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Serum Iron, Ferritin, Erythroferrone and their Inter-Correlation In Adult Cigarette Smokers: A Case-Control study

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Abstract

Cigarette smoking is one of the leading causes of lung and blood cancers worldwide. A higher risk of pulmonary injury is associated with elevated lung iron and ferritin levels. Erythroferrone (ERFE) is a newly discovered tumor necrosis factor (TNF) superfamily member produced by erythroblasts during erythropoiesis. Eventually, it suppresses hepcidin during enhanced erythropoietic activity. The current study investigated the relationship between serum ferritin, iron, and erythroferrone concentrations among adult smokers. This study included 76 selected adult cigarette smokers and 48 healthy individuals (non-smokers) as a control group. The enzyme-linked immunosorbent assay (ELISA) and chemiluminescent immunoassay (CLIA) biochemical techniques were used to accurately measure serum iron, ferritin, and erythroferrone. Cigarette smokers (CS) had a mean age of 42.75±2.92 years, while healthy control (HC) subjects had a mean age of 37.36±2.7 years. The findings have shown a significant increase in serum iron, ferritin, and EREF levels in CS (119± 4.30, 131.1±6.58, and 75.02±2.09) compared with HC (98.67±7.84, 85.50±4.12, and 65.74±2.25) respectively. This study has shown a significant positive correlation between serum EREF (P=0.0072, r=0.4089) and the duration of smoking <20 years and a moderate association between serum iron (P=0.1907, r=0.4511) and the duration of smoking <40 years. For the first time, this outcome shows increased serum EREF and overexpression by erythroblasts in subjects associated with smoking habits, along with increased serum iron and ferritin levels. An indicative correlation is found between EREF overexpression, iron overload, and hyperferritinemia with reference to the duration of smoking. The study also illustrates the interconnection between iron metabolism perturbations and ERFE overexpression induced by cigarette smoking.

Keywords: Erythroferrone, Ferritin, Iron, Cigarette Smoking, Duration of smoking

مصل الحديد والفيريتين والإريثروفيرون وترابطهم المتبادل في مدخني السجائر البالغين: دراسة الحالات والشواهد

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الخلاصة

تدخين السجائر هو أحد العشرة الأكثر فتكًا على مستوى العالم وهو عامل خطر مستقل معروف للعديد من الأمراض، بما في ذلك سرطان الرئة والدم. يرتبط ارتفاع مخاطر الإصابة الرئوية بارتفاع مستويات الحديد والفيريتين في الرئتين. الإريثروفيرون هو أحد أفراد عائلة TNF الذي تم اكتشافه حديثًا، والذي تنتجه خلايا الدم الحمراء أثناء تكوين الكريات الحمر الذي يثبط هرمون الهيبسيدين أثناء نشاط الكريات الحمر المعزز. بحثت الدراسة الحالية في العلاقة بين تركيز فيريتين المصل والحديد والإريثروفيرون بين مدخني السجائر البالغين. اشتملت الدراسة الحالية على 76 مدخن سجائر بالغ تم اختيارهم، بالإضافة إلى 48 شخصًا أصحاء كمجموعة ضابطة. تم استخدام تقنيات الكيمياء الحيوية المستندة إلى ELISA و CLIA لقياس دقيق لمصل الحديد والفيريتين والإريثروفيرون. كان متوسط عمر مدخني السجائر 45.85 ± 2.92 سنة وكان الأشخاص الأصحاء 36.66 ± 2.70. تظهر النتائج زيادة كبيرة معنوية في مستويات الحديد و الفيريتين و الإريثروفيرون في مدخني السجائر (119 ± 4.30، 131,1±6.58، 75.29 ± 2.09) بالمقارنة مع الضوابط الصحية (7,84±98,67، 85,50±4,12، 65,74±2,25) على التوالي. أظهرت هذه الدراسة وجود ارتباط إيجابي معتدل معنوي لمصل الاريثروفيرون (*P*=0.0072، r=0.4089) مع مدة التدخين أقل من 20 سنة وعلاقة متوسطة مع حديد المصل (*P*=0.1907، r=0.4511) مع مدة أقل من 40 سنوات. هذه الدراسة هي الأولى من نوعها التي تبلغ عن زيادة الاريثروفيرون في الدم والإفراط في التعبير عن طريق خلايا الدم الحمراء في الأشخاص الذين يعانون من عادات التدخين جنبًا إلى جنب مع زيادة مستويات الحديد والفيريتين في الدم. هناك علاقة بين الإفراط في إفراز الاريثروفيرون والحمل الزائد للحديد و زيادة فريتين الدم حسب مدة التدخين. توضح هذه الدراسة العلاقة بين اضطرابات التمثيل الغذائي للحديد مع فرط إفراز الاريثروفيرون الناجم عن تدخين السجائر.

1. Introduction

Cigarette smoking is one of the top global risk factors causing various life-threatening diseases. Eventually, it increases the risk of atherosclerosis, coronary artery disease, and peripheral vascular diseases [1-3]. Furthermore, numerous studies have shown that smoking is a significant risk factor for developing chronic obstructive gastrointestinal disorders [4], cancers [5], pulmonary disease [6], pancreatitis [7], and other autoimmune disorders [8]. Tobacco smoke carries more than 4000 compounds that provoke harmful effects on human health, including free radicals, carbon monoxide, and nicotine. Nicotine stimulates clot formation in the coronary arteries, increases endothelial dysfunction, and weakens the vascular activity. Any increase in the carboxyhaemoglobin level leads to hypoxia, and it also controls sub-endothelial oedema as it alters the vascular permeability and accumulation of lipids [9]. Hepatocytes are considered the primary site of iron storage in the body, with most iron bonded to ferritin [10]. Iron is a crucial micronutrient for normal cell function; however, excessive iron levels in the lungs are also associated with a higher risk of pulmonary injury [11]. The origin of the iron in cigarette smoke inhalation causes to build up in the lungs is unverifiable or imprecise [12]. Studies have reported that smoking raises the iron concentration in alveolar macrophages and bronchoalveolar lavage fluid to exceedingly high levels, regularly disrupting iron homeostasis in the lungs [12,13]. Additionally, cigarette smoking contributes to an increase in overall oxidative stress on lung tissues in chronic cigarette smokers [14]. Erythroferrone (ERFE) is a TNF superfamily member, identified as a glycoprotein hormone whose production is stimulated by exogenous or endogenous erythropoietin (EPO) hormone and is produced by erythroblasts during erythropoiesis [15, 16]. ERFE is considered a cytokine and erythroid regulator similar to growth differentiation factor-15 (GDF-15), which acts through the smad-dependent TGF-β/BMP signalling pathway

[17]. ERFE suppresses hepcidin during enhanced erythropoietic activity, increasing iron availability to form new erythrocytes [16]. According to Kautz et al., ERFE expression and serum protein concentration increase in mice subjected to EPO medication or haemorrhage, indicating that ERFE inhibits hepcidin and the resultant decline in hepcidin augments iron transport for enhanced erythropoiesis [18]. Another study by Delaney *et a*l. reported that maternal serum ERFE was associated with erythropoietic demand during pregnancy [4]. The most significant aim of this study is to provide further inspections about the recently identified ERFE, which is still being investigated by scholars to reveal its different functions in the body, whereas limited studies are available at the time of this study. In addition, the measurement of blood ERFE levels and their association with iron and ferritin status in adult cigarette smokers have not yet been studied. Considering the contemporary public health implications of the adverse effects of cigarette smoking on health, the current study stresses the potential impacts of smoking on serum erythroferrone, ferritin, and iron status among adult cigarette smokers. This study also investigated the correlations between the study's biochemical parameters and the duration of cigarette smoking.

2. Materials and Methods

2.1. Study subjects

In Soran, 76 patients were randomly chosen to participate in the study from 29 January to 20 February 2022. A control group of 48 healthy individuals who did not smoke cigarettes was also included. The cigarette smoker (CS) participants were questioned about their gender, age, and smoking history (duration of smoking). While the health control (HC) participants were interviewed about their gender and age and confirmed not having any systemic or chronic illnesses.

2.2. Sample collection

The study participants had blood drawn (5 mL), collected in gold-top serum separator tubes (Gold-Top SST), let stand at room temperature for 15 minutes, and then centrifuged at 3500 rpm for 15 minutes. Serum samples were transferred immediately to pre-labeled and patient-coded Eppendorf tubes and stored at -20 °C until the day of the analysis.

2.3. Measurement of biochemical parameters

The serum level of iron was measured *via* the iron direct method (Ferene) (Giesse Diagnostics Srl, Italy) by spectrophotometric technique. Serum ferritin level was measured using the Maglumi 800 fully automated chemiluminescent immunoassay (CLIA) analyzer (Snibe, China). The human erythroferrone (ERFE) ELISA Kit (SunLong Biotech, China) was used to determine serum erythroferrone levels using the sandwich enzyme-linked immunosorbent assay (ELISA) technique.

2.4. Statistical methods

The GraphPad Prism programme, version 8, was used to conduct the statistical analyses. Visual (histograms) and analytical tests (Shapiro-Wilk and Kolmogorov-Smirnov) were used to examine the data to ascertain if the variables were regularly distributed. A P-value of 0.05 was used to determine statistical significance. Using an independent *t*-test, the serum levels of ferritin, iron, and ERFE in cigarette smokers and healthy controls were compared. A Spearman correlation analysis was also performed to study the relationship between biological markers and the duration of smoking.

3. Results and Discussion

3.1. Study subject demography

In this study, the average age of cigarette smoker participants (CS) was 45.85±3.25 years. Regarding the gender of study subjects, most cigarette smokers (CS) were males: 60, representing 78% of the CS, and 16 females, representing 22% of participants. However, in comparison to the healthy control group (HC), in which the mean age was 37.36 ± 2.7 and the number of male participants was 34 (70%) compared to 14 females (30%).

Table 1 : Depicts the demographic information of cigarette smokers and control groups in the mean ± SE

Studied Groups	Males	Females	Total	*P* value
	Cigarette smokers			
Number (%)	60 (78)	16 (22)	76 (100%)	0.4024
Age (Years)	41.38±3.45	50.33±3.06	45.85±3.25	
Duration of smoking (Years)	17.28±2.87	30±4.90	19.83±2.60	
	Healthy controls			
Number (%)	34(70)	14(30)	48 (100%)	0.1911
Age (Years)	39.98±3.20	33.22±5.30	36.66±2.70	

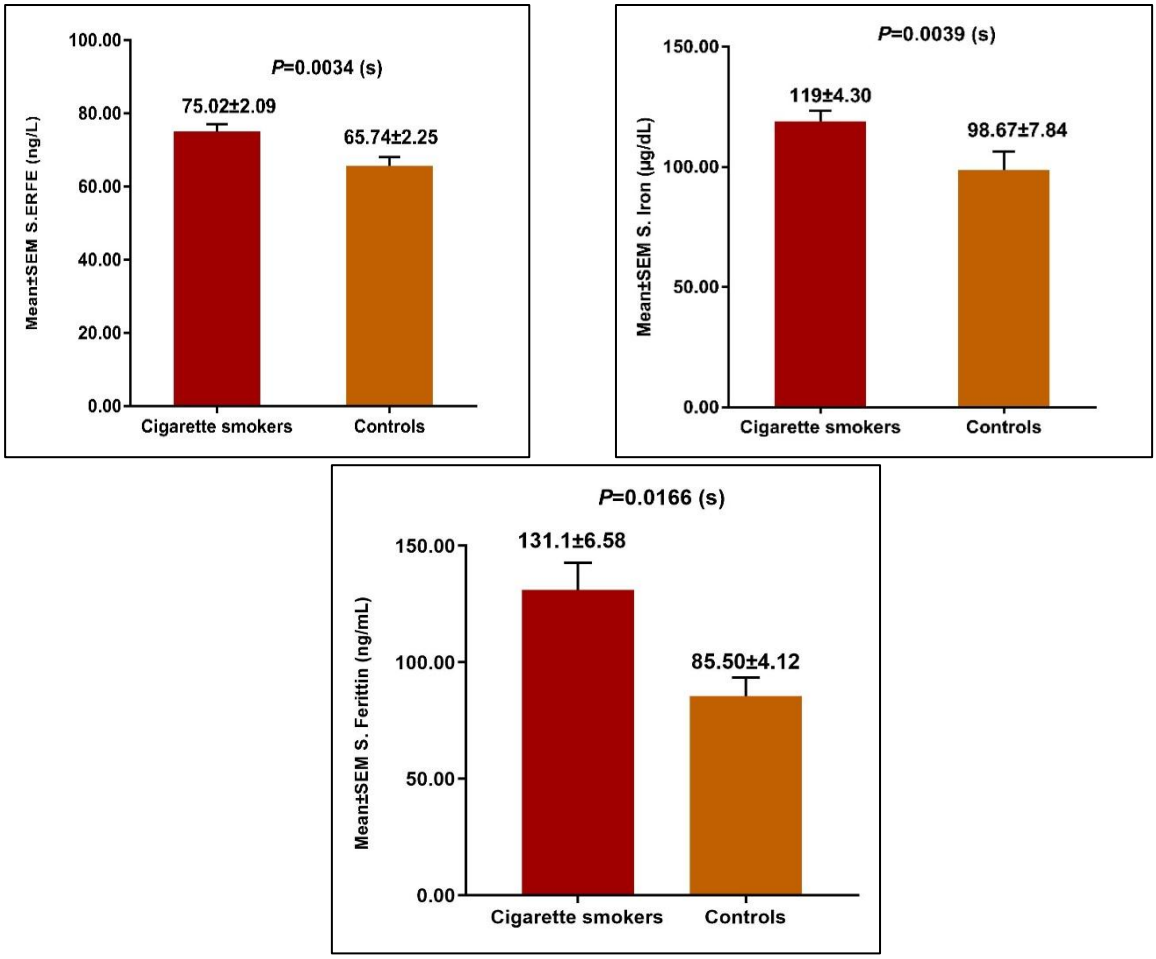

Figure 1: The mean ± SE value of A. iron, B. ferritin, and C. EREF concentration in sera samples of control and cigarette smokers

Table 2 : The Mean ± SE and range, and the *P* value of study parameters in control and cigarette smoker groups

Study groups			
	Cigarette smokers	**Control**	***P* value***
Iron (ug/dL)			0.0039
Range	57-326	54-313	
Mean±SE	119±4.30	98.67±7.84	
Ferritin (ng/mL)			0.0166
Range	8.29-829.3	3.33-237.1	
Mean±SE	131.1±6.58	85.50±4.12	
EREF (ng/L)			0.0034
Range	41.02-129.8	44-91.35	
Mean±SE	75.02±2.09	65.74±2.25	

**P* value < 0.05 considered to be significant

3.2. Serum levels of studied parameters in cigarette smokers and control groups

3.2.1. Serum level of iron

This study discovered an elevated serum iron concentration in the cigarette smokers (CS) group, as illustrated in Figure 1A. There was a significant ($P<0.05$) elevation of serum iron levels in the CS compared to the control group. It is important to note that smoking contains a number of oxidants and prooxidants that might cause the production of free radicals [19]. It also decreases vitamin C levels, increasing the risk of iron deficiency, which causes anemia due to reduced iron absorption [20]. Iron accumulation systemically and locally in the lungs makes any disproportion in iron homeostasis obvious. This alteration is directly linked to oxidative stress and oxidative tissue damage [21]. The hepcidin-ferroportin axis controls systemic iron homeostasis, which is regulated by inflammation *via* inflammatory cytokines (IL-6), iron storage *via* circulation and tissue iron, or erythroid regulators such as erythroferrone [22]. Furthermore, the *in vivo* study by Ghio *et al*. [12] revealed that exposure to cigarette smoking alters iron homeostasis. The study indicated that iron accumulation is linked to the particulates in the smoke, and the tar phase is responsible for the disturbance of iron homeostasis in the lungs and throughout the body. It also affects the oxidative stress resulting from tissue inflammation caused by smoking. Likewise, another study by Ghio and Hilborn [21] implied that iron homeostasis is linked to airway blockage.

3.2.2 Serum level of ferritin

The results presented in Figure 2B show an obvious and statistically significant ($P<0.05$) increase in the serum ferritin level among CS compared to the control subjects. This finding is compatible with the previous result by Lee *et al*. [11], as they reported that hyperferritinemia (a high ferritin level) is interrelated with the escalation of cigarette smoking. Serum ferritin is a critical clinical biomarker for evaluating iron status. Ferritin is an intracellular, positive acute-phase protein that can store and release iron. Inflammation causes the ferritin level to rise, which causes an overabundance of iron rather than reflecting iron storage [23,24]. Among smokers, a high ferritin level has been associated with an increased risk of acute myocardial infarction [25]. The relationship between cigarette smoke and ferritin is explained by the cigarette smoke-induced release of iron from ferritin by cigarette smoke aqueous extracts [26]. Interestingly, elevated serum ferritin levels were identified as a mortality risk factor for cigarette smoking and inflammatory conditions [27].

3.2.3 Serum level of Erythroferrone

Erythroferrone (ERFE) is a peptide hormone and a recently identified erythroid regulator that suppresses hepcidin during erythropoiesis stress [18]. Consequently, the current study is focused on this recently discovered erythroid regulator to investigate its relationship with tobacco consumption. Figure 2C shows a statistically significant (P<0.05) increase in serum ERFE levels in cigarette smokers compared with the control group. The significantly high serum ERFE level and its overexpression by erythroblasts were also reported by Almousawi and Sharba [15], as their study indicated that the serum EREF level was higher in anemia and beta-thalassemia patients. In contrast to the current study findings, Inomata *et al*. [28] reported a decreased level of EREF in hepatitis C virus-infected patients, which is linked to a decrease in hepcidin levels. ERFE is a newly identified member of the tumor necrosis factor and transforming growth factor-beta superfamilies which have been proven to repress hepcidin expression [17]. Iron homeostasis, which is maintained by ERFE, ensures that there is just the right amount of iron for biological functions while preventing excessive iron accumulation, which can damage many tissues. Iron bioavailability in mammalian systems is regulated by the interplay of hepcidin, an iron-regulatory hormone, and ferroportin, a protein that functions as both a hepcidin receptor and a cellular iron exporter. Hepcidin inhibits iron mobilization from storage and dietary iron absorption by lowering iron export to the plasma *via* ferroportin. That is because more iron is needed for growing erythroblasts to produce heme and hemoglobin during increased erythropoiesis. Recent studies revealed that the ERFE hormone produced by erythroblasts inhibits the formation of hepcidin in hepatocytes, increasing dietary iron absorption and mobilizing iron from reserves [29]. The results of the current study suggest that erythroferrone (ERFE) is likely to serve as a helpful diagnostic biomarker of inefficient erythropoiesis and a desirable target for its systemic effects. ERFE regulates the hepcidin level as the primary erythroid regulator hormone, controlling plasma levels and total body iron status. Recent mechanistic studies have shown that ERFE inhibits bone morphogenetic protein signaling in hepatocytes, which in turn lowers the hepcidin transcription factor. Even in patients not receiving blood transfusions, pathological overproduction of ERFE by an increased population of erythroblasts results in iron overload and inhibits hepcidin during inefficient erythropoiesis [30]. This exploratory study is the first to investigate the EREF role and erythroblast overexpression among cigarette smokers in relation to iron and ferritin levels. This study findings show that cigarette smokers have statistically high serum EREF concentrations and erythroblast ERFE overexpression, which is associated with iron overload and hyperferritinemia. However, due to cultural considerations, the data on female cigarette smokers was much lower than that on male smokers.

3.5. Intercorrelation of serum levels of iron, ferritin, and erythroferrone in CS according to the duration of smoking

The current study found a significant (P>0.05) positive correlation between serum iron and ferritin levels in cigarette smokers and smoking durations over three time periods (20 years, between 20 and 40 years, and >40 years). Furthermore, there is a significant moderate positive correlation (P=0.0072, r=0.4089) between serum EREF and the duration of smoking (<20 years) among cigarette smokers; however, this correlation is slightly weakened as the duration of smoking increases (P=0.0713, r=0.3594; and P=0.496, r=0.2453) for (>20 to <40 years) and >40 years of smoking, respectively. The intercorrelation graphs for each parameter being studied are illustrated in Figure 2. The current results reveal that the serum iron and ferritin levels are positively correlated with the duration of smoking, with P-values of more than 0.05 (Table 2). Furthermore, the current data show that EREF levels rise in association with an increase in serum iron and iron overload in cigarette smokers. These results can be explained by the fact that the participants who exhibit iron overload are expected to have low

serum hepcidin and high ERFE levels [31]. Remarkably, in this research, serum iron and ferritin levels gradually increase as the duration of smoking increases, and smoking intake increases from <20 years to >40 years of tobacco consumption among adult cigarette smokers, accompanied by the gradual increase of ERFE expression. Accordingly, this finding is comparable to the study by Al-Mousawi and Sharba [15], in which they argued that there is a negative correlation between ERFE levels and clinical parameters (HB, HCT, MCH, MCV, iron, and ferritin) in anemia and beta-thalassemia individuals. In this study, the disturbances in iron metabolism involving iron overload and hyperferritinemia caused by the lengthy duration of smoking can be justified by smoking-induced hypoxia among cigarette smokers [32]. According to Coffey *et al.* [33], a high level of EREF expression by erythroid cells is sufficient to decrease hepcidin responsiveness to iron loading and generate systemic iron overload, as evidenced by elevated plasma iron concentrations and hepatic tissue iron storage in a recent mouse study paradigm. Indeed, these results indicate a crucial biochemical association of EREF overexpression with iron overload and hyperferritinemia among adult cigarette smokers.

Table 3 : The *P* value and *r* square of iron, ferritin, and EREF levels in cigarette smokers

Duration of smoking	Iron		Ferritin		Erythroferrone	
	r square	*P*-value	*r* square	*P*-value	*r* square	*P*-value
< 20 years	0.2861	0.0663	0.258	0.0990	0.4089	0.0072
20-40 years	0.06911	0.7373	0.3654	0.0664	0.3594	0.0713
> 40 years	0.4511	0.1907	0.2696	0.4512	0.2453	0.4946

**P* value < 0.05 considered to be significant

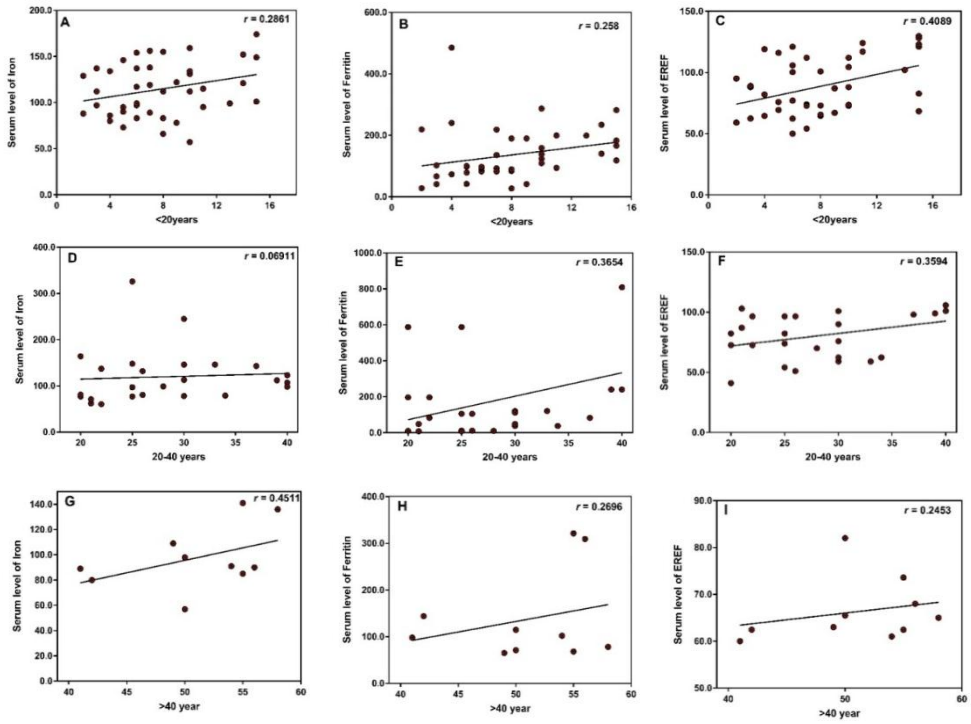

Figure 2 : Correlation graphs of serum iron, ferritin, and EREF with the duration of smoking in three periods (A, B, and C). The periods A, B, and C are less than 20 years of smoking, while the periods D, E, and F are between 20 and 40 years of smoking, and the periods H, I, and J are more than 40 years of smoking.

4. Conclusion

One of the highest risk factors causing pulmonary injury associated with elevated lung iron and ferritin levels is consistent cigarette smoking, which is globally identified as one of the top risk factors for various cancer types. The findings of the current study are consistent with the idea that smoking has negative effects on iron, ferritin, and EREF status. This study closely examines the increased serum erythroferrone (EREF) and its overexpression by erythroblasts in subjects with smoking habits, along with increased serum iron and ferritin levels. Serum EREF is shown to be an accurate biomarker of iron status. Consequently, the study concludes that there is a positive correlation between iron overload, hyperferritinemia, and EREF overexpression according to the duration of smoking. Smoking-induced ERFE overexpression is probably associated with iron metabolism disruption. Moreover, ERFE plays a vital role in erythropoiesis and iron body status, which are interrelated in cigarette smokers. This topic requires further comprehensive and systematic research.

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Author contributions

Parwin J. Jalil wrote the paper with assistance from Akam J. Mustafa. Akam J. Mustafa and Parwin J. Jalil created the theoretical framework, ran the statistical analysis, paraphrased the article, and proofread it. Jabbar H. Ade also proofread and language-edited the manuscript. The experimental works were performed by Akam J. Mustafa and Anjam J. Mustafa. The final version of the manuscript was edited by Parwin J. Jalil, Akam J. Mustafa, Jabbar H. Ad, and Anjam J. Mustafa. All authors discussed the findings and contributed to the final paper version, and writers provided critical comments and helped shape the research, data analysis, and manuscript.

Data availability

The data generated and analyzed in the present study is available from the corresponding author upon request.

Ethics approval and informed consent

Students from Soran University and Erbil Polytechnique University provided blood samples and information with their permission. The Faculty Council (Faculty of Science, Soran University) and the Faculty of Science, Soran University Deanery authorized the project (Code 1/1/354, Date: 6 April 2022).

Conflicts of interest

The authors have stated that they have no conflicts of interest.

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