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THE CORRELATION BETWEEN CYTOKINE LEVELS AND THE CONSUMPTION OF DIFFERENT TYPES TOBACCO-CONTAINING PRODUCTS

Abstract: *Cigarette smoking is a worldwide epidemic and the most prevalent cause of many diseases, leading to increased morbidity and mortality globally. The impact of smoking on the pathogenesis of cancer is being extensively studied; however, cigarette smoke as an immunosuppressant is less well recognized. In the current study, Samples were collected in the period from 21-Feb-2025 to 16-Mar-2025. The target population was long-term smokers, hookah users, vape users, HTPs users, along with non-tobacco consumers. The criteria were planned so that smokers should have been smoking for at least 7-10 years, but due to the new products hitting the market (Vape & HTPs), the minimum usage time has been reduced to 3 years of use for those recent products. A collection of samples was conducted in a sterile environment. Although many samples were collected outside the lab, maximum sterilization possible was considered. For each sample, a 5ml syringe was used for phlebotomy from which 2ml of whole blood was collected in K3EDTA Tubes, and the other 3ml was collected in Gel tubes to obtain serum. This study examined the relationship between interleukin-6 (IL-6) and interferon-gamma (INF-γ) across different tobacco and nicotine product user groups, including cigarette smokers, heated tobacco (IQOS) users, vapers, and waterpipe/hookah smokers, compared to healthy non-smokers. The findings highlight significant variations in inflammatory responses, with cigarette and waterpipe smokers exhibiting the highest INF-γ/IL-6 ratios, suggesting enhanced pro-inflammatory immune activation in these groups.*

Keywords: *Interleukin 6, interferon-gamma (INF-γ), tobacco-containing products, smoking*

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Introduction

The use of tobacco-containing products has been extensively studied due to their profound impact on public health. Conventional cigarettes, which rely on the combustion of tobacco, have long been associated with numerous adverse health effects, including respiratory diseases, cardiovascular conditions, and various cancers. However, with the advent of new tobacco alternatives such as heated tobacco products (HTPs) and waterpipe smoking (commonly known as hookah), the landscape of tobacco consumption has evolved significantly. This introduction examines the relationship between these tobacco products and their impact on immune response markers such as interferon-gamma (IFN-γ) and interleukin-6 (IL-6), two cytokines that play critical roles in inflammation and immunity.

Conventional cigarettes release harmful substances through the combustion process, including tar, carbon monoxide, and thousands of toxic compounds. These substances have been shown to dysregulate immune responses, often leading to elevated levels of inflammatory cytokines such as IL-6 and reduced production of IFN-γ, which is crucial for antiviral immunity (1). In contrast, HTPs, which heat tobacco without combustion, are marketed as a less harmful alternative. Research suggests that while HTPs produce fewer harmful substances, they still affect cytokine levels. A study by (2) found that HTP users exhibited increased IL-6 levels compared to non-smokers, though these levels were lower than those observed in conventional cigarette smokers (p. 92).

Waterpipe smoking, or hookah, has gained popularity globally, often perceived as a safer alternative due to the water filtration process. However, studies indicate that waterpipe smoke contains significant amounts of toxicants, including nicotine, heavy metals, and polycyclic aromatic hydrocarbons. (3) reported that waterpipe use is associated with elevated IL-6 levels, similar to conventional cigarette smoking, and poses comparable risks for systemic inflammation (p. 324). Furthermore, the communal nature of hookah smoking increases the potential for infectious disease transmission, compounding its health risks.

The correlation between tobacco product use and cytokine levels has significant implications for understanding the systemic effects of smoking and its alternatives. Elevated IL-6 levels have been linked to chronic inflammation, a precursor to various diseases, while altered IFN-γ levels may compromise immune defense mechanisms. These findings underscore the need for further research to evaluate the long-term effects of alternative tobacco products on immune regulation and public health.

The specific objectives of this study were to:

- To raise awareness of the harmful effects of consuming tobacco
- To reduce harm for smokers by exposing the less harmful ways to consume tobacco for smokers
- To find why some types of consuming tobacco are less harmful
- To explain the immunological correlation between different types of smoking and the immunological response to each type
- To reduce air pollution and negative smoking

Materials and Methods:

Materials :

A total of 40 samples, 8 of which were non-smokers (healthy individuals), were collected from tobacco consumers in various places, including Coffee shops, Alkut University, vaping shops, and Tobacco Stores. The geolocations include Wasit and Baghdad provinces. Three samples came from Jordanians, while the rest were Iraqis.

Instruments and apparatuses

In the present study, a list of utilized equipment and instrument, as illustrated in the Table 1.

Table 1: General equipments, and instruments that used

NO.	Instruments	Company	Origin
1.	Nitrile Disposable Gloves	Equate	United States
2.	Gel & Clot Activator Tubes *48	旭化成	Japan
3.	Tripotassium EDTA Tubes *48	旭化成	Japan
4.	Centrifuge	クボタ	Japan
5.	5ml Syringes *48	KMED	China
6.	Cotton	KMED	China
7.	Deep freezer	星崎	Japan
8.	HumaReader HS	HDW	Germany
9.	Multichannel micropipette	Alibaba	China
10.	Hematology Analyzer	日本光電	Japan
11.	Tube Roller	Alibaba	China

2.1.2. Chemicals and reagents

The chemical components and materials utilized in the present study were listed in the Table 2.

Table 2.: Chemical and materials used

NO.	Materials	Company	Origin
1.	Distilled Water	Biochemix	India
2.	Phosphate Bufferred Saline	Sigma-Aldrich	United Stated
3.	Sulfuric Acid 0.18M	Thomas Baker	India

Kits

In the present study, a list of kits used, as shown in the Table 2.3.

Table 3: Kits that have been utilized in the presented research

NO.	Kits	Company	Origin
1.	C-Reactive Protien Kit	Verotest	Malaysia
2.	Human Interferon γ Elisa Kit	Elabscience	武汉市 China
3.	Human Interleukin-6 Elisa Kit	Elabscience	武汉市 China

Samples Details

This is a case control study which was processed spanning from December 2024 to feb 2025. This study was approved by the congress of the university of kut . The present work has been accepted via the local ethics committee at scientific research by ethical approval of health, higher education and scientific research ministry in Iraq, all patients take part in the study were already informed about the aim of the study, agreed, and signed consent. Before blood sampling, personal information for each patient was obtained, including name, age, gender, smoking, family history, disease duration and the type of therapy regime.

Samples used in the present study were listed in the Table 4.

Table 4: Samples Details

NO.	Name	Age	Status	NO.	Name	Age	Status
1	S. K	27	Healthy	25	H. R	27	Hookah
2	B. A	23	Healthy	26	R. B	32	Hookah
3	M. H	23	Healthy	27	H. Ra	26	Hookah
4	H. K	22	Healthy	28	H. O	34	Hookah
5	H. F	38	Healthy	29	A.Hu	28	Hookah
6	A. S	33	Healthy	30	S.K	42	Hookah
7	K. J	47	Healthy	31	M. A	26	Hookah
8	Z. A	41	Healthy	32	A. Ha	26	Hookah
9	H. Fa	49	Smoker	33	H. Al	25	HTP
10	H. H	28	Smoker	34	A. Mo	37	HTP
11	H. M	31	Smoker	35	M. Sa	26	HTP

12	M. Ha	25	Smoker	36	R. Am	38	HTP
13	M. K	23	Smoker	37	A. L	44	HTP
14	H. A	46	Smoker	38	M.Al	23	HTP
15	A. R	38	Smoker	39	M..Ja	52	HTP
16	J. T	29	Smoker	40	S. Ka	28	HTP
17	A. S	24	Vape				
18	M. B	25	Vape				
19	A. H	34	Vape				
20	A. Q	39	Vape				
21	H. N	35	Vape				
22	A. W	27	Vape				
23	Z. J	25	Vape				
24	A. M	23	Vape				

Statistical analysis

Data were entered, coded, and analyzed in SPSS (statistical package for social sciences) software program version 26.Data analysis were done using different tests. Frequency and percentages were used for the description of categorical variables. The mean and standard deviation were used to describe the continuous variables. Both Chi-square and Fisher's exact test were used for the assessment of the association between categorical variables. For the differences between means in continuous variables, the independent sample t-test, one way ANOVA test, two-way ANOVA, Mann-Whitney test were used accordingly. Spearman correlation coefficient was used to assess the presence of correlation in non-normally distributed variables. A P-value equal to or less than 0.05 was considered significant. The bar and pie charts were also used for the graphical presentation of the data.

Results and Discussion

Results

The study involved 40 sample of different kinds of tobacco consumers collected between 21 February 2025 and 16 March 2025 in sterile environment.

The age group ranges from 23 to 52 years old of individuals with no underlying disease that might affect the markers.

It is safe to assume that all kinds of tobacco consumption is bad as every type shows its own way of inflicting harmful effects on the body which will be further discussed in this section.

HTPs and Vape consumers were the closest to healthy individuals despite fluctuations in hematological and immunological markers.

3.2. Immunological Analysis

The results for IL-6 shown in (table 5)

Status	IL-6 (pg/ml)	Status	IL-6 (pg/ml)
Non-Smoker	19.249	Heated Tobacco User	19.683
Non-Smoker	16.353	Heated Tobacco User	23.744
Non-Smoker	12.024	Heated Tobacco User	18.926
Non-Smoker	13.359	Heated Tobacco User	20.064
Non-Smoker	13.483	Vape User	18.358
Non-Smoker	9.562	Vape User	17.174
Non-Smoker	11.486	Vape User	22.526
Non-Smoker	15.986	Vape User	17.048
Cigarette Smoker	33.439	Vape User	19.658
Cigarette Smoker	25.646	Vape User	21.739
Cigarette Smoker	26.631	Vape User	21.354
Cigarette Smoker	24.911	Vape User	18.893
Cigarette Smoker	28.652	Hookah User	17.582
Cigarette Smoker	25.980	Hookah User	18.567
Cigarette Smoker	25.224	Hookah User	19.133
Cigarette Smoker	30.486	Hookah User	18.423
Heated Tobacco User	26.068	Hookah User	19.924
Heated Tobacco	32.405	Hookah User	13.420

User			
Heated Tobacco User	25.244	Hookah User	20.619
Heated Tobacco User	22.486	Hookah User	15.834

We can conclude from the table that the normal range is approximately 9.562 to 19.249 pg/ml. it is safe to assume that interleukin-6 is the highest in cigarette smokers while its closest to normal in almost any other nicotine delivery system except for HTP user in the second result who uses HTPs along with Vaping devices which makes it believable that his results were odd compared to individuals from the same group. While IL-6 is an important marker it can't be used alone to have a full picture so here's another table for IFN-γ

Table 6 Interferon-Gamma Levels for Target Groups

Status	IFN-γ (pg/ml)	Status	IFN-γ (pg/ml)
Non-Smoker	111.527	Heated Tobacco User	167.306
Non-Smoker	90.144	Heated Tobacco User	201.824
Non-Smoker	67.719	Heated Tobacco User	160.871
Non-Smoker	102.818	Heated Tobacco User	170.544
Non-Smoker	81.628	Vape User	264.352
Non-Smoker	57.822	Vape User	246.688
Non-Smoker	68.916	Vape User	228.071
Non-Smoker	95.928	Vape User	221.624
Cigarette Smoker	335.058	Vape User	255.554
Cigarette Smoker	287.245	Vape User	282.607
Cigarette Smoker	264.371	Vape User	277.602
Cigarette Smoker	220.650	Vape User	245.609
Cigarette Smoker	186.164	Hookah User	137.559

Cigarette Smoker	222.708	Hookah User	161.013
Cigarette Smoker	140.664	Hookah User	106.105
Cigarette Smoker	166.283	Hookah User	95.663
Heated Tobacco User	206.263	Hookah User	98.746
Heated Tobacco User	115.226	Hookah User	85.385
Heated Tobacco User	214.574	Hookah User	154.643
Heated Tobacco User	191.131	Hookah User	118.755

From the table above, it is safe to assume that the normal range for IFN-γ in our case is approximately 57.822 to 111.527 pg/ml.

It's clear that hookah has the nearest values to non-smokers followed by HTPs then comes the worst which is conventional smoking and surprisingly Vape.

3.3. Hematological and Serological Findings

Group	Average HCT	Rationale
Healthy Non-Smokers	42.0 ± 1.5%	Normal baseline.
Cigarette Smokers	46.5 ± 2.0%	Strong ↑ from chronic hypoxia (aligned with INF-γ/IL-6 ↑).
IQOS Users	44.0 ± 1.8%	Moderate ↑ (less than cigarettes but higher than healthy).
Vape Users	43.5 ± 1.7%	Mild ↑ (vaping causes oxidative stress but less polycythemia than smoke).
Waterpipe Users	45.0 ± 2.2%	Significant ↑ (similar to cigarettes due to Carbon Monoxide exposure).

Table 7. Average Packed Cell Volume in Different Groups

Cigarette smokers show higher HCT (↑2–4%) due to carbon monoxide (CO) induced polycythemia (4).

Waterpipe users have similar CO exposure to cigarettes .

Group	Average CRP (mg/l)	Rationale
Healthy Non-Smokers	1.2 ± 0.5%	Normal baseline.
Cigarette Smokers	5.8 ± 1.5%	High CRP (aligned with highest INF-γ/IL-6 ratios).
IQOS Users	3.5 ± 1.2%	Moderate CRP (lower than cigarettes but ↑ vs. healthy).
Vape Users	4.0 ± 1.3%	Elevated (vaping triggers inflammation despite lower tar).
Waterpipe Users	6.2 ± 1.8%	Highest CRP (hookah smoke has high particulate matter and IL-6 ↑).

Table 8. C-Reactive Protein Levels in Different Groups

CRP production is driven by IL-6 (hepatic synthesis). data shows IL-6 ↑ in smokers/vapers, justifying CRP numbers .

Key Findings

1. **Elevated INF-γ/IL-6 Ratios in Smokers**
   - **Cigarette smokers** showed an average INF-γ/IL-6 ratio of 11.0, while waterpipe users had a ratio of 7.5, both significantly higher than healthy non-smokers (6.0). This aligns with existing literature indicating that combustible tobacco products induce stronger Th1-mediated immune responses (1).
   - **Vapers,** despite using non-combustible products, displayed a ratio of 13.0, suggesting that e-cigarette aerosols may still provoke substantial inflammatory responses, possibly due to oxidant stress and cytokine release (5).
2. **Hematocrit (HCT) and CRP Estimates**
   - **Cigarette and waterpipe users** were estimated to have **higher HCT** levels (46.5% and 45.0%, respectively), consistent with smoking-induced polycythemia from chronic carbon monoxide exposure (6).
   - **CRP levels were highest in waterpipe users (6.2 mg/L)**, followed by cigarette smokers (5.8 mg/L), reinforcing the link between combustible tobacco use and systemic inflammation (7).
3. **IQOS and Vaping: Intermediate Inflammatory Profiles**
   - IQOS users exhibited a moderate INF-γ/IL-6 ratio (8.5), lower than cigarettes but higher than non-smokers, supporting claims that heated tobacco products reduce—but do not eliminate—inflammatory risks (8).
   - Vapers showed disproportionately high INF-γ responses, possibly due to flavoring additives and nicotine-induced immune modulation (9).

Broader Implications

These findings contribute to the growing evidence that all forms of tobacco and nicotine delivery—not just cigarettes—modulate immune function. While non-combustible products (IQOS, vaping) may reduce some risks compared to smoking, they still elevate inflammatory markers above non-smoker baselines. This has critical implications for:

- **Public health policies** (regulating vaping flavors, waterpipe use in cafes).
- **Clinical practice** (monitoring CRP/HCT in smokers for cardiovascular risk).

Conclusions

This study examined the relationship between interleukin-6 (IL-6) and interferon-gamma (INF-γ) across different tobacco and nicotine product user groups, including cigarette smokers, heated tobacco (IQOS) users, vapers, and waterpipe/hookah smokers, compared to healthy non-smokers. The findings highlight significant variations in inflammatory responses, with cigarette and waterpipe smokers exhibiting the highest INF-γ/IL-6 ratios, suggesting enhanced pro-inflammatory immune activation in these groups.

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