher

among smokers, either significantly [18, 28, 31] or non-significantly [32, 41]. The significantly low MCHC levels among smokers in our study suggests hypochromic anemia, might bedue to lack of folic acid, vitamin B12, or thyroid problems [37, 39, 42, 43].

In our study, all leukocyte counts were significantly higher in smokers compared to non-smokers, which was consistent with other studies, either significantly or non-significantly [16, 17, 34, 35, 38, 39, 41]. However, some studies were not consistent with these findings. Some exhibited higher levels of neutrophils and/or monocytes among non-smokers compared to smokers, either significantly or non-significantly [20, 28, 30-33] and others showed an insignificant increase in either eosinophils [33] or lymphocytes [18, 31], all among non-smokers compared to smokers. One study found no significant increase in WBC count among non-smokers [37] and two studies found contradictory results that showed a significant increase in WBC count among smokers compared to non-smokers [44, 45]. Many studies suggest that nicotine may stimulate hormonal secretion (particularly epinephrine and cortisol) which in turn increases the leukocyte count [17]. Nicotine also decreases the vascular activity, promotes endothelial dysfunction, and causes a clot to develop in the coronary arteries [34]. Moreover, the tobacco smoke has an irritant effect on the respiratory (bronchial) tree,leading to inflammation and production of inflammatory markers (especially cytokines) which results in an increase in leukocyte count [17, 18]. Additionally, it is worth noting that smoking cessation is associated with a decrease in leukocyte count [18]. Some authors claim that tobacco cigarette smoking may have atherogenic effect by increasing the number of leukocytes; they also claim that the leukocyte count may constitute the most useful and cheapest simple biological marker of endothelium damage; the occurrence of high leukocyte count in smokers contributes to pathogenesis of smoking related disorders, particularly ischaemic vascular disease. Thus, the increased leukocyte aggregation contributes to microcirculatory obstruction and vascular damage[17]. Many authors assured that the leukocyte count is a reliable indicator for predicting atherosclerosis and cardiovascular disease; their results point out that high leukocyte count in smokers (particularly among males) suggest that they are at higher risk for developing atherosclerosis and cardiovascular diseases compared to non-smokers [17, 39].

Some studies suggest that there are no significant differences in values of RBCs and HCT between smokers and non-smokers; however, statistically significant higher RBC and HCT values were found in male smokers in relation to female smokers [17]. Moreover, Hb and HCT values were significantly raised in smokers having more than 10 cigarettes a day [29]. Other studies propose that RBCs [20] and total WBC count [20, 46] increase

as intensity of smoking increase (i.e. higher RBC and WBC values in heavy smokers than in moderate and mild smokers).
Most previous studies had small sample size [17, 18, 20, 27, 28, 30, 32, 34, 36] with a sample size less than 100 individuals for each of smokers/non-smokers groups, an issue that has been overcome in our study by recruiting a relatively large number of participants (145 for each group).
One drawback of our study is that we did not include female subjects, as the expected number of female smokers is too small for this type of studies. In fact, females in Libya are less willing to volunteer for such studies due to cultural, traditional and social constraints.

5. Conclusion

The findings of our study reveal that cigarette smoking has a significant impact on haematological parameters; Hb level, WBC count, lymphocytes, monocytes, HCT, MCV, and MCH of healthy smokers were all notably higher (P-value < 0.05) than those of the healthy non-smokers. Consequently, this study adds to the growing body of literature evidence that tobacco cigarette smoking has severe adverse effects (both acute and chronic) on most haematological parameters.Similar studies are needed to investigate the effect of smoking cigarettes on other biochemical parameters such as kidney and liver biomarkers.

6. Conflict of interest:

None declared.

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