Macrophage migration inhibitory factor as an indicator of chronic inflammation and metabolic risk in women

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Abstract. Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine with multiple functions in the immune system and other tissues. Physiologically, MIF has been implicated in the regulation of the immune response, in pathogen clearance, in cell regeneration, in the regulation of insulin secretion, and in the regulation of cardio- and neuro-protective effects. Pathologically, high MIF levels have been associated with inflammatory and autoimmune diseases, diabetes mellitus, obesity and cancer. The present study assessed serum MIF levels in women by ELISA. Subsequently, the possible relationship between MIF serum levels and risk factors associated with the development of breast and other types of cancer was evaluated. MIF serum levels in the studied population ranged between 80.7 and 4,790.5 pg/ml. Notably, high MIF serum levels were related to obesity (median 1,604.0 pg/ml; P=0.0197 vs. overweight and P=0.0002 vs. normal body mass index), a high waist-to-hip ratio (WHR; median 1,542.0 pg/ml; P=0.0126 vs. moderate WHR) and hypertension (median 1,582.0 pg/ml; P=0.0234). Also, a weak correlation was observed between MIF levels and aging (P=0.0239, r=0.1558). Furthermore, MIF levels were decreased in women with Breast Imaging Reporting and Data System (BI-RADS) classification 2 (P=0.0189) and 3 (P=0.0023) when compared with the levels in women with BI-RADS classification 1, and no other association was identified with the other cancer risk factors evaluated. In conclusion, the present results indicated that, although increased levels of MIF were not associated with increased breast cancer risk as evaluated by BI-RADS classification or other cancer risk factors, elevated MIF levels were related to states of chronic inflammation such as obesity, a high WHR and aging, indicating that an increase in the serum concentration of MIF may be related to metabolic disease.

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Abbreviations: BMI, body mass index; BI-RADS, Breast Imaging Reporting and Data System; cPLA2, cytosolic phospholipase A2; DC, dendritic cell; ILC2, type II innate lymphoid cells; MIF, macrophage migration inhibitory factor; PMOS, polycystic ovary syndrome; PMOS, polyendocrine metabolic ovarian syndrome; Th, T helper; VAT, visceral adipose tissue; WAT, white adipose tissue; WHR, waist-to-hip ratio

Key words: MIF, obesity, hypertension, aging, metabolism, cancer, cytokine, inflammation, mammogram

Introduction

Macrophage migration inhibitory factor (MIF) is a multifunctional cytokine with important functions in the regulation of the immune system. It is primarily produced by the pituitary gland to counteract the immunosuppressive effects of glucocorticoids and is also secreted by diverse cell types, including immune cells (B and T lymphocytes, monocytes and macrophages), endothelial cells, adipocytes, smooth muscle cells, red blood cells, pancreatic islet β-cells, specialized cells in the nervous system and platelets, thus having autocrine, paracrine and endocrine functions (1-3). Physiologically, MIF has been shown to regulate the immune response and microbial clearance, to participate in cell regeneration and wound healing, to regulate insulin secretion, and to exert neuro- and cardio-protective effects, among others. The best characterized role of MIF involves regulation of innate immunity; it

is constitutively expressed and stored in most immune cells, including macrophages, and is released in response to inflammatory factors such as lipopolysaccharide, exotoxins, hypoxia, angiotensin II, glucose, insulin, ultraviolet B irradiation and glucocorticoids (1,4,5). MIF thus acts as a first responder cytokine with the ability to promote other inflammatory events. MIF can also reduce the effects of glucocorticoids, which normally reduce inflammation, therefore promoting inflammation under stressful conditions (4). After binding to its receptor CD74/44, MIF induces the expression of pro-inflammatory cytokines, such as tumor necrosis factor, IL-6, interferon-γ and IL-1β, and upregulates Toll-like receptor 4, facilitating bacterial recognition (1), or elicits chemotactic responses by binding to CXCR2/4/7, influencing leukocyte recruitment (6). Upon receptor binding, MIF leads to the activation of Src kinase and MAPK/ERK or PI3K/AKT pathways, thus signaling cell proliferation, inflammation, migration, survival and the secretion of angiogenic factors (1,3,4).

MIF has also been reported to be involved in pathological conditions, and is elevated in inflammatory and autoimmune diseases, including rheumatoid arthritis, vitiligo, multiple sclerosis, psoriasis and lupus; in infectious diseases, such as periodontitis, dengue, tuberculosis and sepsis; in diabetes; and in some types of cancer, such as esophageal squamous cell carcinoma, gastric and breast cancer. In addition, polymorphisms associated with an increased regulation of MIF expression have been associated with increased susceptibility to and severity of inflammatory and autoimmune diseases, as well as prostate and gastric cancer (3,5). Notably, MIF has recently been identified as an important mediator in type 2 diabetes mellitus pathogenesis and in obesity (2,7), where high MIF plasma levels have been shown to regulate adipocyte lipid storage, increasing plasma triacylglycerol concentration (2).

Chronic inflammation is defined as an activation of the immune system, triggered by immunogenic compounds, lifestyle-related or environmental factors, which lead to a low-grade, chronic inflammation lacking the typical symptoms of acute inflammation (8). This state has been reported to be associated with states such as aging and in the development of diverse chronic diseases, including cancer and cardiovascular disease (9). Causes of this chronic inflammatory state include chronic infections, cellular senescence and obesity (9).

Obesity has been redefined as a complex systemic disease, thus transcending caloric imbalance, which is driven by metabolic, neuroendocrine, immune and epigenetic dysregulation (10). Clinical obesity is defined as a chronic, systemic illness caused by excess adiposity and characterized by alterations in tissue or organ function, alterations in the individual, or a combination thereof (11). The prevalence of obesity has markedly increased in recent decades, and estimates suggest that ~50% of all adults in the world are currently overweight or obese (8). This high prevalence of obesity poses a global threat to human health, due to its association with metabolic disorders, including insulin resistance and diabetes, metabolic-associated fatty liver disease, atherosclerosis and an increased risk of developing dementia (8). Globally, obesity is slightly more common in women than in men (12), and women are more likely to be affected by obesity due to its effects on endocrine dysfunction, which is related to menstrual irregularities, infertility, polycystic ovary syndrome [PCOS; which is now referred to as polyendocrine metabolic ovarian syndrome (PMOS)], and complications in pregnancy outcomes (13). Moreover, obesity in women increases the risk of developing heart disease, diabetes and breast cancer (12), underscoring the need to implement prophylactic and therapeutic measures to tackle obesity and prevent the development of its associated alterations.

The accumulation of lipids in subcutaneous fat depots, visceral adipose tissue (VAT), liver, muscle and other body sites promotes the release of fatty acids and pro-inflammatory cytokines, inducing a systemic, low-grade, subclinical chronic inflammatory state (14). The white adipose tissue (WAT) is normally populated by immune cells, including macrophages, dendritic cells (DCs), type II innate lymphoid cells (ILC2s), eosinophils, T helper (Th)2 cells and regulatory T cells, which undergo reprogramming during obesity. In obese conditions, infiltrating inflammatory macrophages are increased due to enhanced recruitment, proliferation and differentiation into inflammatory type I macrophages; these macrophages have a key role in maintaining chronic, low-level inflammation, thus decreasing ILC2s, activating DCs, and recruiting Th1 cells and CD8 T lymphocytes (8). One of the cytokines involved in the maintenance of this obesity-related chronic inflammation is MIF, and its circulating levels have been linked to the development of insulin resistance, type 2 diabetes and non-alcoholic fatty liver disease, highlighting the need to delineate and understand the precise role of MIF in metabolic disease.

The present study aimed to assess the possible relationship between serum MIF levels and diverse risk factors related to cancer development in women, to evaluate its possible relationship with breast cancer risk factors or metabolic disease.

Materials and methods

Patients. Blood samples were collected by forearm venipuncture between January and August 2024 from women attending two different Family Medicine Units (Instituto Mexicano del Seguro Social, Unidad de Medicina Familiar #2 and 6) in Puebla, Mexico for routine mammography screening. After collection, blood samples were centrifuged at 1,200 x g for 10 min and sera were collected and stored at 4˚C for 2-3 days and later at -80˚C until analysis. A total of 162 patients, with a median age of 50 years (age range, 20-73 years) were included in the study. All patients who agreed to participate and signed an informed consent form were included. A questionnaire was applied to each patient to collect information about risk-factor exposure. Patients were classified according to the following parameters: BMI was calculated as mass/height (kg/m^2), and in agreement with adult guidelines (11), patients were considered as being underweight (BMI <18.5), normal weight (BMI 18.5-24.9), overweight (BMI 25-29.9) or obese (BMI ≥30). WHR was considered low, <0.85; moderate, 0.85-0.89; or high, >0.89; blood pressure was classified as normal or high, as reported by the patient (previous diagnosis of hypertension); mammary density was classified as A, almost entirely fat; B, breasts with scattered areas of fibroglandular densities; C, heterogeneously dense; or D, extremely dense breasts (15);

Table I. Clinical and sociodemographic characteristics of the studied population (n=162 patients).

Variable	N (%)
Age, years	
22-34	3 (1.85)
35-44	38 (23.46)
45-54	66 (40.74)
55-64	43 (26.54)
65-74	12 (7.41)
BMI (kg/m^2)	
Underweight (<18.5)	1 (0.62)
Normal (18.5-24.9)	38 (23.46)
Overweight (25-29.9)	69 (42.59)
Obese (>30)	54 (33.33)
WHR	
Low (<0.85)	8 (4.94)
Moderate (0.85-0.89)	46 (28.40)
High (>0.89)	108 (66.67)
Mammary density	
A	22 (13.580)
B	71 (43.827)
C	60 (37.037)
D	9 (5.556)
BI-RADS	
1	16 (9.877)
2	75 (46.296)
3	56 (34.568)
4	14 (8.642)
5	0 (0.000)
6	1 (0.617)
Tobacco use (current and past)	
Yes	26 (16.05)
No	136 (83.95)
Alcohol consumption	
Yes	56 (34.57)
No	106 (65.43)
Radiation exposure	
Yes	8 (4.94)
No	154 (95.06)
Age of menarche, years	
<12	45 (27.78)
12-15	117 (72.22)
Age of menopause	
<45 years	26 (16.049)
45-55 years	67 (41.358)
>55 years	5 (3.084)
Still menstruating	64 (39.506)
Breastfeeding (past)	
Yes	125 (77.16)
No	37 (22.84)
Number of births	
0	24 (14.81)
1	27 (16.67)
≥2	111 (68.52)
Contraceptive use/type	
Yes/Oral	13 (8.025)
Yes/Others	65 (40.124)
No	84 (51.852)
Blood pressure	
Normal	127 (78.40)
High	35 (21.60)
Previous mammary lesions	
Yes	57 (35.19)
No	105 (64.81)
Family history of cancer	
Yes	38 (23.46)
No	124 (76.54)
Previous tuberculosis	
Yes	2 (1.23)
No	160 (98.77)

BI-RADS, Breast Imaging Reporting and Data System; BMI, body mass index; WHR, waist-to-hip ratio.

and BI-RADS classification (15,16) was categorized as 1, negative; 2, benign (noncancerous) findings; 3, probably benign finding, with a <2% chance of malignancy; 4, suspicious, with a 2-95% probability of cancer; 5, highly suggestive of malignancy, with a 95% chance of cancer; or 6, known biopsy-proven malignancy, according to the mammography results. Other risk factors were collected from the questionnaire completed by the patients, including age, tobacco use (present and past), alcohol consumption (present and past), number of births, contraceptive use/type, previous breastfeeding, age of menarche/menopause, previous mammary lesions, previous tuberculosis, radiation exposure and a family history of breast cancer. Patient data are shown in Table I.

Ethics approval. The present study was approved by the Local Institutional Review Boards 21038 and 21048, in the Instituto Mexicano del Seguro Social (Puebla, Mexico; approval nos. R-2024-2104-048 and R-2021-2103-011, respectively). The authors certify that the study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments.

Serum MIF level determination. Serum samples were stored at -80˚C until analysis. MIF levels were determined using the Human MIF DuoSet ELISA kit (cat. no. DY289; R&D

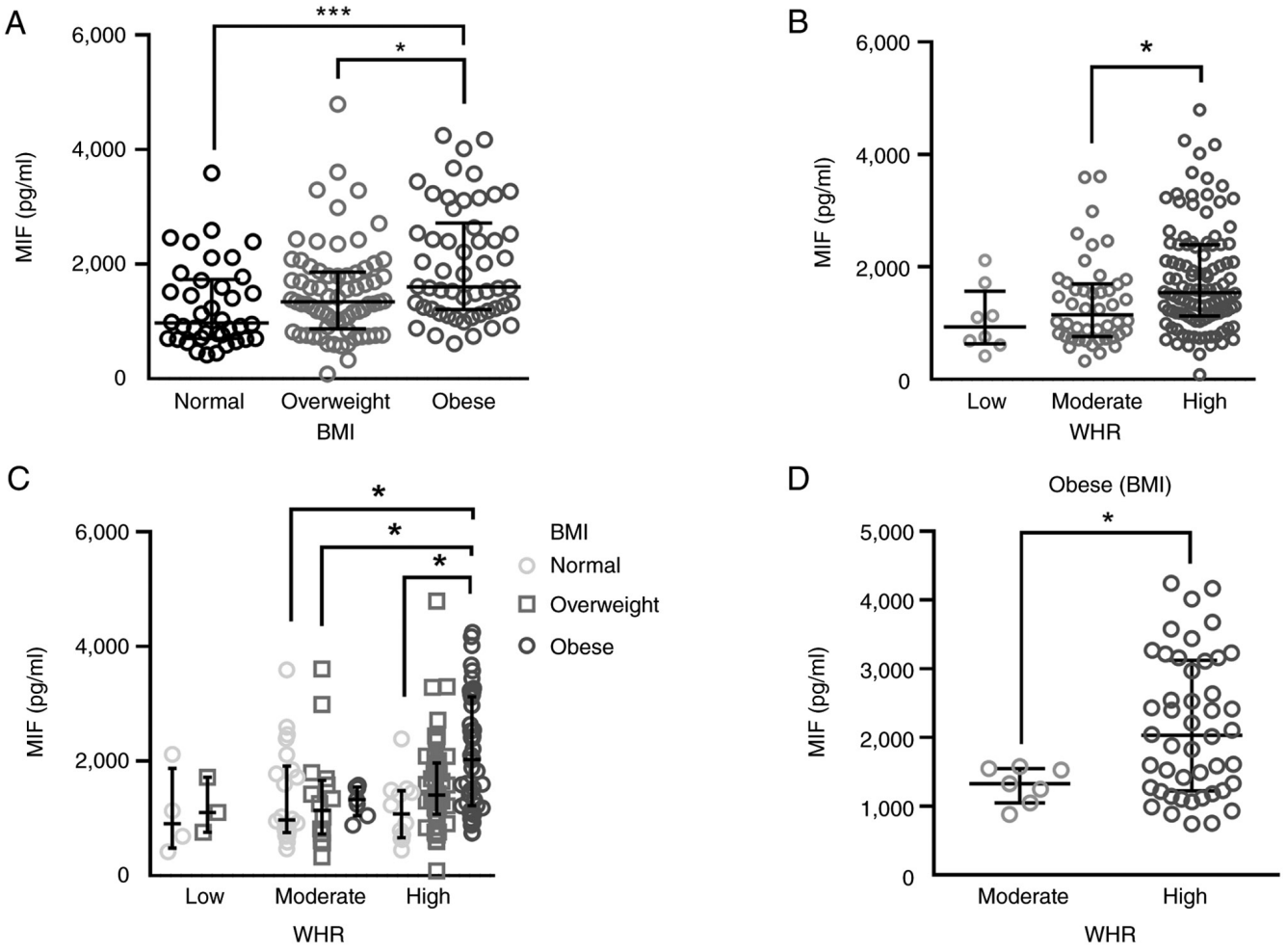

Figure 1. MIF serum levels are elevated in women with a high BMI and high WHR. MIF serum levels were assessed by ELISA in women classified according to their (A) BMI, (B) WHR, (C) in a combination of both and (D) in women with a high BMI (obese) and with a high WHR compared with those with a moderate WHR. The graphs show data from 162 samples; horizontal lines indicate the median value, and the upper and lower small horizontal lines indicate the interquartile range (Q1-Q3) for each category. (D) When two groups were compared, a Mann-Whitney U test was used; (A-C) for more than two group comparisons, a Kruskal-Wallis test with Dunn's post-hoc test for multiple comparisons was used. The graphs show only those pairwise comparisons with a significant value of $P<0.05$. $^{*}P<0.05$, $^{***}P<0.001$. BMI, body mass index; MIF, macrophage migration inhibitory factor; WHR, waist-to-hip ratio.

Systems, Inc.), which uses the sandwich ELISA method and has a limit of detection of 31.2 pg/ml. All procedures were performed in accordance with the manufacturer's instructions. No serum dilution was performed; 40 μl each sample was added to each well, and tetramethylbenzidine was used as chromogenic substrate for horseradish peroxidase in the colorimetric reaction. The absorbance was measured using a BioTek Synergy-4 plate reader (Agilent Technologies, Inc.) at 450 nm, and a reading at 570 nm was used for background subtraction. Serum concentrations of MIF were quantified with a standard curve and expressed in pg/ml.

Statistical analysis. A Kolmogrov-Smimov test was used to analyze data normality. Since data were not normally distributed, a Mann-Whitney U test was used for two-sample comparisons and a Kruskal-Wallis test with Dunn's post-hoc test was used for multiple comparisons. Correlation analysis was performed with a one-tailed Spearman's correlation test. Data in contingency tables were analyzed with a χ^2 test. Data were analyzed using GraphPad Prism 6.07 (Dotmatics). Data in the text are presented as the median and interquartile range (IQR; Q1-Q3) for each parameter mentioned. $P<0.05$ was considered to indicate a statistically significant difference.

Results

MIF serum levels are elevated in women with a high BMI, elevated WHR and hypertension. The present study detected increased levels of MIF in women with a BMI indicative of obesity [median (IQR): 1,604.0 (1,208.0-2,720.0) pg/ml] when compared with those who were overweight [1,342.0 (871.1-1,861.0) pg/ml; P=0.0197] or had a normal BMI [970.0 (703.0-1,731.0) pg/ml; P=0.0002] (Fig. 1A). In addition, women with a high WHR had higher levels of MIF [1,542.0 (1,126.0-2,393.0) pg/ml] than those with a moderate ratio [1,149.0 (761.1-1698.0) pg/ml; P=0.0126] (Fig. 1B). When both indexes of obesity were combined, MIF levels were higher in obese patients with a high WHR [2,030.0 (1,223.0-3,123.0) pg/ml] when compared with patients with a normal BMI but with a high WHR [1,076.0 (664.8-1,484.0) pg/ml; P=0.0146]; or with those with a moderate WHR and a normal BMI

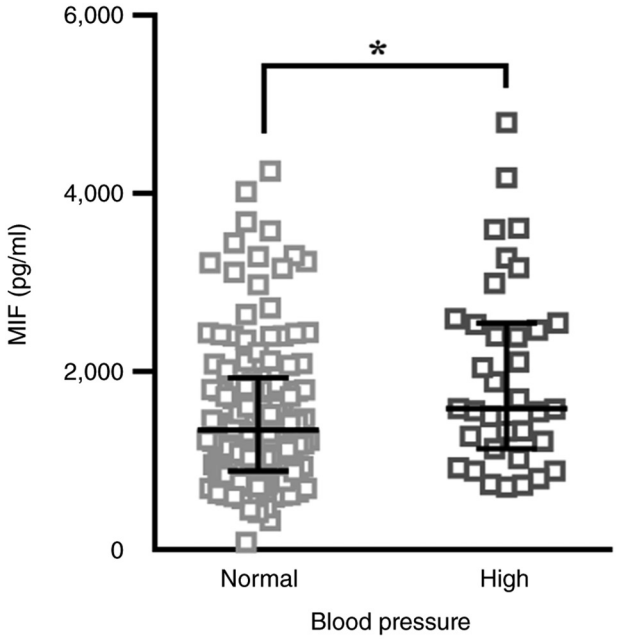

Figure 2. MIF serum levels are elevated in women with hypertension. MIF serum levels were assessed by ELISA in women with high or normal blood pressure. The graph shows data from 162 samples; horizontal lines indicate the median value, and the upper and lower small horizontal lines indicate the interquartile range (Q1-Q3) for each category. Mann-Whitney U-test was used for the pairwise comparison. *P<0.05. MIF, macrophage migration inhibitory factor.

[970.0 (751.8-1,912.0) pg/ml; P=0.0279] or a BMI indicative of being overweight [1,139.0 (725.8-1,665.0) pg/ml; P=0.0284] (Fig. 1C). Notably, when considering only those patients with a high BMI, MIF levels were higher in obese patients with a high WHR [2,030.0 (1,223.0-3,123.0) pg/ml] than in those with a medium WHR [1,328.0 (1,049.0-1,547.0) pg/ml; P=0.0372] (Fig. 1D), suggesting a relationship between MIF levels and a higher metabolic risk.

MIF levels were also elevated in women with hypertension [1,582.0 (1,131.0-2,544.0) pg/ml] when compared with those with a normal blood pressure [1,342.0 (880.7-1,926.0) pg/ml, P=0.0234] (Fig. 2), suggesting a relationship between elevated MIF levels and metabolic complications associated with obesity.

Serum MIF levels are decreased in patients with BI-RADS classifications 2 and 3. BI-RADS is the standard method for mammogram classification. This categorization provides information about the risk of malignancy, from essentially zero (BI-RADS 1) to >95% (BI-RADS 5) (17). Notably, a decrease in MIF levels was observed in patients with BI-RADS classifications 2 [1,468.0 (762.1-2,111.0) pg/ml; P=0.0189] and 3 [1,241.0 (881.4-1,754.0) pg/ml; P=0.0023] when compared with BI-RADS 1 [2,146.0 (1,654.0-3,131.0) pg/ml] (Fig. 3A). In addition, when comparing high and low MIF levels between BI-RADS categories (with the cut-off value set on the median of patients with BI-RADS classification 1), differences were observed among the BI-RADS categories (P=0.0273; Table II).

High mammary density is an important risk factor for breast cancer (18). When comparing MIF levels among patients with different breast densities, an apparent decrease in MIF levels was observed with increasing mammary density, without reaching statistical significance when compared to A, ranging from [1,986.0 (1,071.0-2,655.0) pg/ml in A; 1,488.0 (1,024.0-2,106.0) in B with P=0.7934; 1,329.0 (844.4-1,860.0) pg/ml in C with P=0.2303; and 1,104.0 (823.3-1,689.0) pg/ml in D with P=0.3451] (Fig. 3B). In addition, no differences in frequencies between high and low MIF levels were observed among different classifications of breast density (P=0.0686; Table III). In Table III, the cut-off value for high and low MIF was set as the median MIF level in all samples evaluated. Furthermore, no differences were observed among different classifications of breast density when the cut-off value was set as the median MIF value in patients with mammary density classification A [median 1,986.0 pg/ml; P=0.0956; data not shown].

MIF serum levels increase with aging. Aging has been described as a state related to low-level systemic chronic inflammation (9). In agreement, the current study identified a weak but significant positive correlation (r_S=0.1558, P=0.0239) between increased levels of MIF and increased age (Fig. 4), indicating that MIF may be related to chronic inflammation during aging.

Serum MIF levels are not related to other cancer risk factors. In humans, low levels of systemic chronic inflammation have also been related to age-associated chronic diseases, such as cancer (9). Thus, it was hypothesized that low levels of chronic inflammation would be evident in women exposed to diverse cancer risk factors. However, no relationship was identified between the following risk factors and increased MIF serum levels: Tobacco use (P=0.1260; Fig. 5A), alcohol consumption (P=0.0586; Fig. 5B), number of births (P=0.4330; Fig. 5C), contraceptive use/type (P=0.5279; Fig. 5D and P=0.1876; Fig. 5E), breastfeeding (P=0.8093; Fig. 5F), age of menarche (P=0.3906; Fig. 5G), age of menopause (P=0.1533; Fig. 5H), previous mammary lesions (P=0.5147; Fig. 5I), previous tuberculosis (P=0.4313; Fig. 5J), radiation exposure (P=0.096; Fig. 5K) or a family history of cancer (P=0.6700; Fig. 5L).

Discussion

Recent evidence has shown that MIF has an important role in diverse diseases, including inflammatory, metabolic disease (19) and cancer (1,20,21). The current study identified an association between high serum MIF levels in women and a high BMI, high WHR and hypertension, and revealed a slight correlation between MIF levels and increased age. Furthermore, a decrease in MIF levels was detected in women with BI-RADS classifications 2 and 3, which have a slightly higher chance of having breast cancer when compared with patients classified as BI-RADS 1. Notably, no difference was identified between patients classified as BI-RADS 4, who have a higher chance of having breast cancer, when compared with those classified as BI-RADS 1. These findings indicate that elevated MIF serum levels are related to metabolic disease and chronic inflammation, rather than to exposure to breast cancer risk factors.

MIF has been implicated in obesity-related chronic inflammation, and high levels of MIF and its receptors have been

Table II. Association between MIF serum levels and BI-RADS categories (n=162 patients).

Category	BI-RADS 1, n (%)	BI-RADS 2, n (%)	BI-RADS 3, n (%)	BI-RADS 4, n (%)	P-value
MIF high	8 (50.0)	17 (22.6)	8 (14.3)	3 (21.4)	0.0273[a]
MIF low	8 (50.0)	58 (77.3)	48 (85.7)	11 (78.6)	

MIF serum levels were split into high and low based on the median value in patients with BI-RADS classification 1 (2,146.0 pg/ml). Data were compared using χ^2 test. [a]P<0.05. BI-RADS, Breast Imaging Reporting and Data System; MIF, macrophage migration inhibitory factor.

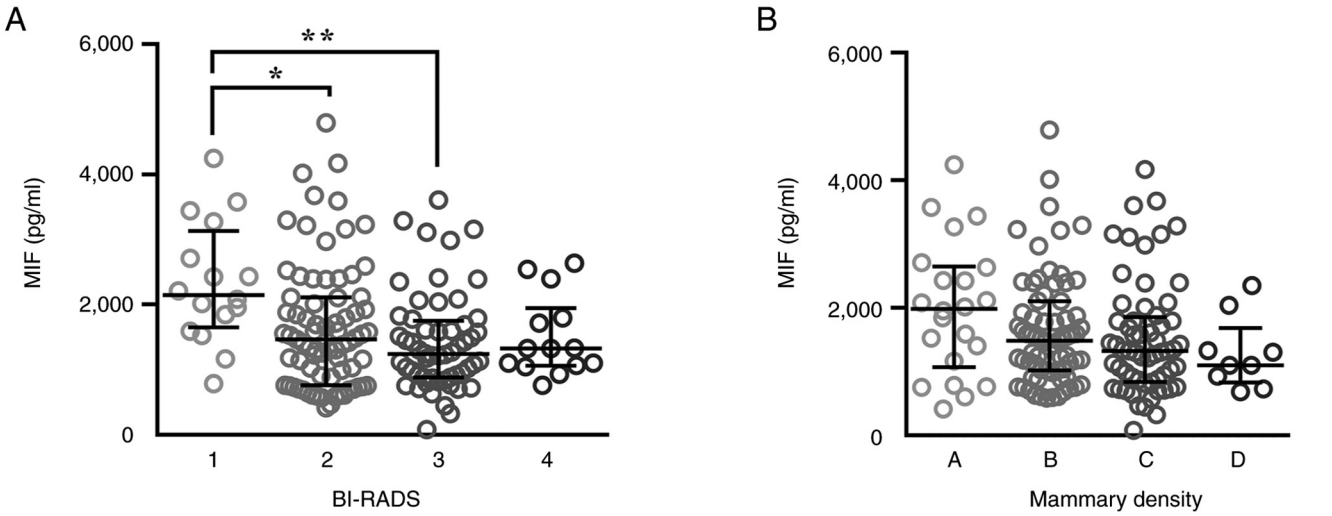

Figure 3. MIF serum levels are decreased in patients with BI-RADS classifications 2 and 3. MIF serum levels were assessed by ELISA in women classified according to (A) BI-RADS classification or (B) mammary density, in agreement with the mammography report. A Kruskal-Wallis test with Dunn's post-hoc for multiple comparisons was used. The graphs show data from (A) 161 and (B) 162 samples; horizontal lines indicate the median value, and the upper and lower small horizontal lines indicate the interquartile range (Q1-Q3) for each category. Only those pairwise comparisons which had a significance value of P<0.05 are shown. *P<0.05, **P<0.01. BI-RADS, Breast Imaging Reporting and Data System; MIF, macrophage migration inhibitory factor.

reported in VAT (22,23). Elevated levels of MIF expression in VAT were initially attributed to infiltrating macrophages, although adipocytes have also been shown to express and secrete MIF, which may contribute to its circulating levels in obese patients (19,24). Thus, in obesity, VAT may be considered an important source of MIF, with possible roles in the maintenance of inflammation in visceral and peripheral tissues. Besides regulating inflammation, MIF has been shown to have an important role in the regulation of metabolism. MIF has been reported to be increased in the plasma of male mice fed a high-fat diet as well as in aged animals (2,25), and it has been shown to favor lipid accumulation during obesity. Notably, blocking extracellular MIF has been reported to decrease obesity in high-fat diet-fed mice (25). Moreover, MIF secretion decreases WAT lipoprotein lipase expression, avoiding circulating triglyceride hydrolysis and lipid accumulation, inducing hypertriglyceridemia (2). These studies underscore an important role for MIF in the regulation of lipid accumulation in adipocytes during obesity, in the promotion of obesity-associated alterations, such as hypertriglyceridemia, and indicate a therapeutic potential of inhibiting MIF in the treatment of obesity and its related co-morbidities.

Previous studies have reported elevated MIF levels in obese patients (19). For example, MIF has been reported to be upregulated in subcutaneous abdominal adipose tissue from obese individuals compared with in lean male subjects (23). Furthermore, the mRNA levels of MIF and one of its receptors, CCR2, have been found to be elevated in VAT when compared with subcutaneous adipose tissue in women (22), and MIF levels have been reported to decrease upon weight loss (19). Thus, circulating MIF levels could reflect adipocyte accumulation and more specifically, VAT accumulation. Accordingly, the present findings revealed that MIF levels were increased in obese patients with a high WHR when compared with those with a moderate WHR, suggesting that high serum MIF levels in women may be related to the accumulation of visceral fat, possibly due to its direct production by VAT.

Visceral fat is considered a marker of ectopic fat deposition. It accumulates in or around organs and impairs their function, increasing disease risk, secreting pro-inflammatory cytokines, homing innate and adaptive immune cells, and has thus been strongly associated with cardiometabolic disease (26,27). Elevated circulating MIF levels have also been related to type 2 diabetes and insulin resistance (19), and, although some discrepancies exist with respect to normal circulating MIF levels (19) or sex-dependent effects, it is generally accepted that MIF is increased in obesity, type 2 diabetes, gestational diabetes and in patients with insulin resistance (28). Moreover,

Table III. Association between MIF levels and mammary density (n=162 patients).

Category	A, n (%)	B, n (%)	C, n (%)	D, n (%)	P-value
MIF high	15 (68.2)	38 (53.5)	26 (43.3)	2 (22.2)	0.0686
MIF low	7 (31.8)	33 (46.5)	34 (56.7)	7 (77.8)	

Serum MIF levels were split into high and low based on the median value in all patients (1,423.0 pg/ml). Data were compared using χ^2 test. MIF, macrophage migration inhibitory factor.

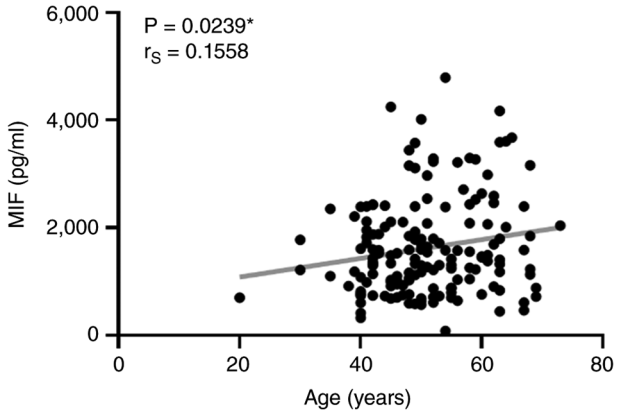

Figure 4. MIF serum levels show a slight correlation with increased age. MIF levels were assessed by ELISA. 162 samples were analyzed using a one-tailed Spearman correlation analysis (P=0.0239; r_S=0.1558). MIF, macrophage migration inhibitory factor.

increased circulating MIF levels have been detected in women with metabolic syndrome but not in men, suggesting a sex-specific effect of circulating MIF in this condition and possibly, in its related complications, such as atherosclerosis or type 2 diabetes (29). In this regard, both the estrogen receptor, estradiol and progesterone have been shown to modulate MIF expression in animal models of inflammatory disease (30,31), suggesting a different regulation of MIF expression in women than in men. Although the current study did not evaluate comorbidities such as type 2 diabetes, elevated levels of MIF were found in women with hypertension, implicating MIF in this obesity-associated comorbidity. Future studies should evaluate the role of MIF in hypertension and its possible association with hypertriglyceridemia, or in other complications related to metabolic syndrome such as type 2 diabetes and atherosclerosis.

MIF has also been demonstrated to be involved in other metabolic disorders affecting women. MIF levels have been shown to be elevated in women with PCOS (32), now referred to as PMOS (33), and MIF has been suggested to be involved in the pathogenesis of PMOS in animal models through the activation of MAPK or NF-κB pathways (34,35). PMOS is characterized by metabolic, reproductive and dermatological alterations, with obesity being prevalent in women with this syndrome and contributing to its severity (33). Thus, the fact that MIF is involved in the pathogenesis of metabolic disorders, together with the present findings of increased MIF in obese women with a high WHR and in patients with hypertension, indicated that further research is needed to identify the precise role of MIF in metabolic disorders and to evaluate its targeting for the treatment of these highly prevalent pathologies.

Circulating MIF levels can bind to its receptors in diverse target cells, activating MAPKs and cytosolic phospholipase A2 (cPLA2). MIF-induced cPLA2 activation promotes the production of inflammatory components, including prostaglandins, arachidonic acid and leukotrienes (3,5). Arachidonic acid release activates JNK/SAPK, which is responsible for inducing the translation of TNF-α mRNA, thus serving a key role in the maintenance of cytokine production and inflammation (5). Chronic elevated MIF levels could contribute to the maintenance of chronic inflammation associated with obesity and its comorbidities.

Diagnosis of clinical obesity should contain clinical confirmation of obesity, plus evidence of reduced organ or tissue function, or notable age-adjusted limitations of day-to-day activities associated with mobility or other basic activities due to obesity. Although BMI has been widely used in the diagnosis of obesity, it is not always an adequate measure for its diagnosis, leading to alternative methods for the clinical assessment of obesity. Thus, excess adiposity should be confirmed by other anthropometric criteria, such as waist circumference or direct fat measurement, to identify individuals at a higher metabolic risk (11). Preclinical and clinical obesity have been defined as conditions where negative health effects might occur, or have occurred, respectively. Moreover, metabolically healthy obese patients have been described, making it imperative to distinguish those patients with a higher metabolic risk (28). In the present study, MIF levels were increased in obese patients who had an elevated WHR and to hypertension. Since two criteria were used to define excess adiposity, one of which was related to visceral fat accumulation, the patients with a high BMI and elevated WHR may be considered as obese with a high metabolic risk, and those with hypertension could be considered as patients where negative health effects have occurred. Thus, MIF may be proposed as an indicator of metabolic risk, and its significance to metabolic risk and for the treatment of obesity-associated complications should be assessed.

The BI-RADS system standardizes breast cancer risk assessment and provides uniformity in mammography reports, where risk of malignancy goes from essentially zero (negative examination, BI-RADS 1), benign findings (BI-RADS 2), <2% chance of malignancy (BI-RADS 3), low-to-high chance (2-95%) of malignancy (BI-RADS 4) to a >95% chance of malignancy (BI-RADS 5). BI-RADS 6 classification is

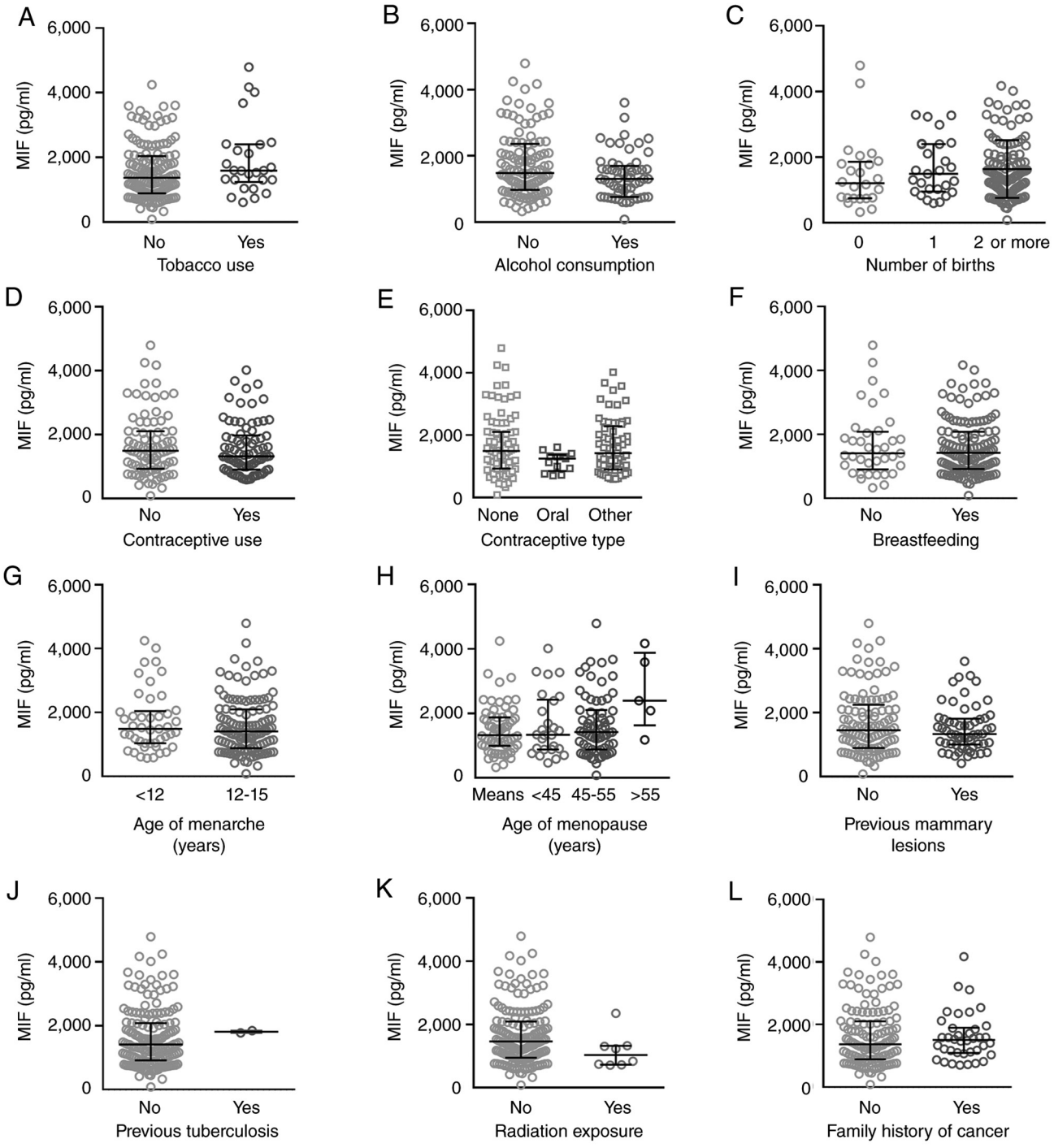

Figure 5. Evaluation of MIF serum levels and their possible association with diverse cancer risk factors. MIF serum levels were assessed by ELISA in women and classified according to (A) tobacco use, (B) alcohol consumption, (C) number of births, (D) contraceptive use, (E) type of contraceptive used, (F) breast-feeding, (G) age of menarche, (H) age of menopause, (I) previous mammary lesions, (J) previous tuberculosis, (K) exposure to radiation and (L) family history of breast cancer. The graphs show data from 162 samples with median and interquartile range (Q1-Q3) for each category. (A, B, D, F, G and I-L) When two groups were compared, a Mann-Whitney U test was used; (C, E and H) for more than two group comparisons, a Kruskal-Wallis test with Dunn's post-hoc test for multiple comparisons was used. No significant differences were revealed ($P<0.05$). MIF, macrophage migration inhibitory factor; mens, menstruating.

used to indicate a pathology-proven malignancy (17). In the current study, women with BI-RADS classifications 2 and 3 had decreased levels of MIF when compared with those with BI-RADS 1 classification. As aforementioned, MIF has been shown to be elevated in breast cancer cells, patients or tissues when compared with healthy women, non-tumorigenic controls or in women with higher stage cancers when compared to those with stage I breast cancer (36,37). In addition, abundant MIF expression in breast tumor tissue has been shown to be associated with markers of favorable prognosis (estrogen and progesterone receptor expression) and with increased overall survival (37). Since higher MIF levels are expected in patients with breast cancer when compared with patients without cancer and the present data shows decreased MIF levels in BI-RADS 2 or 3 patients, increased MIF levels in patients with breast cancer would probably show a more striking difference

when compared to BI-RADS 2 or 3 patients, but this assumption should be tested in the future. The reason as to why MIF levels were decreased in these patients is not clear and could be related to mammary density as discussed in the following paragraph.

When evaluating mammary density, a non-significant trend was observed, similar to the one observed regarding BI-RADS classification. Increased mammary density has been related to an enhanced breast cancer risk, increased breast calcifications and to a higher chance of developing aggressive cancer types. In addition, an increased BMI has been associated with decreased breast density due to increased breast fat (non-dense tissue in a mammogram) and reduced fibroglandular tissue (18,38). Since breast density and BMI are likely to be inversely related (38), the observed changes in MIF concentration with BI-RADS classification, as well as those apparent among mammary density classifications, might be related to the relationship between MIF and obesity. Although this was an unexpected finding, it indicates that high circulating MIF levels are not related to breast cancer risk factors, since they would be expected to be associated with increased BI-RADS classification or to increased mammary density. Moreover, MIF levels were not related to exposure to other breast cancer risk factors. These findings indicate that high levels of circulating MIF may be related to metabolic alterations, rather than to cancer risk factors.

Aging is associated with increased chronic inflammation with local and systemic manifestations (39). Inflammatory cytokines and biomarkers have been shown to increase with age, including C-reactive protein and IL-6, which are associated with a decrease in immune function (39). The present study identified a weak positive correlation between increased MIF levels and increasing age in women. Although MIF levels have been shown to be elevated in aged mice (2), to the best of our knowledge this is the first report showing increased MIF levels in elderly women, connecting MIF to other inflammatory biomarkers associated with aging. Since the current study population comprised women who requested screening mammography, it is important to enforce this conclusion with more data from younger women. Furthermore, it is important to establish a precise role for MIF in the regulation of organ and tissue decline with aging in women, with the aim of effectively targeting this cytokine and decreasing the effects of aging.

Several MIF inhibitors have been described with diverse mechanisms of action (3,4,40), and some natural products such as vanillic acid (41) or sulforaphane (42) have been used in diverse studies to block MIF function. Among MIF-targeting drugs, clinical trials have used drugs that inactivate MIF as an off-target, such as ibudilast, with promising results for multiple sclerosis treatment, but it is unclear to what extent its effects are due to MIF inhibition or to its effects on other targets (phosphodiesterase or TLR4). Also, an anti-oxidized MIF monoclonal antibody has been tested in patients with malignancies, and anti-CD74 monoclonal antibodies have also been tested for patients with systemic lupus erythematosus (43). Therefore, MIF may also be considered a promising target for the treatment of metabolic disorders such as obesity and its related complications, and could potentially decrease age-associated inflammation and its effects in cell and organismal function.

In conclusion, elevated serum MIF levels were shown to be related to states of chronic inflammation such as obesity, high WHR and aging in women. These findings suggest an important relationship between MIF and metabolic disease and VAT accumulation, and indicate a possible implication for MIF in the pathogenesis of obesity and its related comorbidities.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by FVD, KALR, NZB and JHP. LMFNLDG, FDASC and SOPZ were involved in data and sample collection and study supervision. The first draft of the manuscript was written by FVD, KALR, NZB and PM, and all authors commented on previous versions of the manuscript. FVC, KALR, NZB and PM confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Local Institutional Review Boards 21038 and 21048, from Instituto Mexicano del Seguro Social (Puebla, Mexico; approval nos. R-2024-2104-048 and R-2021-2103-011). Two ethics approval numbers are provided since patients were from two different Units and the protocol was registered in both local Review Boards. Written informed consent for participation was obtained from all individual participants included in the study. The authors certify that the study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments.

Patient consent for publication

Written informed consent for publication was obtained from all participants in the study.

Competing interests

The authors declare that they have no competing interests.

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