

PROLACTIN, ESTROGEN, HbA1C AND INTERLEUKIN-12 IN IRAQI SMOKERS SINGLE FEMALES



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BACKGROUND: Smoking disrupts the endocrine system, altering levels of key hormones such as estrogen (E2) and prolactin (PRL). This makes the relationship between smoking and sex hormones an important research topic. IL-12 is a crucial cytokine that enhances immune responses, particularly in promoting T-cell differentiation.

AIM: This study aims to assess the impact of smoking on immune function by analyzing the reduction of pro-inflammatory cytokines (e.g., IL-12) and determining whether hormonal differences exacerbate this effect in females.

MATERIALS AND METHODS: Ninety serum samples were collected from healthy single females, age range (20–29) years old. The samples were divided into three groups: smokers (n=30), secondhand smokers (individuals exposed to tobacco smoke in their environment) (n=30), and non-smokers (control group, n=30). The female smokers were subdivided into 2 sub-groups: the first is based on the rate of smoking per day into heavy and light smokers, and the second group is the duration of smoking years (<4 years) and (≥4 years).

RESULTS: The findings revealed notable variations in the levels of IL-12, PRL, and HbA1c across all the studied groups (p-value <0.05). While, for E2, the (Median=92.68) in the smokers is lower than the control and secondhand smokers groups, the difference is non-significant.

CONCLUSION: Our findings suggest that heavy smoking adversely affects both metabolic health (increases HbA1c level) and hormonal balance in women. Also, smoking does decrease IL-12 levels, which can impair immune responses and affect overall health. While the durations of smoking (short-term vs. long-term) did not lead to notable changes in the outcomes being measured.

KEYWORDS: IL-12; PRL; E2; HbA1c; smoking

ПРОЛАКТИН, ЭСТРОГЕН, HbA1c И ИНТЕРЛЕЙКИН-12 У НЕЗАМУЖНИХ КУРЯЩИХ ЖЕНЩИН ИРАКА

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ОБОСНОВАНИЕ. Курение нарушает эндокринную систему, изменяя уровни ключевых гормонов, таких как эстроген (E2) и пролактин (PRL). Взаимосвязь между курением и половыми гормонами является важной темой исследования. IL-12 — важный цитокин, усиливающий иммунные ответы, особенно способствуя дифференцировке Т-лимфоцитов.

ЦЕЛЬ. Оценить влияние курения на функцию иммунной системы путем анализа снижения провоспалительных цитокинов (например, IL-12) и определить, усугубляют ли гормональные различия этот эффект у женщин.

МАТЕРИАЛЫ И МЕТОДЫ. Было собрано 90 образцов сыворотки от здоровых незамужних женщин в возрасте 20–29 лет. Образцы разделили на три группы: курящие (n=30), подвергшиеся пассивному (вторичному) курению (лица, подвергающиеся воздействию табачного дыма в окружающей среде) (n=30) и некурящие (контрольная группа, n=30). Курящие женщины были дополнительно разделены на 2 подгруппы: по интенсивности курения в сутки — тяжелые и легкие курильщицы; и по длительности курения — <4 лет и ≥4 лет.

РЕЗУЛЬТАТЫ: Выявлены значимые различия в уровнях IL-12, PRL и HbA_{1c} между исследуемыми группами (p<0,05). Что касается E2, медиана в группе курящих (медиана=92,68) ниже, чем в контрольной и группе пассивного курения, однако разница статистически незначима.

ВЫВОДЫ: Результаты свидетельствуют, что тяжелое курение негативно влияет как на метаболическое здоровье (повышает уровень HbA_{1c}), так и на гормональный баланс у женщин. Кроме того, курение снижает уровень IL-12, что может ослаблять иммунные ответы и отрицательно влиять на общее состояние здоровья. Длительность курения (короткий срок vs. длительный) существенно не изменяла изучаемые показатели.

КЛЮЧЕВЫЕ СЛОВА: IL-12; PRL; E2; HbA1c; курение

BACKGROUND

The relationships between smoking and circulating sex hormones in premenopausal women have not been thoroughly investigated [1]. Although factors like demographics, alcohol intake, and physical activity, have been linked to levels of endogenous sex hormones in both premenopausal and postmenopausal groups, the evidence regarding the effect of smoking on sex-steroid hormone levels has been less consistent [2, 3].

Cigarette smoking, a common addictive behavior and lifestyle choice in modern culture, involves inhaling and exhaling smoke from burning tobacco and other substances in cigarettes [4]. Health consequences of smoking include conditions like peptic ulcer and periodontitis, also, several types of cancers including those of the lung and bronchial tubes (moreover, larynx, oral, and bladder cancer). Also smoking can cause stroke, cataracts, atherosclerosis, emphysema, asthma, coronary and myocardial infarction, leukemia, infertility in both women and men [5].

Smoking remains the primary cause of higher mortality rate and is associated with numerous diseases, including those related to reproductive issues, with its prevalence driven by growing social and economic pressures [6]. In the United States, Smoking accounts for 140,000 premature deaths from cardiovascular disease (CVD) each year, making up about 30% of all deaths associated with smoking [7].

Smoking affects ovarian endocrine function and is associated with an earlier onset of menopause. Research on in vitro fertilization cycles has indicated that cigarette smoking significantly diminishes ovarian reserve and results in less response to ovarian stimulation at a younger age [8]. Additionally, smoking during pregnancy has been linked to harmful changes in the placenta and fetus, with pregnant smokers experiencing higher rates of low birth weight and perinatal and neonatal mortality [9]. However, as far as we know, the impact of smoking on human endometrium is still not well understood.

Chronic smoking disrupts the balance of the endocrine system, leading to elevated levels of cortisol, growth hormone, sex hormone-binding globulin, and prolactin (PRL). This disruption can impair fertilization in both men and women [10].

Acute cigarette smoking leads to a significant rise in PRL secretion, with this increase correlating with elevated plasma nicotine concentrations [11]. In contrast, smoking nicotine-free cigarettes does not result in changes to PRL levels [11]. Notable increases in PRL levels have been observed following opioid blockade [12]. However, this response is markedly weaker in smokers than in non-smokers, as dopamine typically inhibits PRL secretion, and opioids enhance dopamine release, which further suppresses PRL levels. This suggests that smokers may exhibit a diminished response to opioid-induced dopamine release or experience disruption in the interaction between dopamine and PRL [12].

Previous research has indicated that the levels of placental estriol, human placental lactogen (HPL), and β -human chorionic gonadotropin (β -HCG) are inversely correlated with the daily number of cigarettes smoked [13]. These alterations may be significant, as these placental hormones can impact fetal brain development.

Smoking not only weakens the beneficial effects of estrogen (E2) on hot flushes, urogenital symptoms and lipid metabolism, but it may also impair estrogen's protective role against osteoporosis and possibly cardiovascular diseases. This is mainly due to dose-dependent increases in liver clearance, which are linked to lower E2 levels, a pattern observed primarily with oral E2 administration [14]. It is not advisable to increase the dose in smokers to counteract the reduced therapeutic efficacy, as this may result in the production of potentially mutagenic E2 metabolites, which would raise the risk of breast cancer. Since the beneficial effects of E2 appear to remain intact when administered transdermally in smokers, this method is recommended for them [14].

Cigarette exposure exacerbates insulin resistance [15], even with secondhand exposure contributing to this effect in a dose-dependent manner, thereby increasing the likelihood of developing metabolic syndrome [15]. On the other hand, quitting smoking may also induce changes that promote insulin resistance, depending on the fluctuations in body weight [16]. Additionally, smoking leads to an increase in visceral fat independent of changes in BMI, which helps explain the paradoxical increase in metabolic risk associated with visceral adiposity, despite overall weight loss [17].

Tobacco smoking can lead to autoimmune and inflammatory through various mechanisms. These include changes in genes and their expression, increased oxidative stress, and the production of free radicals [18, 19]. As a result, smoking leads to an increase in the proliferation of T and B cells, a decrease in the function of immune-suppressing T regulatory (Treg) cells, and elevated levels of pro-inflammatory substances like IL-1 beta, IL-8, IL-6, and tumor necrosis factors. Additionally, smoking enhances the expression of chemotactic cytokines [20, 21, 22].

Inflammatory cytokines, such as Interleukins and chemokines play a vital role in the communication, regulation, and activation of immune cells, as well as for mediating inflammatory responses. These processes are profoundly influenced by exposure to tobacco smoke. Previous and existing researches have concentrated on the link between tobacco smoking and various diseases. For instance, smoking is recognized for modifying the concentration and activation of particular white blood cells, such as leukocytes, leading to increased levels of inflammatory markers like C-reactive protein (CRP) and IL-6 [23]. However, further investigation is needed to understand the changes in these cytokines in serum following smoking cessation.

IL-12, primarily produced by dendritic cells, monocytes, macrophages, and B cells, can activate various immune cell subsets that recognize and destroy cancer cells, and enhance immunity during the cancer immunity cycle [24]. This pro-inflammatory cytokine interleukin (IL)-12 is essential for initiating and progressing of the Th-1 response, which is marked by the production of interferon-gamma (IFN- γ) by different T cells and natural killer cells [25]. IL-12 is released early in the immune response and is essential for regulating both innate and adaptive immunity, in addition to playing mediating anti-tumor activity [26, 27].

RESEARCH AIM

Building on our previous findings of reduced IL-12 levels in smokers, the current study aims to examine the impact of cigarette smoke on IL-12, PRL, E2, and HbA1c levels in healthy single female smokers. The reason for selecting the «single females» in this study is to minimize confounding from spouse smoking. Married women may have household exposure, making it difficult to distinguish between secondhand and active exposure. Focusing on single women allows us to study secondhand smoking from environmental sources like family, workplaces, and public area.

MATERIALS AND METHODS

Place and period of the research

Place of the research. National Diabetes Center laboratories, Mustansiriyah University.

Period of the research. From (September to October) 2024.

Populations under study (one or more)

Population "90": the first 30 females were smokers, the second 30 females were secondhand smokers "non-smokers but living with a smoker persons" and the third group was 30 female non-smokers (control group). The first group (smoker females) was subdivided into two sub-groups: the first based on the rate of smoking per day into heavy and light smokers, the second group based on the number of smoking years (<4 years) and (≥ 4 years).

Inclusion criteria: Single Females, age range were (20–29) years old.

Exclusion criteria: Married Females, Not Healthy, having Diabetes.

Study design and patients

A Randomized case-control study (The term 'randomized' was inaccurately used, as the study followed a case-control design without a formal randomization process) was performed with ninety serum samples obtained from healthy single females within the age range 20 to 29 years, attending to the NDC from (August to October 2024). The significant influence of menstrual cycle regularity, cycle phase, oral contraceptive, and pregnancy on PRL levels is acknowledgeable. To minimize hormonal variability, the study exclusively included non-pregnant women who were not using contraceptives. Additionally, menstrual cycle regularity was considered in participants selection to insure consistency in hormonal profile. The samples were divided into three main groups, the first 30 females were smokers, the second 30 females were Secondhand Smokers "non-smokers but living with a smoker persons" and the third group was 30 female non-smokers (control group). The first group (smoker females) was subdivided into two sub-groups: the first based on the rate of smoking per day into heavy and light smokers, the second group based on the number of smoking years (<4 years) and (≥ 4 years). Our data collection focused on smoking duration rather than the exact number of cigarettes smoked per day.

Methods

Five milliliters of venous blood was drawn by using a disposable syringe (5cc) from all participants, (2ml)

into an EDTA tube for the HbA1c test, 3ml into a gel tube and centrifuge for ten minutes at 3000 rpm for serum obtaining and divided into two Eppendorf tubes one for the measurement of hormone immediately and the other storage at -20 C degree until IL-12 measurement. The level of serum IL-12 in the patients and control groups was determined by using the MyBioSource (USA) enzyme-linked immunosorbent assay (ELISA) kit and following the manufacturer's instructions. Body Mass Index (BMI) was calculated by using the equation $BMI = \text{weight (Kg)} / \text{height (m}^2\text{)}$. The levels for HbA1c were measured by using (HbA1c kit-Germany) and (Cobas c111) device, while the levels of both E2 (Estradiol III kit-Germany) and PRL (Elecsys Prolactin kit-Germany) were measured in (ng/ml) by using (Cobas e411) device.

Statistical analysis

Statistical data were analyzed with the Statistical Package for the Social Sciences (SPSS) version 26.0 software (SPSS Inc, Chicago, IL, USA). For evaluating the significance levels between the three groups in this study Kruskal-Wallis H test was used, while the Mann-Whitney U test was used to calculate the level of significance between the two group's patients and control. Results are presented as median, minimum, and maximum. Spearman test was used for correlation. A result was considered statistically significant if the p-value was below 0.05.

Ethics review

Before the commencement of this trial, all patients provided their written and dated consent for their participation. Furthermore, this study received ethical approval from the ethics committee of the National Diabetes Center at Mustansiriyah University in (September 2024), Ensuring adherence to the guidelines set forth in the 1964 Declaration of Helsinki, along with any subsequent revisions or similar ethical standards.

RESULTS

Ninety samples were collected from healthy single females aged 20 to 29 years. The samples were categorized into three primary groups: first group consisted of control (non-smokers), second group included secondhand smokers (non-smokers living with smokers), and the third group comprised active smokers. The latter group was further subdivided into two categories based on daily smoking rates: heavy smokers and light smokers. Additionally, a second subdivision was made according to the duration of smoking, distinguishing between those with 2 to 3 years and those with 4 to 6 years of smoking experience. The results indicated significant differences in the levels of IL-12, PRL, and HbA1c across all groups studied ($p\text{-value} < 0.05$). Detailed statistics, including median values, interquartile, and p-values of the study parameters between all the groups are presented in Table 1.

In the correlation results between study parameters in the control group and secondhand smokers group, only E2 and PRL showed a significant correlation with ($p=0.000$) (Table 2, 3), while in the smoker's group, BMI showed a significant correlation with HbA1c, and E2 with PRL $p=(0.032, 0.000)$ respectively (Table 4).

Table 1. Median, interquartile, and p-values of the study parameters between all the groups

Metabolites	Controls (Median, Min-Max)	Secondhand smokers (Median, Min-Max)	Smokers (Median, Min-Max)	P-value
BMI	22.1 (19.5–23.7)	22.7 (20.1–23.7)	22.1 (20.5–23.8)	0.920
IL-12 (pg/ml)	272.9 (217.7–330.9)	241.6 (186.4–320.5)	200.9 (182.7–275.3)	0.011
E2 (pg/ml)	98.2 (68.9–244.8)	109.4 (98.7–126.9)	92.7 (78.2–128.9)	0.153
PRL (ng/ml)	20.3 (9.53–37.3)	15.7 (9.58–22.1)	8.5 (7.35–29.6)	0.015
HbA1c %	4.5 (4.30–4.93)	5.15 (4.50–5.63)	5.90 (5.30–6.23)	0.000

Note: *significant at the 0.05 level

BMI — body mass index; IL-12 — interleukin 12; E2 — estradiol; PRL — prolactin; HbA1c — glycated hemoglobin.

Table 2. Correlation results between study parameters in the control group

		BMI	IL-12	E2	PRL	HbA1c
BMI	Correlation Coefficient	1.000	0.150	0.192	0.194	0.118
	Sig. (2-tailed)		0.430	0.310	0.304	0.535
IL-12	Correlation Coefficient	0.150	1.000	0.044	0.127	0.118
	Sig. (2-tailed)	0.430		0.818	0.505	0.535
E2	Correlation Coefficient	0.192	0.044	1.000	.862**	0.075
	Sig. (2-tailed)	0.310	0.818		0.000	0.695
PRL	Correlation Coefficient	0.194	0.127	.862**	1.000	0.037
	Sig. (2-tailed)	0.304	0.505	0.000		0.846
HbA1c	Correlation Coefficient	0.118	0.118	0.075	0.037	1.000
	Sig. (2-tailed)	0.535	0.535	0.695	0.846	

Note: **. Correlation is significant at the 0.01 level (2-tailed).

BMI — body mass index; IL-12 — interleukin 12; E2 — estradiol; PRL — prolactin; HbA1c — glycated hemoglobin.

Table 3. Correlation results between study parameters in secondhand smokers group

		BMI	IL-12	E2	PRL	HbA1c
BMI	Correlation Coefficient	1.000	0.123	-0.112	-0.170	-0.299
	Sig. (2-tailed)		0.516	0.555	0.368	0.108
IL-12	Correlation Coefficient	0.123	1.000	0.266	0.246	-0.047
	Sig. (2-tailed)	0.516		0.155	0.189	0.804
E2	Correlation Coefficient	-0.112	0.266	1.000	.971**	0.042
	Sig. (2-tailed)	0.555	0.155		0.000	0.824
PRL	Correlation Coefficient	-0.170	0.246	.971**	1.000	0.064
	Sig. (2-tailed)	0.368	0.189	0.000		0.738
HbA1c	Correlation Coefficient	-0.299	-0.047	0.042	0.064	1.000
	Sig. (2-tailed)	0.108	0.804	0.824	0.738	

Note: **. Correlation is significant at the 0.01 level (2-tailed).

BMI — body mass index; IL-12 — interleukin 12; E2 — estradiol; PRL — prolactin; HbA1c — glycated hemoglobin.

Table 4. Correlation results between study parameters in the smokers group

		BMI	IL-12	E2	PRL	HbA1c
BMI	Correlation Coefficient	1.000	-0.097	0.172	0.122	.392*
	Sig. (2-tailed)		0.608	0.363	0.521	0.032
IL-12	Correlation Coefficient	-0.097	1.000	0.032	0.123	-0.125
	Sig. (2-tailed)	0.608		0.866	0.516	0.509
E2	Correlation Coefficient	0.172	0.032	1.000	.932**	0.181
	Sig. (2-tailed)	0.363	0.866		0.000	0.338
PRL	Correlation Coefficient	0.122	0.123	.932**	1.000	0.154
	Sig. (2-tailed)	0.521	0.516	0.000		0.417
HbA1c	Correlation Coefficient	.392*	-0.125	0.181	0.154	1.000
	Sig. (2-tailed)	0.032	0.509	0.338	0.417	

Note: *. Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

BMI — body mass index; IL-12 — interleukin 12; E2 — estradiol; PRL — prolactin; HbA1c — glycated hemoglobin.

Table 5. Median, interquartile, and P-values of the study parameters in the smokers group (heavy and light).

Metabolites	Heavy smokers (Median, Min-Max)	Light smokers (Median, Min-Max)	P-value
BMI	21.4 (20.3–23.2)	23.6 (21.0–24.0)	0.065
IL-12	222.0 (183.7–297.6)	200.3 (173.1–255.8)	0.351
E2	84.2 (77.7–93.2)	128.7 (84.6–149.2)	0.007
PRL	8.10 (6.50–8.60)	11.8 (8.4–33.1)	0.014
HbA1c	6.2 (5.6–6.4)	5.5 (4.8–6.0)	0.006

Note: *significant at the 0.05 level. BMI — body mass index; IL-12 — interleukin 12; E2 — estradiol; PRL — prolactin; HbA1c — glycated hemoglobin.

Table 6. Median, interquartile, and P-values of the study parameters in the smokers group (> 4 and ≥4).

Metabolites	< 4 (Median, Min-Max)	≥4 (Median, Min-Max)	P-value
BMI	21.6 (20.3–23.9)	22.4 (20.9–23.7)	0.74
IL-12	238.7 (191.3–320.6)	192.8 (181.8–241.0)	0.141
E2	84.6 (78.1–98.7)	96.6 (78.2–140.4)	0.330
PRL	8.3 (7.3–11.0)	9.1 (7.1–37.9)	0.443
HbA1c	5.9 (5.5–6.3)	5.6 (5.1–6.2)	

Note: *significant at the 0.05 level. BMI — body mass index; IL-12 — interleukin 12; E2 — estradiol; PRL — prolactin; HbA1c — glycated hemoglobin.

In the first division of the smoker subgroup, which categorized participants as heavy and light smokers, the levels of E2, PRL, and HbA1c exhibited significant differences, with p-values of 0.007, 0.014, and 0.006, respectively (see Table 5). Conversely, in the second division, which was based on the duration of smoking, no significant differences were observed among the study parameters (refer to Table 6).

DISCUSSION

Despite that, the p-value for E2 levels in all of the study groups showed non-significant differences (>0.05), but the (Median=92.68) in the smokers group is lower than the control and secondhand smokers groups (Table 1). Cigarette smoke contains numerous harmful chemicals that can disrupt endocrine function, potentially altering estrogen metabolism and production, and leading to lower E2 levels. While this study did not find significant differences

in E2 levels, other research suggests that smoking can lead to lower E2 levels [14], which may increase the risk of reproductive issues, osteoporosis, cardiovascular diseases, and other health problems. Smoking may accelerate the metabolism of estrogens in the liver. Enzymes induced by tobacco constituents can lead to faster breakdown of hormones, potentially lowering circulating levels [28].

The relationship between PRL and E2 is complex and can be bidirectional, meaning that changes in one hormone can influence the levels of the other (see the correlation in Tables 2, 3, 4). As shown in (Table 1), PRL level showed significant differences between all study groups (P-value=0.015). PRL is lowered in the secondhand smokers (Median=15.7) also, is diminished in the smokers females group (Median=8.5) as in Table 1. The majority of addictive substances that raise dopamine levels in the brain also act to inhibit prolactin secretion. This includes cocaine [29], amphetamine [30] and methylphenidate [31, 32]. Some animal

studies have demonstrated that the acute nicotine administration regardless of whether it is delivered intravenously or intraperitoneally, consistently produces dose-dependent increases in PRL levels. [33].

In 2010, Xue Y., *et al.* found that the average nicotine cigarette smoking resulted in significant and prolonged increases in PRL levels [11]. Contrary to that study, our results revealed that smoking correlates with PRL reduction Table 1. Although this study focused on analysis PRL levels as a continuous variable, categorizing participants based on the presence of hyperprolactinemia could provide additional insights into the effects of active and secondhand smoking on this hormone. Analysis the frequency of hyperprolactinemia among different groups is an important area for future research, which may help in understanding the potential clinical implications of smoking exposure.

In smokers, levels of pro-inflammatory cytokines, including IL-12, T-cell, and specific regulators of Natural Killer cell (NK-cell), are reduced. This decline may be attributed to the immunosuppressive effect of smoking and an increased risk of periodontitis [34]. Altering these cytokine levels due to smoking could potentially impact clinical features and treatment responses. Consequently, lower IL-12 levels in female smokers could impair their immune responses, making them more susceptible to infections and potentially increasing their risk for autoimmune diseases and certain cancers [35]. The results of our study showed a lower level of IL-12 in smoking females compared to control and secondhand smoker individuals (p -value<0.05) Table 1.

Smokers with diabetes often encounter great difficulties in managing insulin dosing and controlling the disease compared to non-smokers [36]. Remarkably, the previous studies have shown that cigarette smokers have higher levels of HbA1c and a 30–40% increased risk of developing type 2 diabetes compared to non-smokers [37, 38].

In 2018, Choi *et al.* demonstrated that smoking is likely associated with elevated HbA1c levels within the general population [39]. Our findings further corroborate this, revealing a significant increase in HbA1c levels among both secondhand and active female smokers compared to the control group ($p=0.000$) Table 1. These results indicate that smoking has a substantial impact on HbA1c levels, highlighting its potential role in impairing glycemic control [40]. As the increased HbA1c levels among smokers was observed in this study, it is important to consider that smoking is associated with increased hemoglobin levels due to chronic carbon monoxide exposure. Therefore, the observed elevation in HbA1c may not solely reflect alterations in carbohydrates metabolism but could also be influenced by changes in total hemoglobin concentration. More studies about both hemoglobin and HbA1c together are needed.

Smoking and diabetes risk are multifaceted. Smoking has been shown to contribute to insulin resistance, primarily through increased oxidative stress and systemic inflammation. Nicotine and other toxic compounds in the cigarette can trigger the release of pro-inflammatory cytokines, including IL-12 which may impair insulin signaling pathway. Also, smoking is associated with hormonal imbalance, such as altered PRL and E2 levels, which may further disrupts glucose metabolism.

Chronic exposure to tobacco smoke has also been linked to beta-cell-dysfunction, leading to impaired glucose secre-

tion and increased HbA1c levels. Furthermore, smoking-induced endothelial dysfunction and increased abdominal adiposity may exacerbate metabolic disturbances, increasing the risk of diabetes and its complications [41].

According to our study sub-group, between heavy and light smokers females, the E2 concentrations show a significant difference ($p=0.007$) and give the lowest levels in Heavy smokers (Median=84.23) Table 5. Current smoking was linked to an elevated WHR (waist-to-hip ratio), a risk factor for increased inflammation, and a potentially metabolically obese phenotype [42]. Additionally, smokers showed higher levels of androgens like testosterone and androstenedione, which are linked to a heightened risk of breast cancer and hyperandrogenism [43]. As previous studies findings, in the body, testosterone can be converted into estradiol through a process called aromatization. If testosterone levels are excessively high, the body may not effectively convert it to estradiol, or other mechanisms might lead to altered production or metabolism [44].

Also, PRL shows significant differences between heavy and light smokers ($p=0.014$), with lowest levels in heavy smokers females (Median=8.1) Table 5. Estradiol generally stimulates the production of prolactin, while testosterone may have inhibitory effects on prolactin secretion. Therefore, changes in E2 and testosterone can influence PRL levels [45, 46].

While for HbA1c levels, there was a significant difference between heavy and light smokers ($p=0.006$) Table 5. With the highest level in Heavy smokers females (Median=6.2). This result aligned with Vlassopoulos *et al.*, (2013), who found that HbA1c was higher in smokers by 0.25 SDs (0.08%), and 0.38 SDs higher (0.14%) in heavy smokers than non-smokers ($p<0.001$ both) [46]. See also [40–48].

According to the duration of smoking sub-group, No notable differences were observed among the study parameters (Table 6), which means that varying durations of smoking (e.g., short-term vs. long-term) did not lead to notable changes in the outcomes being measured.

One important consideration is the influence of lifestyle factors linked to smoking. Female smokers may have different diets, higher psychoactive substance use, or greater abdominal obesity, which could affect PRL, E2, HbA1c, and IL-12 levels. As this study lacked detailed data on these factors, Future research should account for these confounders to better isolate smoking's effects.

Directions for further research

Future research should focus on understanding the mechanisms through which smoking disrupts hormonal balance and metabolic health in women, particularly regarding estradiol, prolactin, and HbA1c levels. Longitudinal studies are needed to assess the long-term effect of smoking on metabolic disorders, such as the development of cardiovascular diseases, and type 2 diabetes, with particular attention to immune responses and hormonal changes that differ between sexes. Additionally, examining how smoking cessation can help revise these disruptions and evaluating smoking cessation programs that are tailored to the specific needs of each gender, would provide valuable insights into improving health outcomes for women. This research could help refine strategies for early detection and intervention to mitigate the adverse effects of smoking on women's health.

CONCLUSION

Smoking has a profound adverse effect on public health females, disrupts hormonal balance, leading to alterations in E2 and PRL levels, which can impact reproductive health and contributes to metabolic disturbances through inflammation and insulin resistance, potentially increasing diabetes development risk.

Also, smoking does decrease IL-12 levels, which can impair immune responses and affect overall health. Furthermore, our results revealed that smoking does increase HbA1c levels. Persistently elevated HbA1c levels may raise the risk of developing type 2 diabetes and its related complications, including cardiovascular disease and other metabolic disorders.

Significant differences in hormonal and metabolic markers between heavy and light female smokers were observed. Heavy smokers had lower estradiol (E2) levels ($p=0.007$, Median=84.23) and lower prolactin (PRL) levels ($p=0.014$, Median=8.1), potentially due to elevated testosterone levels that may inhibit PRL secretion. Additionally, significantly higher in heavy smokers ($p=0.006$, Median=6.2), indicating poorer glycemic control. These findings suggest that heavy smoking adversely affects both metabolic health and hormonal balance in women.

This underscores the importance of addressing smoking cessation as a critical component of improving overall health outcomes in women. Moreover, all smoking women should undergo regular screenings for diabetes

to monitor their glycemic control and mitigate associated health risks.

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