UNIVERSIDAD AUTÓNOMA DE SAN LUIS POTOSÍ

FACULTAD DE MEDICINA

Centro de Investigación en Ciencias de la Salud y Biomedicina (CICSaB)

EVALUACIÓN DE BIOMARCADORES DE RIESGO CARDIOVASCULAR EN POBLACIÓN EXPUESTA A ARSÉNICO (As) Y PLOMO (Pb)

TESIS QUE PRESENTA

M. C. ALEJANDRA GONZÁLEZ BRAVO

PARA OBTENER EL GRADO DE DOCTOR EN CIENCIAS BIOMÉDICAS BÁSICAS

DIRECTOR DE TESIS
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Agosto 2024

CREDITOS INSTITUCIONALES

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Evaluación de biomarcadores de riesgo cardiovascular en población expuesta a arsénico (As) y plomo (Pb)

Resumen

Las enfermedades cardiovasculares (ECV), son un grupo de trastornos que afectan al corazón y a los vasos sanguíneos, representan la principal causa de mortalidad y morbilidad, tanto a nivel mundial como en nuestro país. La condición patológica que los inicia es un proceso conocido como disfunción endotelial. En este contexto, se ha demostrado que existen factores de riesgo asociados al desarrollo de ECV, como el tabaquismo, la diabetes, la hipertensión arterial, la obesidad; y recientemente se ha confirmado que la exposición a compuestos tóxicos ambientales, como el arsénico y el plomo, representan un factor importante que puede desencadenar el inicio de este proceso patológico, dada su capacidad de generar especies reactivas de oxígeno. Por otro lado, los determinantes genéticos han sido reconocidos como factores de riesgo críticos en el inicio, desarrollo y establecimiento de enfermedades cardiovasculares, un ejemplo son los polimorfismos de un solo nucleótido (SNP), por ejemplo, diversas investigaciones han demostrado una asociación entre el polimorfismo del factor de crecimiento transformante β1 (TGF-β1) (C509T; rs1800469) y un mayor riesgo de ECV. Aunado a lo anterior, también se ha demostrado que tanto el arsénico como el plomo, inducen alteraciones a nivel epigenético (cambios en la estructura química del ADN), siendo la modificación de la expresión de ciertos ARN no codificantes (microARN) uno de ellos, debido a evidencias recientes sugieren que es capaz de influir en la expresión de microRNA (miRNA) lo que

afecta la función celular e influye en el riesgo de desarrollar ciertas enfermedades; además de que los miRNA responden a numerosos agentes tóxicos ambientales, lo que indica su utilidad como posibles biomarcadores de exposición. Entre los miRNA que se han asociado a la exposición a metales pesados se encuentran miR-126 y miR-155, los cuales aumentan o disminuyen su expresión con la exposición al plomo. Debido al gran impacto que generan las ECV la identificación adecuada de personas con alto riesgo de desarrollarlas es un gran desafío para su prevención. En este sentido, varias investigaciones científicas han propuesto numerosos biomarcadores no invasivos y de bajo coste para detectar poblaciones vulnerables; sin embargo, su utilidad en individuos expuestos a contaminantes ambientales y con predisposición genética (polimorfismo), no ha sido completamente investigada. Por tanto, el objetivo de este trabajo doctoral fue evaluar la concentración de biomarcadores de riesgo cardiovascular en población adulta expuesta a arsénico (As) y plomo (Pb).

Metodología

Se realizó un estudio transversal, de conveniencia, no probabilístico. La población de estudio fueron habitantes mayores de edad, ambos sexos, residentes de la comunidad Las Palmas, municipio de Tamuín, SLP. Esta localidad es considerada zona de riesgo debido a la cercanía de la comunidad con dos termoeléctricas y una planta cementera. La aceptación para participar en la investigación se obtuvo mediante la firma del consentimiento informado de la población de estudio. Se registraron las medidas antropométricas de cada individuo, así como se colectaron dos muestras de sangre y una de orina, las

cuales se resguardaron a -80°C y -20°C, respectivamente hasta su uso. Se midieron los parámetros bioquímicos (glucosa, triglicéridos, colesterol, HDL), empleando un método enzimático colorimétrico y un espectrofotómetro UV/Vis. Los niveles de As urinario y Pb en sangre se determinaron mediante la técnica de espectrofotometría de absorción atómica. Los biomarcadores de riesgo cardiovascular: ADMA y FABP4 se evaluaron mediante inmunoensayo enzimático tipo sándwich (ELISA), la expresión de miRNA-126 y miRNA-155 en plasma se determinaron mediante RT-qPCR y finalmente se analizó la presencia del polimorfismo TGF-β C509T (rs1800469), en las muestras de sangre y empleando la técnica de RT - PCR y usando sondas TaqMan. Las pruebas estadísticas empleadas fueron prueba de kolmogorov-smirnov, Shapiro wilk como pruebas para determinar la normalidad de los datos, y posteriormente se realizó una clasificación de la población en dos grupos (baja y alta exposición) utilizando como referencia las NOM-047-SSA1-2011 y NOM-199-SSA1-2000, se utilizó la prueba T-Student y prueba U de Mann-Whitney para la comparación de los grupos. Finalmente se empleó la prueba de Kruskal Wallis para la comparación de los grupos expuestos al contaminante ambiental con o sin presencia del polimorfismo. El análisis de datos se realizó utilizando GraphPad Prism 5.01 (GraphPad Software Inc. La Jolla, CA, EE. UU.).

Resultados

La población de estudio estuvo conformada por 53 adultos, con un intervalo de edad de 18 a 87 años, de éstos, el 64% son mujeres. Con una media de peso corporal de 68.9 kg y más del 70% de los individuos presentó un IMC por encima

del valor de referencia. Respecto a los parámetros bioquímicos la población presentó niveles medios ± desviación estándar de triglicéridos y colesterol LDL de 125.6 ± 70.04 mg/dL y 94.3 ± 14.01mg/dL, respectivamente. Los niveles urinarios de As oscilaron entre 11.5 y 175 µg/g de creatinina, con una media de 43.9 µg/g creatinina y los niveles de Pb en sangre tuvieron una media ± desviación estándar de 7.6 ± 5.8 µg/dL. La frecuencia alélica para el polimorfismo TGF-β C509T fue de 0.37 y 0.63 para los alelos C y T, respectivamente, por lo que más del 80% de los participantes tiene la presencia del polimorfismo analizado. Los niveles séricos medios de ADMA y FABP4 fueron 1.35 ± 0,35 µmol/l y 26.9 ± 19.40 ng/ml, respectivamente. Por su parte, los niveles séricos circulantes de miR-126 presentaron niveles de 1,28 ± 0,7; mientras que miR-155 presentó niveles de 1,68 ± 1,2. Se detectaron asociaciones estadísticamente significativas ($p< 0,05$) entre los biomarcadores de exposición (As y Pb) y los biomarcadores de riesgo cardiovascular (ADMA y FABP4), así como asociaciones significativas entre miR-126 y los niveles de plomo en sangre y entre miR-155 y los niveles de plomo y triglicéridos en sangre.

Conclusiones

Se detectó una asociación positiva entre los niveles séricos de biomarcadores predictivos de ECV (ADMA, FABP4 y AIP) y los altos niveles de exposición al arsénico y plomo (factor ambiental) y el polimorfismo TGF-β C509T (factor genético). Además, nuestros hallazgos sugieren una interacción gen-ambiente sobre los niveles de los biomarcadores de ECV evaluados en esta investigación. En consecuencia, la población evaluada podría presentar riesgo de desarrollar

eventos cardiovasculares. Sin embargo, se necesita investigación adicional para aclarar mejor estas asociaciones, particularmente con estudios epidemiológicos en población abierta.

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ORIGINAL ARTICLE

Influence of arsenic exposure and TGF-β gene single nucleotide polymorphisms (gene-environment interaction) on cardiovascular risk biomarkers levels in Mexican people from San Luis Potosi, Mexico

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Abstract

Objective Cardiovascular diseases (CVD) are the primary cause of death worldwide. Therefore, this investigation aimed to evaluate the effect of arsenic exposure and genetic polymorphism (rs1800469) on cardiovascular risk biomarkers levels in a Mexican adult population.

Methods Then, a cross-sectional study was completed, including 155 adults. Urinary arsenic (UAs) levels were analyzed as an exposure biomarker, and assessed cardiovascular risk biomarkers were: serum ADMA and FABP4 concentrations and atherogenic indices (Castelli risk index and atherogenic index of plasma). Gentrification of TGF-β C509T polymorphism (rs1800469) was determined by real-time polymerase chain reaction (PCR) using TaqMan probes.

Results UAs levels ranged from 11.5 to 175 µg/g creatinine. The allele frequency for TGF-β C509T polymorphism was 0.37 and 0.63 for the C and T alleles, respectively. Mean serum ADMA and FABP4 levels were 1.35 ± 0.35 µmol/L and 22.0 ± 9.50 ng/mL, respectively. Also, statistically significant associations ($p < 0.05$) were detected between the exposure biomarker (UAs) and the cardiovascular risk biomarkers (ADMA and FABP4). Similarly, a strong relationship was detected between the TGF-β C509T polymorphism and assessed cardiovascular risk biomarkers (ADMA and FABP4). In addition, a gene-environmental interaction was found.

Conclusion Our findings suggest a gene-environment interaction with an enhanced effect on CVD biomarkers.

Keywords ADMA · Arsenic · Atherogenic index of plasma · Cardiovascular diseases · FABP4 · TGF-β C509T polymorphism

Introduction

Cardiovascular diseases (CVD) are recognized as the primary cause of morbidity and mortality worldwide [1]. In this line, multiple risk factors are associated with the development of CVD, such as smoking, diabetes, arterial hypertension, obesity, genetic determinants, and environmental factors, among others [1]. Concerning environmental risk factors, several studies have shown a positive association between human exposure to several toxic compounds and an incremented susceptibility to the development of cardiovascular diseases [2]. Recently, Nuvolone et al. found a strong association between exposure to high concentrations of inorganic arsenic via drinking water (> 10 µg/l) and the development of cardiovascular diseases in a population-based cohort [2]. In the central region of Mexico, where San Luis Potosí state is located, high levels (> 25.0 µg/L; Mexican guideline) of inorganic arsenic have been found in groundwater samples. Besides, groundwater supplies approximately 50% of the drinking water in this region of Mexico. Therefore, an elevated proportion of people living in San Luis Potosi state are exposed to concerning concentrations of inorganic arsenic through drinking water consumption [3].

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Also, genetic determinants have been recognized as critical risk factors in the onset, development, and establishment of cardiovascular diseases [4]. For example, emerging epidemiological investigations have demonstrated a strong association between the transforming growth factor-β1 (TGF-β1) polymorphism (C509T; rs1800469) and an increased risk for CVD [5, 6]. The TGF-β protein is involved in inflammatory response modulation, cell proliferation, immune response, matrix synthesis, and tissue repair, among others [6]. The TGF-β1 gene in human cells is located on chromosome 19q13 with seven exons that encode a peptide of approximately 390 amino acids [6, 7]. Besides, several single nucleotide polymorphisms (SNPs) have been detected in this gene, as previously demonstrated elsewhere [7]. The C509T TGF-β polymorphism is found in the promoter region of the gene at nucleotide 509 (a substitution of cytosine for thymine) [7]. The C → T substitution is associated with an increment in the production of the TGFβ1 protein [8]. Moreover, the augmented expression of the TGFβ1 cytokine is related to an increased risk of developing several diseases, including CVD [9, 10].

The World Health Organization (WHO) has established that the best tactic to combat CVD is to prevent it [11]. Then, early and precise recognition of high-risk individuals is imperative to reduce these diseases. In this regard, several scientific investigations have proposed numerous non-invasive and low-cost biomarkers to detect vulnerable populations. [12, 13]. However, their utility in individuals exposed to environmental pollutants and with a genetic predisposition (polymorphism) has not been fully investigated.

Although the C509T TGF-β polymorphism and arsenic exposure have been associated independently with CVD, the interplay between both factors (gene-environment interaction) on cardiovascular risk in different populations has been poorly investigated. Therefore, this investigation aims to evaluate the effect of inorganic arsenic exposure and the C509T TGF-β polymorphism (gene-environment interaction) on cardiovascular risk biomarkers levels in an adult population from San Luis Potosí, Mexico.

Results and discussion

Anthropometric measurements, clinical characteristics, and biochemical profiles

Table 1 describes the anthropometric measurements, clinical characteristics, and biochemical profiles obtained from the enrolled participants. Approximately 64% of participants were women, and the age range was: 18–87 years (mean = 50.5 ± 20.0). Overweight and obesity were detected in approximately 70% of the included population (mean

Table 1 General characteristics of the population

Variables	Mean (SD*)	Median	Range	% > reference value#
Age (years)	50.5 (20.0)	53.0	18.0–87.0	–
Weight (Kg)	68.5 (15.0)	67.0	43.0–100	–
Height (cm)	155 (9.50)	155	140–185	–
Waist/hip index	0.95 (0.05)	0.90	0.80–1.10	91.0
Body mass index (kg/m^2)	28.0 (5.00)	27.5	19.5–40.5	–
(*Z* score) Normal (18.5–24.9)	22.0 (1.80)	22.0	19.5–25.0	30.0
Overweight (25.0–29.9)	27.5 (1.50)	27.5	25.0–30.0	39.0
Obesity (> 30)	34.5 (3.50)	34.5	30.0–40.5	30.0
Diastolic blood pressure (mmHg)	72.5 (10.0)	76.0	55.0–90.0	10.0
Systolic blood pressure (mmHg)	120 (15.0)	118.0	100–145	20.0
Glucose (mg/dL)	94.5 (37.0)	87.0	59.0–220	15.0
Triglycerides (mg/dL)	135 (70.0)	125	87.0–260	33.0
Total cholesterol (mg/dL)	165 (45.0)	175	75.0–275	25.0
HDL cholesterol (mg/dL)	41.5(35.0)	35.0	17.5–200	15.0
LDL cholesterol (mg/dL)	27.5 (14.5)	25.5	5.00–78.0	0.0
Urinary arsenic (μg/g creatinine)	45.0 (30.0)	40.0	11.5–175	55.0

Data are represented as arithmetic mean with standard deviation (SD) and median with range from minimum to maximum. #Reference value. Waist/hip index = 0.8, Systolic blood pressure = 130 mmHg. (James et al. [16]). Diastolic blood pressure = 90 mmHg (James et al. [16]). Glucose = 110 mg/dL. (Iii et al. [17]). Triglycerides = 150 mg/dL. (Iii et al. [17]). Total cholesterol = 200 mg/dL. (Iii et al. [17]). HDL cholesterol = 45 mg/dL. (Iii et al. [17]). LDL cholesterol = 100 mg/dL. (Iii et al. [17]).—variables without reference values

BMI = 28.05 kg/m^2). Also, all of the participants had a waist/hip index greater than 0.8. Both parameters (waist/hip and BMI) are considered important risk factors in cardiometabolic disease development [14]. Respecting SBP and DBP, levels ranged from 100.0 to 145 mmHg for SBP; and for DBP from 55.0 to 90.0 mmHg. For biochemical analyses, 33% of the participants had TG levels above 150 mg/dL, while 25% had serum TC concentrations above 200 mg/dL. Besides, HDL cholesterol levels ranged from 17.5 to 200 mg/dL [15].

Urinary arsenic determination

The results of urinary arsenic concentrations are shown in Table 1. A mean urinary arsenic level of 45.0 µg/ g creatinine was found in the assessed population (range: 11.5–175 µg/g creatinine), and 55% of enrolled individuals have urinary arsenic levels above the reference value in Mexico (35 µg/g creatinine) [18]. According to this guideline, the assessed population was classified into two groups, low exposure (≤ 35 µg/g creatinine) and high exposure (> 35 µg/g creatinine). Mean values of urinary arsenic of 23.5 ± 6.50 µg/g creatinine and 58.5 ± 32.0 µg/g creatinine were detected for low and high exposure groups, respectively. Also, the mean urinary arsenic concentration detected in the high exposure group (58.5 ± 32.0 µg/g creatinine) is higher than the prevention limit value recommended by the CDC (Center for Disease Control) in the USA of 50 µg/g creatinine [19]. Regarding urinary arsenic levels in national surveys performed around the world, the 2003–2014 National Health and Nutrition Examination Survey (NHANES) in the USA found a mean urine arsenic level of 8.50 µg/g creatinine in individuals aged 20 years and older [20]. The French National Nutrition and Health Study (ENNS) quantified a mean concentration of urinary arsenic of 8.90 µg/g creatinine in adults aged 18–30 years [21]. For studies carried out in Mexico, Sánchez-Rodríguez et al. found mean urinary arsenic levels of 19.0 µg/g creatinine in people aged 18–53 years old in the northern region of Mexico [22]. Similar results were found by Gamboa–Loira et al., a mean urinary arsenic level of 20.2 µg/g creatinine of arsenic in urine was detected in women aged 18 years and older from the following Mexican states: Chihuahua, Coahuila, Durango, Nuevo Leon, and Sonora [23]. In conclusion, high exposure levels to arsenic were detected in this research.

TGF-β polymorphism (C509T)

In this study, the allele frequencies (C509T gene polymorphism) were 0.37 and 0.63 for the C and T alleles, respectively (Table 2). Regarding the genotypic frequencies, 14% of the assessed population were homozygous for the C allele (CC genotype), 40% were homozygous for the T allele (TT genotype), and 46% were heterozygous for the C509T gene polymorphism (CT genotype) (Table 2). The χ^2 test for Hardy–Weinberg equilibrium was used to assess the difference between expected and observed allele frequencies [24]. No significant deviation in the C/T allele frequencies was observed from the Hardy–Weinberg equilibrium ($\chi^2 = p > 0.05$).

Table 2 Genotypic and allelic frequencies of the TGF-β C509T polymorphism in the assessed population

Genotype	%
CC	14
CT	46
TT	40
Allele	Frequency
C	0.37
T	0.63

The Transforming Growth Factor-β (TGF-β) proteins family is essential in intercellular communication processes in multicellular organisms [25]. However, the proteins impact a broad range of signaling pathways that influence numerous cellular processes [26]. These involve but are not restricted to, embryogenesis, tissue responses to injury, regulation of the immune system, carcinogenesis, cell proliferation, extracellular matrix degradation and synthesis, and cell migration [27]. Besides, the alterations in the production and/or function of TGF-β proteins have been associated with several human diseases, including CVD [28]. In this line, genetic polymorphisms of crucial proteins in different signal pathways have been related to CVD. Some genetic polymorphisms located in the TGF-β gene are associated with CVD development, including L10P, R25P, and T869C [5]. Concerning the C509T polymorphism situated in the TGF–β promoter region, several studies have shown that the presence of the T allele is associated with an overproduction of the TGF-β protein and an increased risk in the development of CVD [29]. Recently, Koch et al. demonstrated a higher risk of myocardial infarction for the carriers homozygous of the T allele (TT genotype) compared to those homozygous for the C allele (CC genotype) and heterozygous (CT genotype) in an adult German population [30]. Peng et al. concluded a case–control study in a Chinese population (aged 42 to 76 years), an increased T allele frequency in patients at risk of atherosclerotic stroke compared to the control group was found [31]. Although several studies have demonstrated strong associations between the C509T polymorphism and high risk of cardiovascular diseases, insufficient studies have been performed to evaluate synergic effects between the presence of the C509T polymorphism in populations exposed to environmental contaminants (gene-environment interaction) (Fig. 1).

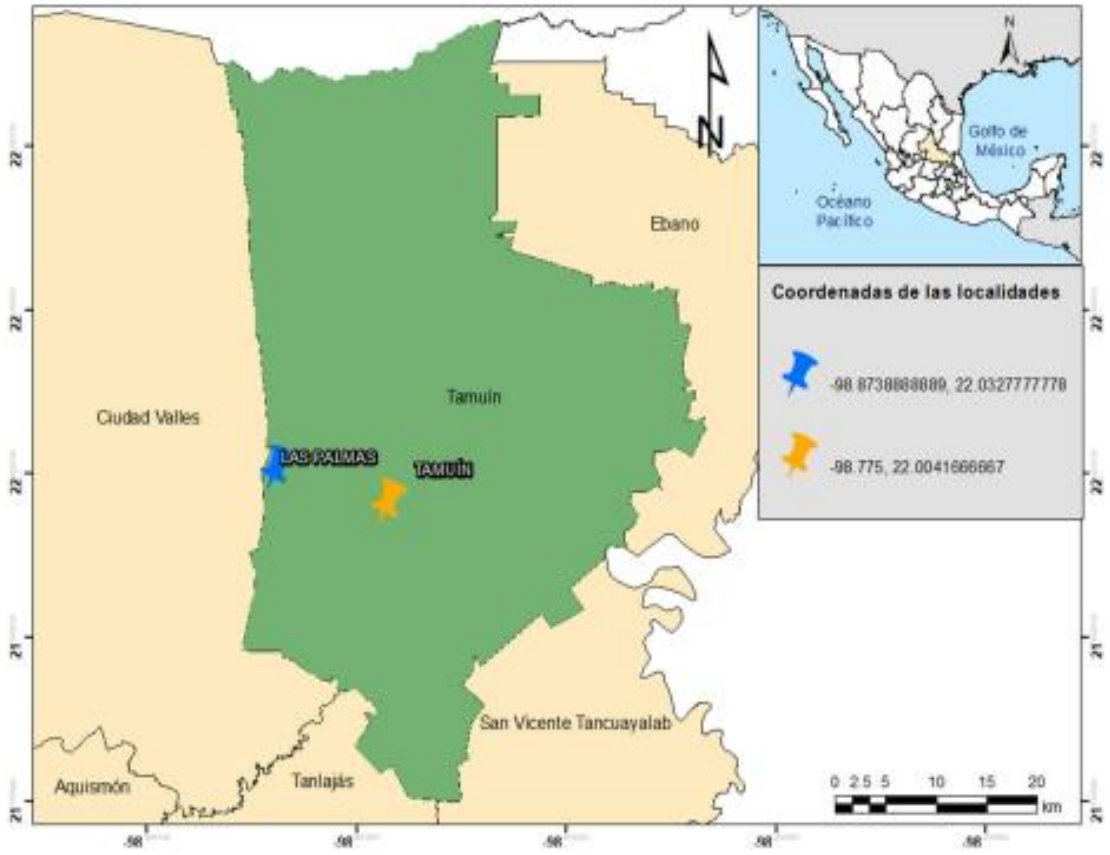

Fig. 1 The geographical location of the Ejido "Las Palmas", Tamuin municipality, San Luis Potosi, Mexico

Table 3 Assessed CVD biomarkers in the enrolled population

CVD Biomarkers	Mean (SD)	Median	Range
ADMA	1.35 (0.35)	1.20	0.20–3.60
FABP4	22.0 (9.50)	22.5	4.10–96.5
Atherogenic index of plasma (AIP)	0.50 (0.20)	0.55	− 0.20 to 1.15
Castelli's risk index (CRI)	5.10 (2.25)	4.80	1.25–11.5

Data are represented as arithmetic mean with standard deviation (SD) and median with range from minimum to maximum

Serum CVD biomarker concentrations

Mean circulating levels of ADMA and FABP4 were 1.35 ± 0.35 µmol/L and 22.0 ± 9.50 ng/mL (mean ± standard deviation), respectively (Table 3). In addition, approximately 60% of assessed women had levels above the guideline suggested for serum ADMA (0.71 µmol/L) [32]. Concerning serum FABP4 concentrations, nearly 55% of participating women had serum FABP4 levels higher than 18.6 ng/mL (proposed cut-off value) [33]. ADMA levels were significantly higher ($p < 0.05$) in the high exposure group (1.45 ± 0.75 µmol/L) compared to serum ADMA concentrations detected in the low exposure group (0.75 ± 0.35 µmol/L) (Fig. 2A). Similar results were found when serum FABP4 levels were explored, significantly higher levels ($p < 0.05$) were quantified in the high exposure group (30.0 ± 10.0 ng/mL) in comparison to mean FABP4 concentrations detected in individuals categorized in the low exposure group (16.0 ± 8.50 ng/mL) (Fig. 2B). Besides, when serum ADMA levels were analyzed according to TGF-β C509T polymorphism (CT and TT genotypes were combined assuming to have a dominant allele effect), significantly higher levels ($p < 0.05$) were detected in people with the CT/TT genotype (1.25 ± 0.30 µmol/L) compared to ADMA concentrations in individuals with the CC genotype (0.65 ± 0.35 µmol/L) (Fig. 3A). Similarly, significantly higher ($p < 0.05$) serum FABP4 levels were found in the CT/TT genotype (24.0 ± 9.00 ng/mL) group, contrasted to the CC genotype (18.0 ± 9.00 ng/mL) groups (Fig. 3B). Also, a multivariate linear regression analysis was accomplished,

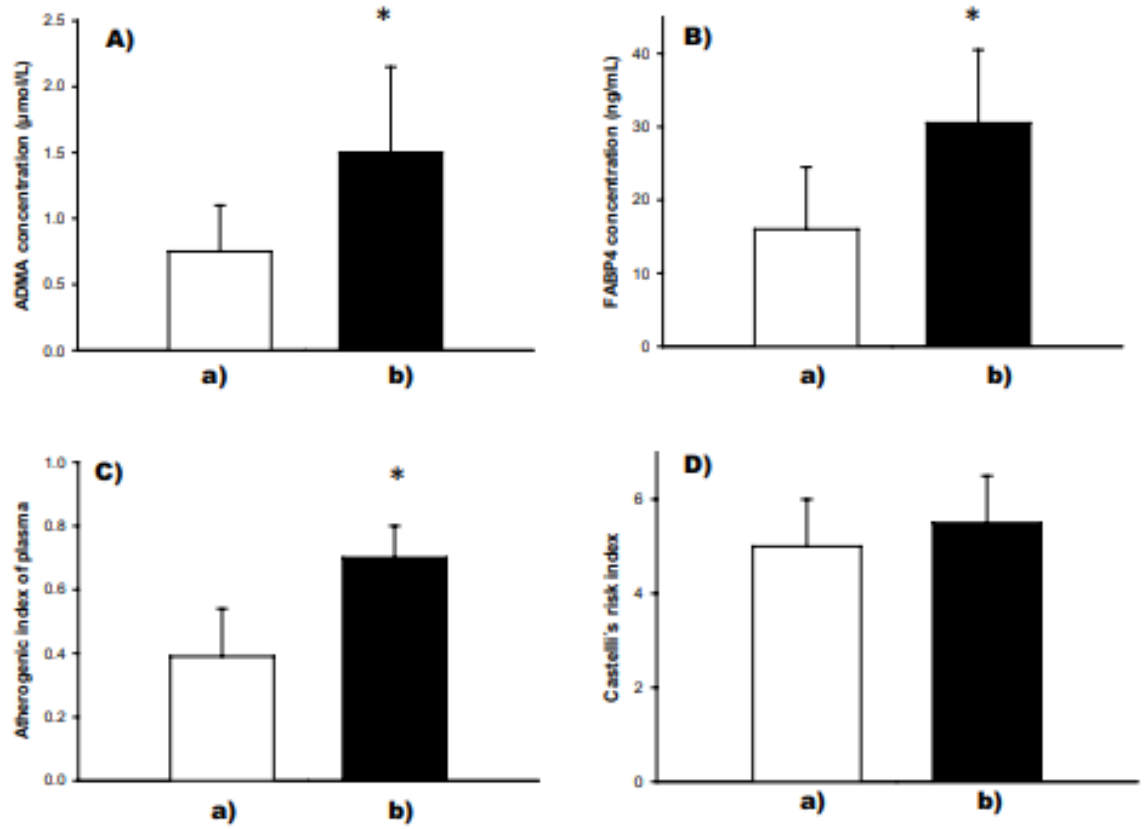

Fig. 2 Assessed CVD biomarkers in enrolled population by exposure levels. **A** Serum ADMA concentrations; **B** Serum FABP4 concentrations; **C** Atherogenic index of plasma; **D** Castelli´s risk index. (a) Low exposure group; (b) High exposure group. *$p < 0.05$

and all assessed variables were included in the models (Table 4). Significant predictors ($p < 0.05$) for serum ADMA concentrations were urinary arsenic levels, TGF-β C509T polymorphism, and waist/hip ratio (Table 4). Regarding FABP4 analysis, significant predictors ($p < 0.05$) for serum FABP4 levels were TGF-β C509T polymorphism and waist/hip ratio (Table 4).

In this regard, an increasing number of emerging scientific research have recognized ADMA as usefulness and reliable biomarker in the early identification of high-risk individuals to develop CVD [34], as serum ADMA levels are increased in different adverse clinical circumstances linked with cardiovascular events. For example, Avci et al. conducted a case–control study in healthy normal-weight, and obese Turkey individuals. Significant higher ADMA levels were detected in the obese groups compared to ADMA concentrations quantified in the healthy individuals. Besides, mean ADMA levels in subjects categorized as obese were higher than 0.71 µmol/L, a guideline considered safe [35]. Also, a recent cross-sectional study developed in Bosnian individuals suggests an augmented cardiovascular risk for diabetic people compared to healthy subjects, as serum ADMA concentrations were higher in diabetic individuals (1.81 ± 0.15 µmol/L) compared to controls subjects (0.62 ± 0.15 µmol/L) [36]. Gamil et al. 2020 performed an investigation that aimed to evaluate serum ADMA levels in patients with diagnosed essential hypertension and paired control subjects in a Sudanese population. Mean serum ADMA concentrations were significantly higher in individuals with essential hypertension (0.69 ± 0.33 µmol/L) than ADMA levels detected in the healthy group (0.62 ± 0.24 µmol/L) [37]. Furthermore, Gasecka et al. 2021 found significantly higher mean levels of serum ADMA (0.60 ± 0.10 µmol/L) in patients after acute myocardial infarction (AMI) in comparison to serum ADMA concentrations detected in healthy controls (0.54 ± 0.08 µmol/L) [38].

Regarding FABP4, numerous recent investigations have demonstrated its key role as a cardiovascular biomarker in different populations [39]. Yeung et al. 2007 examined

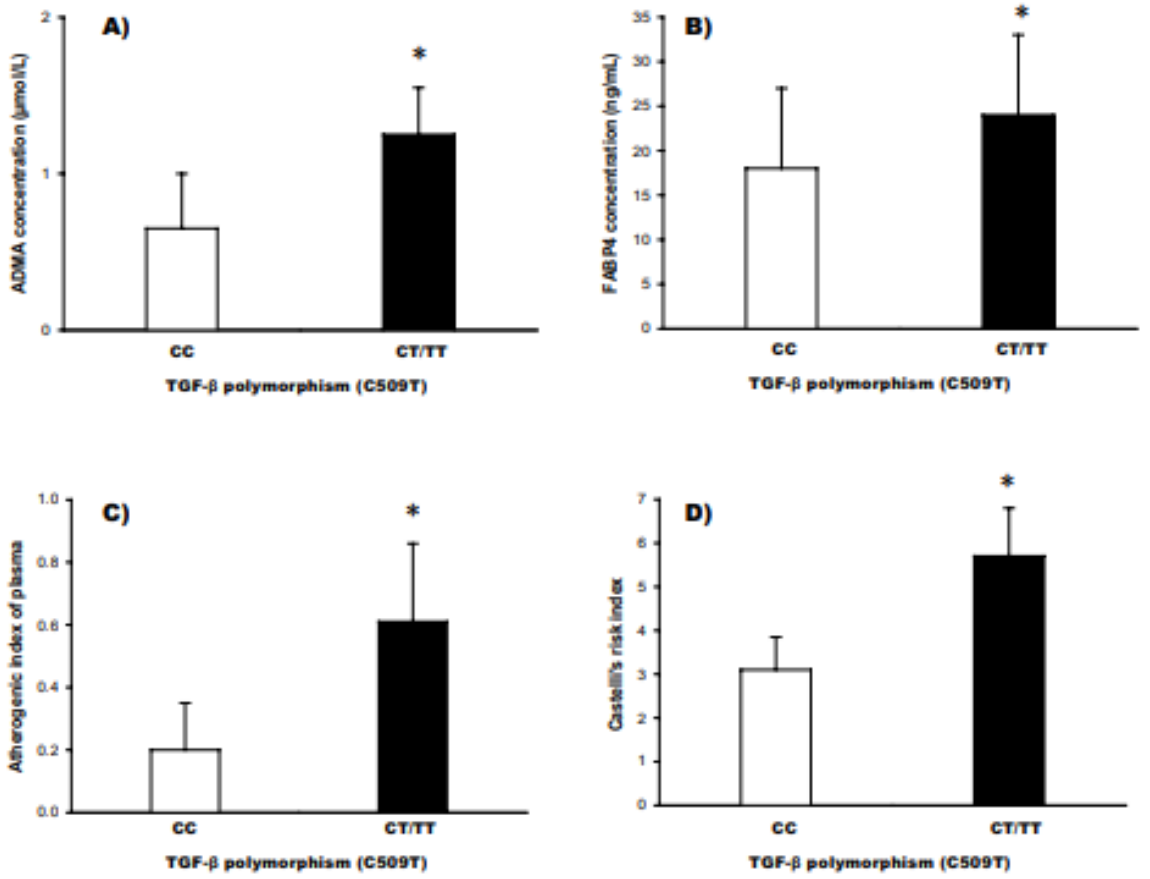

Fig. 3 Assessed CVD biomarkers in enrolled population according to TGF-β C509T genotype. **A** Serum ADMA concentrations; **B** Serum FABP4 concentrations; **C** Atherogenic index of plasma; **D** Castelli´s risk index. *$p < 0.05$

Table 4 Significant predictors of serum ADMA and FABP4 concentrations in the forward multiple linear regression model

Dependent variable	Atherogenic variables	β	SE	p	R^2 of the model
ADMA	Constant	2.20	0.80	0.01	0.0001
(µmol/L)	Arsenic (µg/g creatinine)	0.01	0.0035	0.002	
	Waist/hip ratio	2.25	0.60	0.0003	
	TGF-β polymorphism	0.45	0.10	0.0005	
FABP4	Constant	45.0	17.5	0.01	0.0005
(ng/mL)	Waist/hip	4.45	16.28	0.0001	
	TGF-β polymorphism	3.97	2.34	0.025	

All assessed variables in this investigation (Table 1) were included in the multiple linear regression analysis

β, standardized regression coefficients; SE, standard error; C, estimated intercept value

the association between serum FABP4 levels and the well-recognized atherosclerotic biomarker, the carotid intima-media thickness (IMT). The investigation was developed in a women Chinese population. The findings showed a significant correlation between both markers (FABP4 and IMT), supporting the idea that FABP4 can be used as a reliable biomarker in the identification of high-risk individuals to develop CVD [40]. Ota et al. performed a case–control study to assess the association between serum FABP4 concentrations with hypertension in a Japanese population. Higher

serum FABP4 levels were detected in patients with essential hypertension (22.5 ng/ml) compared to levels found in healthy individuals (15.0 ng/ml) [41]. Also, Kajiya et al. demonstrated higher serum FABP4 levels in CAD (coronary artery disease) patients (23.4 ng/ml) compared to FABP4 concentrations in healthy individuals (15.0 ng/mL), the findings in Kajiya et al. indicate that FABP4 plays a critical role in the development of CVD such as CAD [42].

Atherogenic indices

For Castelli's risk index (CRI), a mean value of 5.10 ± 2.25 (mean $\pm$ standard deviation) was estimated (Table 3). Besides, approximately 50% of enrolled individuals are at high risk of CVD, according to the CRI guideline (CRI > 4.5, high risk) [43]. Regarding the atherogenic index of plasma (AIP), a mean value of 0.50 ± 0.20 was found (Table 3). Also, nearly 50% of participants are classified as at high risk according to AIP guideline values (AIP > 0.21) [44]. Next, the enrolled population was categorized according to urinary arsenic levels as follows: low exposure group (< 35 µg urinary arsenic/g creatinine) and high exposure group (> 35 µg urinary arsenic/g creatinine). Significant higher AIP values ($p < 0.05$) were found in the high exposure group (0.60 ± 0.15) compared to AIP levels detected in the low exposure group (0.40 ± 0.25) (Fig. 2C). No significant ($p > 0.05$) differences were observed for CRI values when low and high exposure groups were contrasted (Fig. 2D). Also, significant differences ($p < 0.05$) were perceived through TGF-β C509T polymorphism, higher AIP values were detected in individuals with the CT/TT genotype (0.61 ± 0.25) compared to AIP value found in people with the CC genotype (0.20 ± 0.15) (Fig. 3C). Similar results were detected when CRI was analyzed through TGF-β C509T polymorphism, CT/TT genotype (5.7 ± 1.10), and CC genotype (3.1 ± 0.75) (Fig. 3D). A multivariate linear regression analysis was completed to evaluate predictive variables for AIP and CRI. However, the performed models for both dependent variables were not significant ($p < 0.05$).

A useful tactic in the battle against CVD is the early and accurate detection of high-risk individuals. In this sense, some atherogenic indices have been suggested as effective alternatives in the risk prediction of CVD events. For example, Niroumand et al. demonstrated in a cross-sectional study (Irani population; $n = 1000$) that AIP could be used as a sensible and predictive CVD biomarker in the general population, specifically when other risk factors are not modified [45]. Also, Cai et al. in a case–control investigation (Chinese population; $n = 5387$) have shown that AIP is an independent and effective predictor biomarker for cardiovascular diseases [46]. Besides, the study by Cai et al. suggests that AIP is a better biomarker to predict CVD risk than traditional lipid biomarkers such as TG, TC, LDL, atherosclerosis index (AI), lipoprotein combined index (LCI), among others [46]. Regarding Castelli´s risk index, Bhardwaj et al. completed a case–control investigation on an Indian population that aimed to evaluate atherogenic indexes such as Atherogenic Coefficient (AC), AIP, and CRI to the identification of high-risk individuals for Coronary Artery Disease (CAD). The results showed significantly higher values of CRI in cases (angiographically diagnosed patients of CAD) compared to controls (healthy volunteers). The findings suggest that CRI could be employed for the recognition of high-risk individuals to develop CAD [47]. Similarly, Azeez completed cross-sectional research on a Nigerian population; the study aims to confirm the association between atherogenic indices (AC, CRI, and AIP) and 10-year cardiovascular risk. The data revealed a significant and robust association between CRI and cardiovascular risk [48].

Gene–environment interaction

Finally, to evaluate a gene-environment interaction, the study participants were categorized into four groups as follows (Fig. 4): (a) low exposure-CC genotype; (b) low exposure-CT/TT genotype; (c) high exposure-CC genotype; and (d) high exposure-CT/TT genotype. Regarding ADMA concentrations (Fig. 4A), significantly higher levels ($p > 0.05$) of this molecule (2.10 ± 0.50 µmol/L) were found in the (d) group compared to other groups: 0.90 ± 0.25 µmol/L for (a) group; 1.10 ± 0.40 µmol/L for (b) group; and 0.85 ± 0.20 µmol/L for (c) group. Similar results were observed for serum FABP4 levels (Fig. 4B) and AIP values (Fig. 4C). Significantly higher values for both CVD biomarkers were detected in the (d) group contrasted to values found in all other groups. No significant differences were determined between groups when CRI was analyzed (Fig. 4D). Those findings suggest a gene-environment interaction. As suggested by Ottman et al., gene-environment interplays are defined as "a different impact on disease risk of an environmental exposure in persons with different genotypes" [49]. Recent data have confirmed that the gene-environment interaction (as detected in this study) is a key factor that increased the sensitivity to different diseases [50]. In this line, He et al. detected an increased risk of breast cancer (BC) in individuals exposed to Bisphenol A with the GG genotype (GG) of CYP19A1 enzyme (rs1902580 polymorphism) contrasted to BC risk detected in individuals exposed to Bisphenol A with GA-AA genotype [51]. Similarly, Farzan et al. developed scientific research to evaluate a gene-environment interaction in individuals from Araihazar, Bangladesh. Farzan et al. evaluated the effect of inorganic arsenic exposure and CYBA gene polymorphism (rs3794624; intron 1, G383A) on the blood pressure of the registered participants. Higher blood pressure levels were found in people exposed to arsenic plus GA-AA genotype contrasted to individuals with the GG genotype

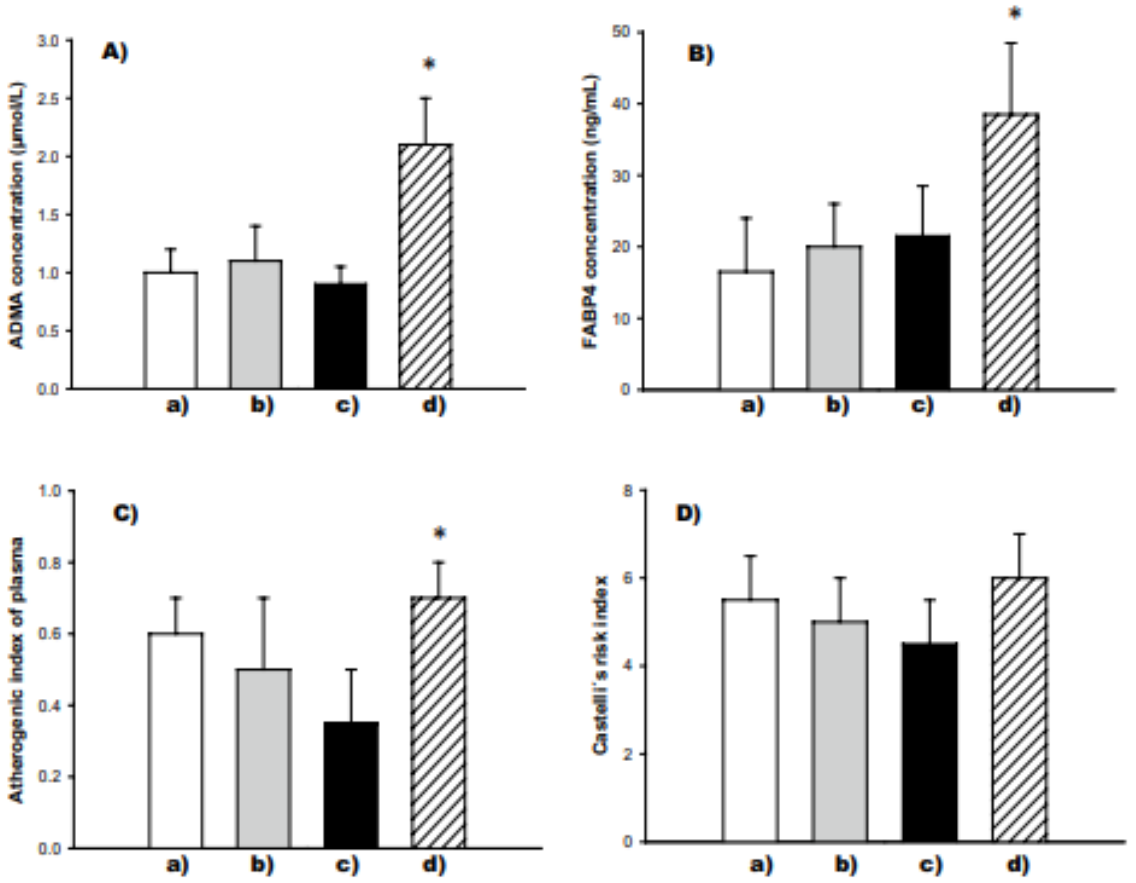

Fig. 4 Gene-environment interaction (arsenic exposure and TGF-β polymorphism (C509T). **A** Serum ADMA concentrations; **B** Serum FABP4 concentrations; **C** Atherogenic index of plasma; **D** Castelli´s risk index. (a) low exposure/CC genotype; (b) low exposure/CT genotype and TT genotype; (c) high exposure and CC genotype; and (d) high exposure/CT genotype and TT genotype. *$p < 0.05$

(no polymorphic allele) and exposed to inorganic arsenic [52]. Although the findings in this research suggest a gene-environment interaction, supplementary investigations are necessary to support the results found in this study.

Study limitations

The main limitations of this research were: the investigation design (cross-sectional study) and causality cannot be declared with this study strategy. The size of the evaluated sample is small to draw convincing conclusions (low statistical power). Also, we focused on a single chemical (inorganic arsenic); an increasing number of studies have demonstrated that human populations are exposed throughout their lifetimes to a composite mixture of different environmental pollutants [53]. Besides, as noted previously, several genetic TGF-β polymorphisms associated with an increased risk of CVD have been described. In our investigation, only the TGF-β C509T polymorphism was assessed. Lastly, the age range of individuals enrolled in this research was very high (18.0–87.0 years); additional studies are necessary focusing on different age groups, as cardiovascular risk is higher in old individuals. Despite all limitations, the results found in this investigation are significant and an initial step to complete large-scale epidemiological studies to evaluate the real influence of the gene-environment interaction depicted in this study on human health.

Materials and methods

Population

A cross-sectional study was developed in the "Las Palmas" community in Tamuin, San Luis Potosi, Mexico (Fig. 1), and

155 individuals (aged 18–87 years old) were incorporated in this research. Previous environmental analysis performed by our research group demonstrated high levels of arsenic in the air, soil, and water in the region, as thermoelectric and cement industries are located near the community (unpublished data). The recruitment individual's strategy was as follows: (1) a face-to-face interview with eligible individuals was completed to explain the objectives and goals of the study; (2) enrolled individuals signed informed consent of voluntary participation; (3) all participants answered a questionary to document personal characteristics such as age, education, occupation, income, alcohol consumption, smoking status, and diagnosed diseases, among others. In addition, anthropometric and clinical measures [weight, height, waist circumference (WC), hip circumference (CP), systolic blood pressure (SBP), and diastolic blood pressure (DBP)] were acquired. Body mass index (BMI) and waist/hip ratio were estimated using anthropometric measurements. The bioethics committee of the Secretary of Health of the State of San Luis Potosi analyzed and approved the execution of this research.

Urine and blood sampling

Urine samples were obtained from all included participants and were used to analyze the exposure biomarker (total urinary arsenic). Urinary samples were collected in sterile plastic containers (arsenic-free) according to the National Health and Nutrition Examination Survey (NHANES) instructions [54]. Immediately after sampling, urine samples were placed on ice and transported to the laboratory. Then, urine aliquots of approximately 10 mL were prepared and stored at − 80 °C until their analysis. In addition, an anticoagulated blood sample was required and used for TGF-β1 (C509T polymorphism) genotyping analysis, ethylenediaminetetraacetic acid (EDTA) was employed as an anticoagulant. A second blood sample was collected using a container without anticoagulant and utilized for quantification of glucose, triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, and assessed CVD biomarkers (ADMA and FABP4). Both blood samples were obtained from the ulnar artery, as described previously by our research group [55]. The blood samples were acquired from all enrolled participants after overnight fasting.

Biochemical profile quantification

The blood sample without anticoagulant was centrifuged at 1200 × g for 10 min, and serum samples were obtained. Then, serum levels of glucose, TG, TC, and HDL cholesterol were quantified using commercially available kits, as already described by our research team [56]. Serum concentrations of the low-density (LDL) cholesterol were estimated using the Friedewald Eq. (1).

$$\mathrm{LDL} = \mathrm{TC}(\mathrm{HDL} + \mathrm{TG}) \tag{1}$$

The Lyphochek Assayed Chemistry Control (Bio-Rad, Hercules, CA, USA) was analyzed as external quality control. An accuracy of approximately 90–110% was achieved for all analytes evaluated (glucose, TG, CT, and HDL).

Arsenic determination

Urinary arsenic concentrations (UAs) were quantified as described elsewhere [57]. Briefly, urine samples (5 mL) were digested using a microwave-assisted method. First, urine samples were thawed at 25 °C, diluted with HNO_3 conc (5 mL), and heated at 200 °C; 350 psi for 30 min. Then, the resulting samples (digested urine) were mixed with equal volumes of KI 1%, HCL conc, and water, and a reduction step [As(V) → As (III)] was performed at 80 °C for 15 min. Determination of total inorganic arsenic in urine was completed using an atomic absorption spectrophotometer with hydride generation (Perkin-Elmer model Analyst 100; Waltham, MA). A standard reference material (SRM 2670) from the National Institute of Standards and Technology (NIST) was used for quality control. An accuracy between 95 and 105% was obtained after SRM 2670 was analyzed. Urine creatinine level was determined following the Jaffe method [58] and used to correct urine arsenic levels.

TGF-β C509T genotypification

Anticoagulated blood samples were used for the genomic DNA (deoxyribonucleic acid) isolation using the kit Genomic DNA purification (PROMEGA, Wisconsin, USA). The isolated DNA samples were frozen at − 80 °C until their analysis. Then, DNA (20 ng/μL) and predesigned TaqMan single nucleotide polymorphism (SNP) probes were used for genotypification analysis (TGF-β C509T polymorphism; rs1800469). The genotypification assays were completed in a StepOne Real-Time PCR system (Applied Biosystems, CA, USA). The analyses were performed according to procedures described previously by our research group [59].

Atherogenic indices estimation

Also, the Castelli risk index (CRI) and atherogenic index of plasma (AIP) were estimated as previously described elsewhere [12], CRI and AIP were calculated as described in Eqs. (2) and (3), respectively [60, 61].

$$\mathrm{CRI} = \frac{\mathrm{TC}}{\mathrm{HDL}} \tag{2}$$

$$AIP = \log\left(\frac{TG}{HDL}\right) \quad (3)$$

Serum ADMA and FABP4 determination

Serum FABP4 and ADMA levels were quantified employing an ELISA (enzyme-linked immunosorbent assay) kit according to the manufacturer's guidelines (R&D Systems, No. DFBP40, Minneapolis, USA., and MyBiosource No. MBS264847 California, USA., respectively). Briefly, ELISA microplate wells were precoated with human anti-ADMA or anti-FABP4 monoclonal antibodies. Serum samples and standards (included in the ELISA kit) were added to the ELISA microplate wells, and a first incubation period was completed (37 °C for 60 min). After incubation time, a washing step (washing buffer) was performed. Later, ELISA microplate wells were treated with streptavidin-HRP (horseradish peroxidase enzyme) reagent and incubated at 37 °C for 30 min. After a washing phase, tetramethylbenzidine (TMB) reagent was added to ELISA microplate wells, and a subsequent incubation period at 37 °C for 30 min was completed. Next, an acidic chemical solution was employed to stop the reaction in the ELISA wells. Finally, serum FABP4 and ADMA levels were quantified using an Eon microplate spectrophotometer (BioTek Instruments, Winooski, VT, USA). The analytical sensitivity of the applied methodology was approximately 0.05 µmol/L. Negative and positive controls included in the ELISA kit were assessed as quality control. FABP4 and ADMA levels for controls were in the concentration range recognized by the manufacturer.

Statistical analyses

Data normality was estimated via the Shapiro–Wilk test. Also, we completed an exploratory analysis of the assessed variables for the data description. Mean, standard deviation (SD), and range are shown for continuous variables, and percentages for categorical variables. Registered individuals were grouped into two categories (low and high exposure) according to urinary arsenic guidelines in Mexico (NOM-047-SSA1-2011). Then, the t-student test was used to compare atherogenic indices values and serum FABP4 and ADMA levels between exposure groups (low and high exposure). Also, a one-way ANOVA (analyses of variance) test followed by the Tukey test were completed to evaluate differences between assessed CVD biomarkers values according to TGF-β (C509T) genotypes. In addition, multivariate linear regression analyses were completed to evaluate the causal association between the exposure biomarker and the TGF-β polymorphism (C509T) and effect biomarkers (ADMA, FABP4, and atherogenic indices), adjusting for possible confounders. $p < 0.05$ was considered statistically significant. Data analyses were performed using GraphPad Prism 5.01 (GraphPad Software Inc. La Jolla, CA, USA).

Conclusion

In this research, an association between increased serum levels of well-recognized predictive CVD biomarkers (ADMA, FABP4, and AIP) and high arsenic exposure levels (environmental factor) and TGF-β C509T polymorphism (genetic factor) was detected. Besides, our findings suggest a gene-environment interaction with an enhanced effect on CVD biomarkers. Consequently, the assessed population exhibits a high risk of CVD events. Then, using the precautionary principle, intervention risk reduction programs are mandatory to safeguard the integrity of the people living in the assessed areas.

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Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Alejandra González-Bravo, Myrna Lizbeth López-Ramírez, Ángeles C. Ochoa-Martínez, Leticia Carrizales-Yáñez, and Salvado I. Martínez-Bernal. The first draft of the manuscript was written by Ivan N. Pérez-Maldonado. All authors read and approved the final manuscript. This project was funded by the CONACYT (Consejo Nacional de Ciencia y Tecnología México). FORDECYT. Grant No. 309519.

Declarations

Conflict of interest Alejandra González-Bravo, Myrna L. López-Ramírez, Ángeles C. Ochoa-Martínez, Leticia Carrizales-Yáñez, Salvador I. Martínez-Bernal and Ivan N. Perez-Maldonado declare that we have no conflict of interest.

Ethical approval and consent to participate The bioethics committee of the Secretary of Health of the State of San Luis Potosi analyzed and approved the execution of this research. Also, enrolled individuals signed informed consent of voluntary participation.

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Evaluation of the expression levels of circulating miRNAs (miR-126, miR-155) in a population exposed to lead (Pb) in San Luis Potosi.

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Abstract

Objective. Cardiovascular diseases (CVD) are the leading cause of death worldwide. Therefore, this research aimed to evaluate the expression of circulating miRNA (miR-1265, miR-155) levels in a lead-exposed population. Methods. Then, a cross-sectional study was completed, including 55 adults. Blood lead levels were analyzed as a biomarker of exposure and were determined by atomic absorption spectrophotometry. The expression levels of miRNAs were carried out using the polymerase chain reaction (qRT-PCR) technique. Results: Blood lead levels had a mean ± standard deviation of 7.6 ± 5.8 µg/dL. For its part, circulating serum levels of miR-126 presented levels of 1.28 ± 0.7; while miR-155 presented levels of 1.68 ± 1.2. In addition, significant associations were found between miR-126 and blood lead levels; as well as between miR-155 and blood lead levels and triglyceride levels. Conclusion. The results obtained in this research demonstrate the epigenetic alteration of the expression levels of both miRNAs and its possible relationship with exposure to high levels of lead in the Mexican population.

Keywords. cardiovascular diseases · lead · miRNA's

Introduction

Exposure to environmental contaminants, including contamination by heavy metals as lead (Pb), has been increasing, due to the development and increase in mining, industrial and agricultural activities that have favored a wide distribution in the environment (1,2,3); which represents a serious global public health problem (3), according to data from the World Health Organization (WHO), exposure to environmental pollution causes 7 million premature deaths, mainly due to non-communicable diseases (4). For decades it has been seen that prolonged exposure and accumulation of heavy metals, particularly lead, cause serious consequences for human health such as abnormalities in growth and development, carcinogenesis, neuromuscular defects, mental illnesses and failures in the metabolic activities (5,6,7); however, recent studies suggest that lead, among other heavy metals, are also capable of affecting the organism, causing alterations at the epigenetic level, (8, 9) which are defined as changes in the chemical structure of DNA that do not alter its DNA sequence coding (10,11), with respect to epigenetics three common alterations have been studied, including DNA methylation, histone modifications and non-coding RNA expression (microRNA, long non-coding RNAs, small RNAs, circular RNAs) (12, 13). Regarding microRNAs, they are a single-stranded structure of 18 to 22 nucleotides and play a key regulatory role in gene expression and therefore in various human diseases (14, 15). In recent years, various studies aim to apply miRNAs for diagnostic and therapeutic applications in diseases, including those caused by exposure to heavy metals, such as cardiovascular and pulmonary diseases, due to recent evidence suggests that it is capable of influencing the expression of microRNA (miRNA) which affects cell

function and influences the risk of developing certain diseases (16,17,18); in addition to the fact that miRNAs respond to numerous environmental toxic agents, which indicates their usefulness as possible biomarkers of exposure (19). Among the miRNAs that have been associated with exposure to heavy metals are miR-126 and miR-155, which increase or decrease their expression with exposure to lead. (20, 21, 22, 23).

Therefore, this research aimed to evaluate the expression of circulating levels of miRNA (miR-1265, miR-155) in a population exposed to lead.

Results and discussion

Anthropometric measurements, clinical characteristics, and biochemical profiles

Table 1 describes the anthropometric measurements, clinical characteristics, and biochemical profiles obtained from the enrolled participants. Approximately 64% of participants were women, and the range for age was 18 to 87 years (mean = 50.6 ± 20.1). Overweight and obesity were detected in 70 % of the enrolled population (mean BMI = 28.05 kg/m2). Also, 80 % of participants had a waist/hip index greater than 0.8, and both parameters (BMI and waist/hip) are considered important risk factors in the development of cardiometabolic diseases (23, 24). For blood pressure (SBP and DBP), measurements ranged from 100.0 mmHg to 142.0 mmHg; and from 55.0 to 90.0 mmHg for SBP and DBP, respectively. Regarding the biochemical analyses, 32% of the participants had triglyceride levels above 150

mg/dL, while 25% had serum cholesterol concentrations above 200 mg/dL. Besides, HDL-C levels ranged from 17.7 – 201.4 mg/dL (25, 26, 27).

Blood lead determination

Regarding the blood lead concentrations of the participants (Table 1), a mean ± standard deviation of 7.6 ± 5.8 µg/dL (range 34.5 – 1.5 µg/dL) was found, with these results it was detected that more than 50% of the population presented blood lead levels above the reference value in Mexico (≥5 µg/dL) (NORMA OFICIAL MEXICANA NOM-199-SSA1-2000) (30). According to this guide, the population evaluated was classified into two groups, low exposure (<5µg/g creatinine) and high exposure (≥5 µg/g creatinine). The low exposure group presented a mean ± standard deviation of 3.22 ± 1.19 µg/dL; on the other hand, in the group classified as high exposure, a mean ± standard deviation of 10.59 ± 5.84 µg/dL was found. The results were compared with values from guides and international organizations; the exposure limit value established by the World Health Organization (WHO) (36) and the Centers for Disease Control and Prevention in the United States (37) is ≥5 µg/dL; therefore, when compared with our results, 58% of the population is above this reference value. In the 2015-2016 National Health and Nutrition Examination Survey (NHANES) in the USA, an average blood lead value of 0.92 µg/dL was reported in adults (38), that is, in our study was found a concentration of lead 6 times. For its part, in Mexico various studies have been carried out on blood lead levels in different populations, one of them is the one presented by Hernández-Ochoa, et al., in 2005, they carried out a cross-sectional study in the Lagunera Region, northwest of Mexico to evaluate the impact of

exposure to environmental lead, finding mean blood lead levels of 9.3 µg/dL. (39); another study is the one presented by Guerra-Tamayo, et al., in 2003, to a group of women residing in Mexico City, reporting blood lead levels of 9.4 – 9.6 µg/dL; compared to these studies 28% of our population is above the reported values (40). In conclusion, high exposure levels to lead were detected in this research.

MiRNA's expression

Table 2 shows the circulating serum levels of miRNAs; miR-126 presented levels of 1.28 ± 0.7; while miR-155 presented levels of 1.68 ± 1.2. As mentioned above, participants were classified according to their blood lead concentrations (low exposure group and high exposure group). Figure 3 shows the expression levels of miR-126 found in each group, significantly lower miR-126 values ($p<0.05$) were found in the high exposure group compared to participants in the low exposure group. Regarding the expression levels of miR-155, significantly higher expression levels ($p<0.05$) were found in the high exposure group (Figure 4). Next, simple linear correlation analyzes were performed to evaluate the relationships between the evaluated variables and the quantified miRNAs (miR-126, miR-155), a significant negative association was detected between the expression levels of miR-126 and the levels of lead in blood ($r= -0.33$, $p<0.05$). miR-155 expression levels, significant positive associations were found between triglyceride levels ($r= 0.347$, $p<0.05$) and blood lead levels ($r= 0.39$, $p<0.05$).

Since recent years, different research has shown that epigenetic alterations play an important role in the appearance and development of non-communicable diseases (41, 42); in particular, it has been suggested that miRNAs represent a

crucial mechanism of epigenetic modification that can affect the cellular function both locally and at a distance and that are related to the development of various diseases, including CVD (43, 44), which is why their use as predictive biomarkers of these diseases has been increasing. Various investigations have explored an increasing number of circulating miRNAs as new candidate biomarkers for potential use in the diagnosis and prognosis of CVD, thanks to their specific expression pattern, their rapid release in body fluids, their remarkable stability in plasma , and the fact that cardiovascular diseases can cause significant changes in the expression level of specific miRNAs in the body (45, 46, 47); in addition, there is increasing evidence that shows that epigenetic markers, such as the expression of miRNAs, are influenced by environmental pollution, such as exposure to heavy metals such as lead, because it has been proven that lead induces cellular necrosis and produces oxygen free radicals, the latter being the main activators of signaling pathways that induce inflammation and promote the production of large amounts of inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β and IL-6 (48, 49, 50). In this context, the miRNAs evaluated (miR-126, miR-155) have been related to cardiovascular events and have been suggested as prognostic biomarkers in the initiation and development of atherosclerosis processes (51, 52, 53). miRNA-126 is a short non-coding RNA that is derived from the gene Like Domain Multiple 7 (EGFL7), is highly expressed in the heart, liver and lungs, and plays an important role in atherosclerosis pathways by regulating function of endothelial cells and improve endothelial regeneration; therefore, low levels have been observed in patients with cardiac ischemia and hypertension (54, 55, 56). Several articles have linked

decreased expression of this miRNA to the development of atherosclerosis, as well as increased risk adverse cardiac and cerebrovascular events.

Furthermore, lower miR-126 expression may indicate greater disease complexity (57, 58, 59). Regarding the relationship between mir-126 expression and lead exposure, there is not much research; our working group in 2021 carried out previous research, in which a lower level of expression can be associated with higher levels of exposure to lead, which could lead to establishing a significant relationship between a low level of expression of miR-126, high levels of lead exposure and the development of CVD (60). On the other hand, miR-155 is a non-coding RNA, encoded by the host gene, MIRHG155, and is highly expressed in activated B and T cells, as well as in macrophages; this molecule represents a multifunctional miRNA and a transcriptional regulator, because there is increasing evidence indicating that it is involved in numerous biological processes, including hematopoiesis, inflammation and immunity, which could open new avenues to attenuate progression of cardiovascular diseases (61, 62, 63). Various investigations have linked high levels of miRNA-155 expression to both high levels of exposure to heavy metals, including lead; a study by Mitra, P., et. al., 2020, found a statistically significant increase in miR-155 expression levels in people exposed to high levels of lead (64), on the other hand, the relationship of this miRNA with the onset and development of CVD has also been demonstrated; in 2017, Faccini, et. al., presented a study in which they evaluated the expression of several miRNAs, including miR-155, highlighting its importance as a biomarker of CVD, in their study, statistically significant levels were reported in the patient group compared to the control group, on the other hand, Qiu, et. al., in 2018 presented a

study where statistically increased expression levels of miR-155 were also observed in people with cardiovascular conditions compared to healthy subjects (65, 66). Finally, in our study we observed that the expression levels of miR-155 and miR-126 in association with risk factors for atherosclerotic lesions may be useful as a new biomarker to evaluate the risk of developing atherosclerosis and other cardiovascular diseases.

Limitations of the study

This study has several limitations, one of the most notable is the low representativeness (a limited number of samples), in addition the type of study (cross-sectional) of this research may cause biases regarding the temporal variation between lead exposure and the levels of the evaluated variables. Additionally, it is possible that other environmental chemical compounds that were not evaluated in this study may have a significant effect on the variables evaluated and quantification of additional chemical compounds associated with CVD events may be necessary. However, the data found in this study are worrying for people who live in scenarios similar to the one studied in this research, because the miRNAs evaluated have been suggested as specific and useful biomarkers to detect populations at high risk of developing diseases cardiovascular. Therefore, the data found in this study must be confirmed in large epidemiological studies.

Materials and methods

Population

A cross-sectional study was developed including 55 adults from the community of "Las Palmas" in the municipality of Tamuin, San Luis Potosi, Mexico (Figure 1). The sampling site was selected according to previous research conducted by our working group, where arsenic and lead levels were evaluated in environmental matrices (unpublished data). Before obtaining blood samples, a conference was held with the population to explain the objectives and goals of the research; voluntary participation and the signing of informed consent were required. Subsequently, a survey was applied to the enrolled population. In addition, anthropometric and clinical measures [weight, height, waist circumference (WC), hip circumference (CP), systolic blood pressure (SBP), and diastolic blood pressure (DBP)] were obtained. Body mass indexes (BMI) and waist/hip ratio were estimated using anthropometric measurements. The bioethics committee of the Secretary of Health of the State of San Luis Potosi analyzed and approved the execution of this research.

Blood sampling

Both blood samples were obtained from the ulnar artery, as described previously by our research group. The blood samples were acquired from all enrolled participants after overnight fasting (24, 25). The first sample was stored in a plastic container doped with EDTA (ethylenediaminetetraacetic acid) as an anticoagulant and was used to quantify blood lead and miRNAs expression levels. The second

sample was stored in a container without anticoagulant and used for the determination of clinical parameters.

Biochemical profile

Serum levels of glucose, triglycerides (TG), total cholesterol (TC), and high-density lipoprotein (HDL) cholesterol fraction were quantified using commercially available kits, as already explained by our research team (24, 25). Serum concentrations of the low-density (LDL) cholesterol fraction were estimated using the Friedewald equation (1).

$$LDL = TC\ (HDL + TG) \quad (1)$$

The Lyphochek Assayed Chemistry Control (Bio-Rad, Hercules, CA, USA) was analyzed as external quality control. An accuracy of approximately 90-110% was achieved for all analytes evaluated (glucose, TG, CT, and HDL).

Lead determination

Blood lead (BLL) levels were quantified according to an analytical method validated by our research team (26). Briefly, 1:1 dilution of blood samples was performed with a matrix modifier (diammonium hydrogen phosphate–Triton X-100 and 0.2% nitric acid; JT-Baker; Center Valley, PA, USA) and mixed vigorously with a mechanical mixer. Next, the BLL determination was performed in a Perkin-Elmer 3110 atomic absorption spectrophotometer coupled to a graphite furnace (Waltham, MA, USA). Certified material obtained from the Centers for Disease Control and Prevention, USA (CDC.WS2H proficiency testing blood; Madison WI,

USA 53707-7996) was tested as external quality control and found a 95% recovery ± 5%. The detection limit of the method was 1.0 µg/dL.

miRNAs analysis

To determine the expression levels of the miRNAs evaluated (miR-126 and miR-155), an analytical procedure previously validated (27). Briefly, a TRIzol-based RNA extraction method was used to obtain serum RNA following the manufacturer's instructions (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). Then RNA concentration of all samples was determined using an Eon microplate spectrophotometer (BioTek, Vermont, USA) (λ = 260 nm). RNA-containing samples were then treated with 5 nmol/L cel-miR-39 (Caenorhabditis Elegans miR-39) (Applied Biosystems, Foster City, CA, USA) as a normalization control in the normalization procedures miRNA determination. Expression levels of the miRNAs tested were determined using TaqMan miRNA qRT-PCR primers and reagents (Applied Biosystems, Foster City, CA, USA). First, RNA samples were reverse transcribed using the TaqMan miRNA Reverse Transcription L Kit and miRNA-specific stem-loop primers (Applied Biosystems, Foster City, CA, USA) to obtain samples of cDNA. Subsequently, cDNA samples (100 ng/µL, 4.5 µL), TaqMan Universal PCR Master Mix L (5 µL), and TaqMan miRNA Assay primers L (0.5 µL) (Applied Biosystems, Foster City, CA, USA) were used in the amplification procedure carried out under the following conditions: 1) denaturation phase (95 ◦C, 10 min); 2) 40 cycles of PCR amplification (95 ◦C, 15 s; and 60 ◦C, 60 s). Finally, the relative expression levels of the miRNAs studied were normalized (cel-miR-39) and expressed as 2− (CT [microRNA] – CT [cel− miR− 39]).

Statistical analysis

Data normality was determined using a Shapiro-Wilk test. Subsequently, a descriptive analysis was developed for all the assessed variables in the study. Participants were categorized into two categories (low and high exposure) according blood lead level guidelines in Mexico (NOM-199-SSA1-2000) (36). Then, the Mann-Whitney U test was used to compare the expression levels of miRNA-126 and miRNA-155 between exposure groups (low and high exposure) respectively. To assess the relationship between miR-126 and miR- 155 with the parameter clinics and anthropometrics, Spearman correlation tests were applied to the dataset of participants. For all analyses, $p<0.05$ was considered statistically significant. Data analysis was performed using GraphPad Prism 5.01 (GraphPad Software Inc. La Jolla, CA, USA).

Conclusion

The results obtained in this research demonstrate the epigenetic alteration of the expression levels of both miRNAs and its possible relationship with exposure to high levels of lead in the Mexican population and the development of CVD. Despite the limitations of the study, the data obtained regarding significant associations may be useful for large-scale epidemiological studies, as well as for the identification of new mechanisms related to cardiovascular damage.

Figure legends

Figure 1. Geographical location of the Ejido "Las Palmas", Tamuin municipality, SLP. Geographic information source: INEGI

Figure 2. Serum expression levels of miR-126 divided into low and high lead exposure groups. Values were normalized to cel-miR-39. *p<0.05.

Figure 3. Serum expression levels of miR-126 divided into low and high lead exposure groups. Values were normalized to cel-miR-39. *p<0.05.

Table 1. General characteristics of the population

Variables	Mean (SD*)	Median	Range	%> reference value[#]
Age (years)	50.6 (20.1)	53	18.0-87.0	-
Weight (Kg)	68.3 (14.8)	67.0	43.0-100.0	-
Height (cm	155.8 (9.3)	155.0	139.0-183.0	-
Waist/hip index	0.94 (0.06)	0.91	0.78-1.07	90.9
Body mass index (kg/m^2)	38.13 (5.27)	27.7	19.3-40.20	-
(Z SCORE) Normal (18.5- 24.9)	22.2 (1.80)	22.2	19.3-24.9	29.4
Overweight (25.0-29.9)	27.5 (1.54)	27.5	25.0-29.8	39.2
Obesity (>30)	34.4 (3.13)	34.3	30.2-40.2	31.4
Diastolic blood pressure (mmHg)	72.71 (1.46)	76.0	55.0-90.0	10.9
Systolic blood pressure (mmHg)	119.4 (1.37)	118.0	100.0-142.0	20.0
Glucose (mg/dL)	94.3 (37.1)	86.9	58.5-222.1	14.5
Triglycerides (mg/dL)	135.5 (71.7)	126.6	87.0-261.0	32.7
Total cholesterol (mg/dL)	167.6 (44.0)	172.3	87.0-261.0	25.4
HDL cholesterol (mg/dL)	41.39(34.3)	32.9	17.7-201.4	14.6
LDL cholesterol (mg/dL)	27.1 (14.3)	25.3	5.05- 78.0	0.0
Blood lead (µg/dL)	7.60 (5.8)	5.93	1.5 - 34.5	

Data are represented as arithmetic mean with standard deviation (SD) and median with range from minimum to maximum. #Reference value. Waist/hip index = 0.8, Systolic blood pressure = 130 mmHg. (James et al. 2013). Diastolic blood pressure = 90 mmHg (James et al. 2013). Glucose = 110 mg/dL. (Iii et al. 2001). Triglycerides = 150 mg/dL. (Iii et al. 2001). Total cholesterol = 200 mg/dL. (Iii et al. 2001). HDL cholesterol = 45 mg/dL. (Iii et al. 2001). LDL cholesterol = 100 mg/dL. (Iii et al. 2001). Blood lead = 5µg/dL - variables without reference values.

Table 2. Serum expression levels of assessed miRNAs in the study population.

MiRNAs	Mean (SD*)	Median	Range
miR-126 ($2^{-\Delta Ct}$)	1.28 (0.72)	1.460	0.082 - 2.57
miR-155 ($2^{-\Delta Ct}$)	1.68 (1.2)	1.491	0.028 - 4.73

Data are represented as arithmetic mean with standard deviation (SD*) and median with range from minimum to maximum.

Table 3. Simple regression analyses between independent assessed variables and evaluated miRNA´s

Molecular marker	Variables	r	95% CI	*p*
miR-126 ($2^{-\Delta Ct}$)	Age	-0.040	-0.33 - 0.25	0.77
	Waist circumference	0.108	-0.19 - 0.38	0.99
	Body mass index	-0.002	-0.29 - 0.29	0.46
	Triglycerides	-0.008	-0.29 – 0.28	0.95
	HDL cholesterol	0.021	-0.26 - 0.31	0.88
	Blood lead	-0.330	-0.56 -0.04	0.02*
miR-155 ($2^{-\Delta Ct}$)	Age	0.138	-0.15 - 0.41	0.34
	Waist circumference	-0.150	-0.42 - 0.15	0.31
	Body mass index	-0.085	-0.37 - 0.21	0.56
	Triglycerides	0.347	0.097 - 0.58	0.03*
	HDL cholesterol	0.028	-0.26 - 0.31	0.85
	Blood lead	0.390	0.11- 0.61	0.006*

* Significant correlations ($p<0.05$)

Figure 1

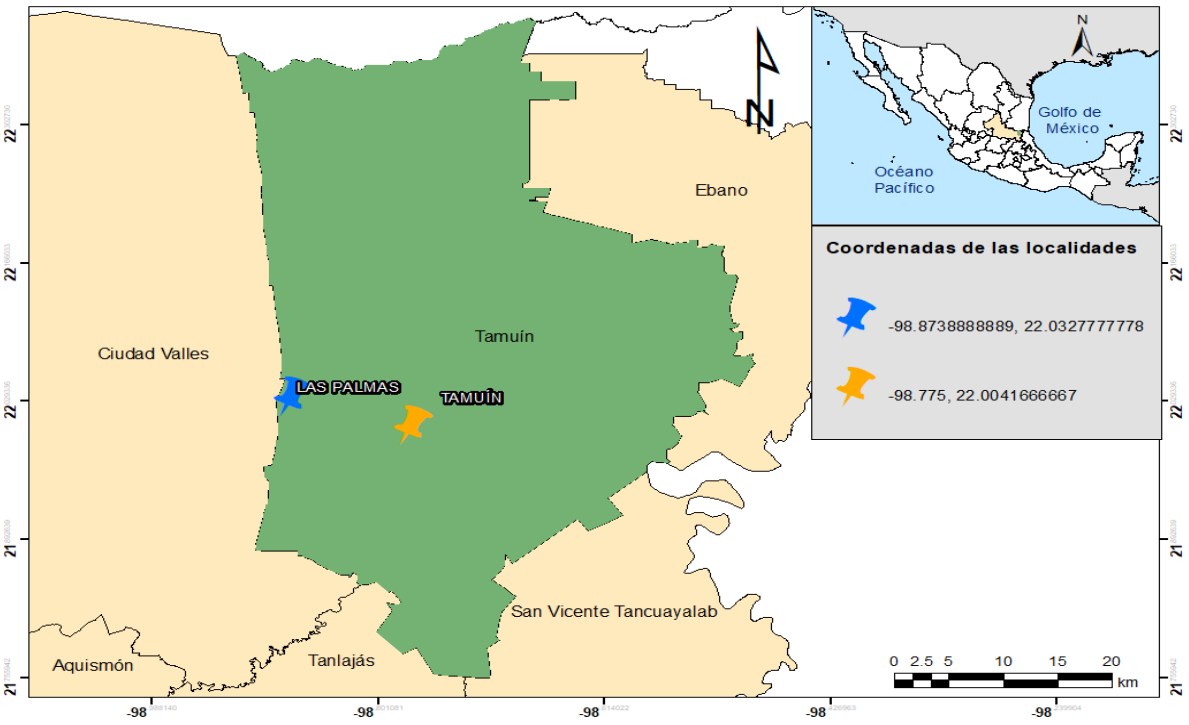


Figure 2

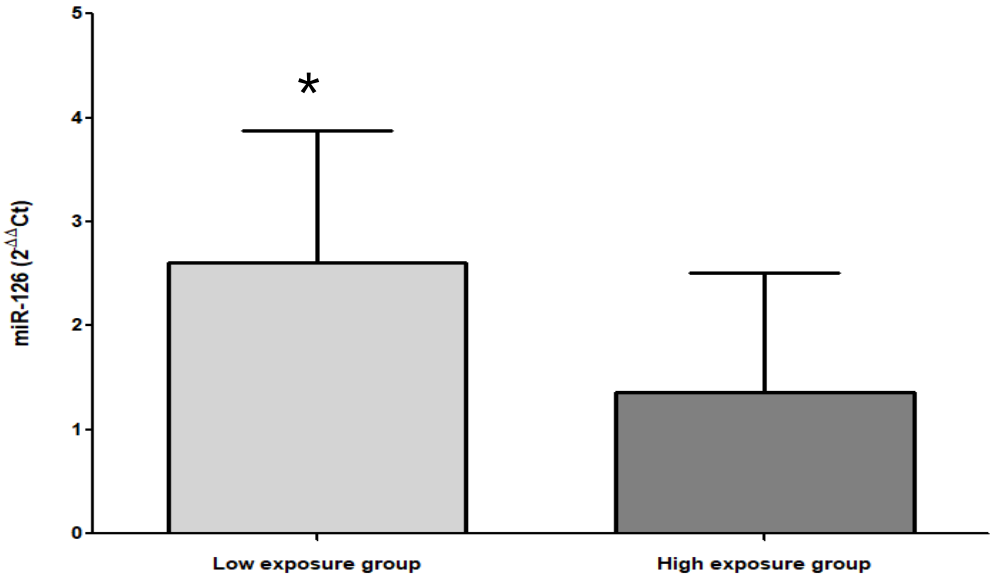
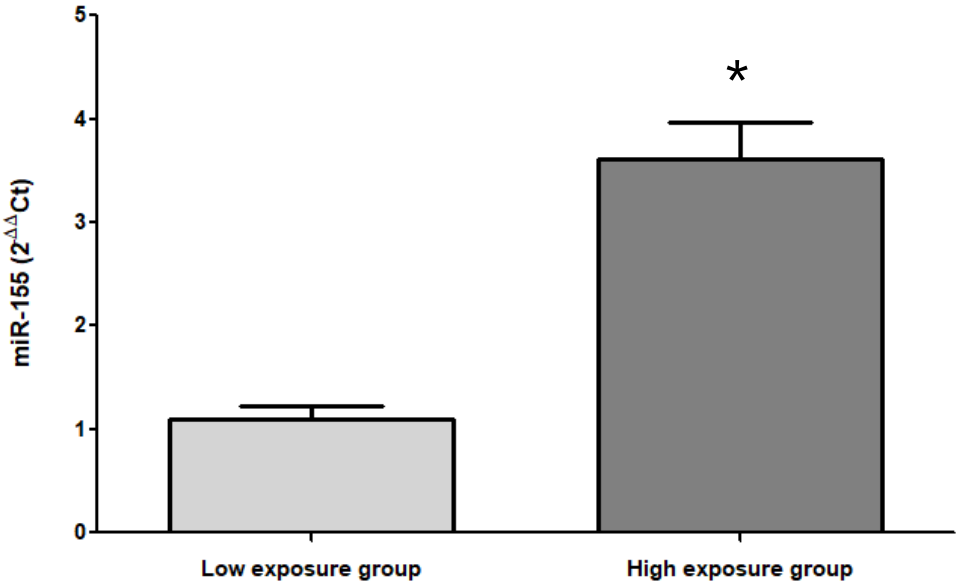
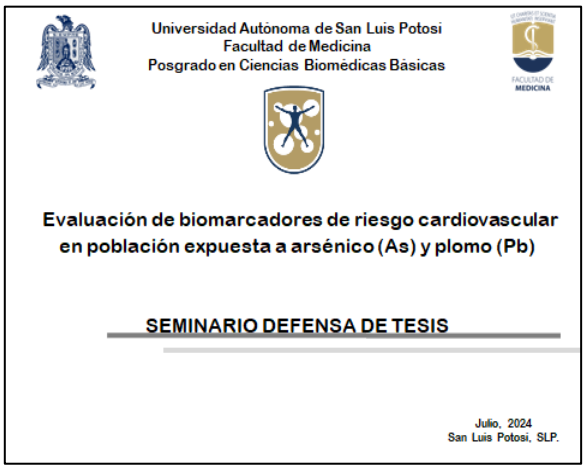
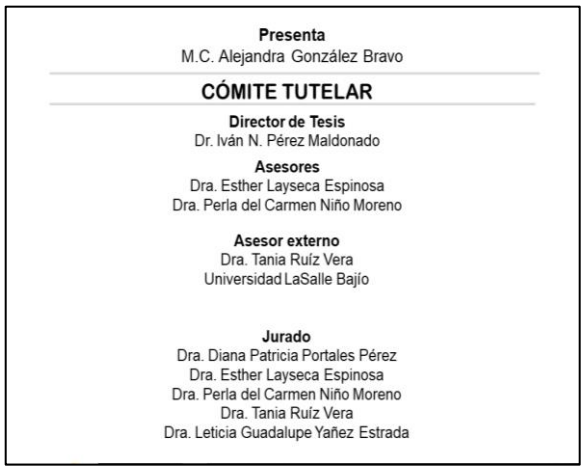


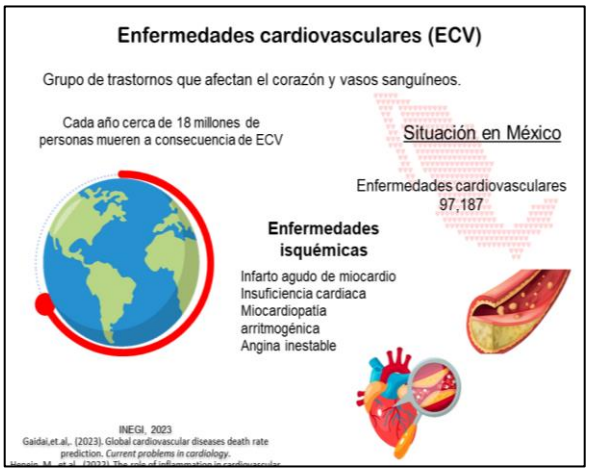
Figure 3

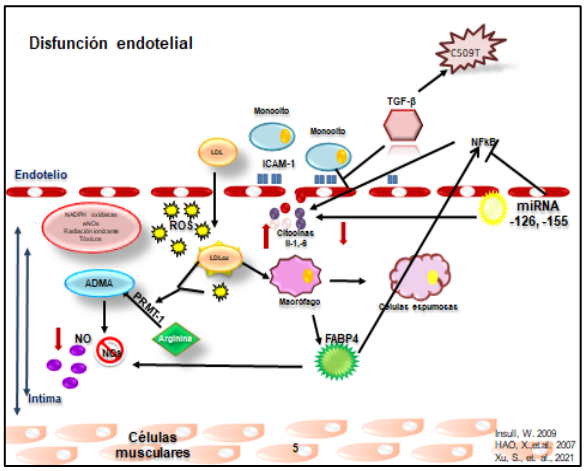

5
4
3
2
1
0
miR-155 ($2^{-\Delta\Delta}$Ct)
*
Low exposure group
High exposure group

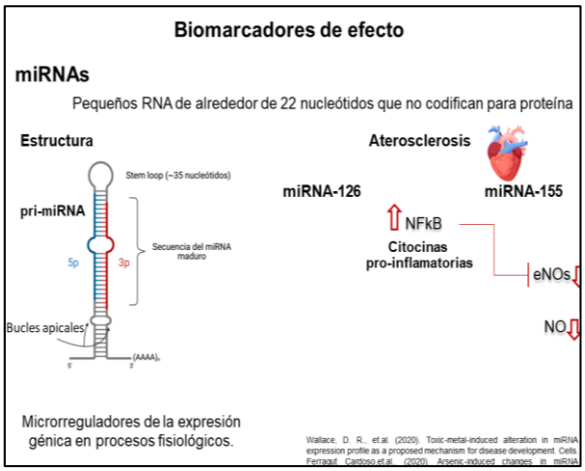
PRESENTACIÓN











Estudio de miRNAs en nuestro grupo de investigación

Lead (Pb) exposure is associated with changes in the expression levels of circulating miRNAS (miR-155, miR-126) in Mexican women

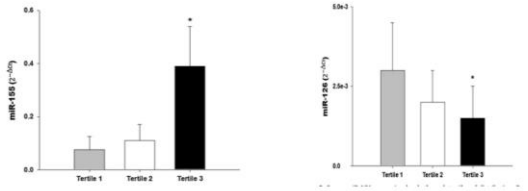
Ochoa-Martínez, et.al. 2021

Table 4
Serum expression levels of assessed miRNAs in the study population.

Variable	Total (mean ± SD) n = 190	T1 [a] (mean ± SD) n = 63	T2 (mean ± SD) n = 64	T3 (mean ± SD) n = 63
miR-155 ($2^{-\Delta\Delta Ct}$)	2.5 E-01 ± 1.8 E-01	1.6 E-01 ± 1.2 E-01	2.6 E-01 ± 1.5 E-01*	3.5 E-01 ± 1.0 E-01*,
miR-126 ($2^{-\Delta\Delta Ct}$)	2.0 E-03 ± 1.0 E-03	2.8 E-03 ± 1.0 E-03	1.9 E-03 ± 0.9 E-03*	1.4 E-03 ± 1.0 E-03*,

Circulating miRNA-126, -145 and -155 levels in Mexican women exposed to inorganic arsenic via drinking water

Ruiz-Vera, et.al., 2019



Ruiz-Vera, T. (2019). Circulating miRNA-126, -145 and-155 levels in Mexican

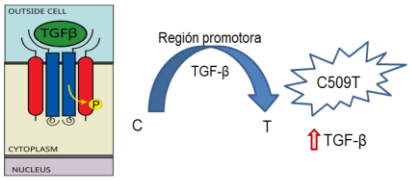
Influencia de factores genéticos Polimorfismos

SNP es una variante genómica en la posición de una base única en el ADN.

Polimorfismos y ECV

Estrés oxidativo | Inflamación | Metabolismo de lípidos

TGF-β (C509T)



Citocina
Modular la respuesta inflamatoria
⇩Expresión de moléculas de adhesión

Singh, N. N.,(2006). The role of transforming growth

Factores de riesgo

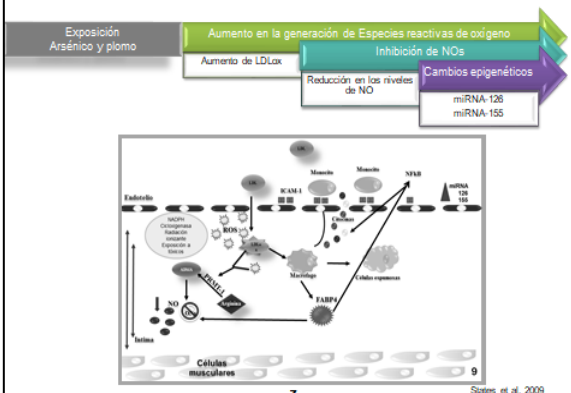
Exposición a metales

Muertes causadas contaminación atmosférica	
Cardiopatía isquémica	40%
Accidente cerebrovascular	40%
Enfermedad pulmonar obstructiva crónica	11%
Cáncer de pulmón	6%
Infección aguda de las vías respiratorias	3%

OMS, 2022
Ohishi M., et. al., 2021
Arredondo Buce, A. E., et. al., 2020

6

Mecanismo de acción de As y Pb



7

States et al. 2009
Andersen O.,et.al., 2002

Exposición a contaminantes ambientales en Las Palmas, SLP

Principales causas de muerte en S.L.P.

No.	DIAGNÓSTICO	TOTALES
1	Enfermedades del corazón	3,434
2	Diabetes mellitus	2,102
3	Tumores malignos	1,866

Comparación de muertes por ECV entre regiones

Las Palmas, Tamuín

Zona agrícola-industrial

Zona de alto grado de exposición

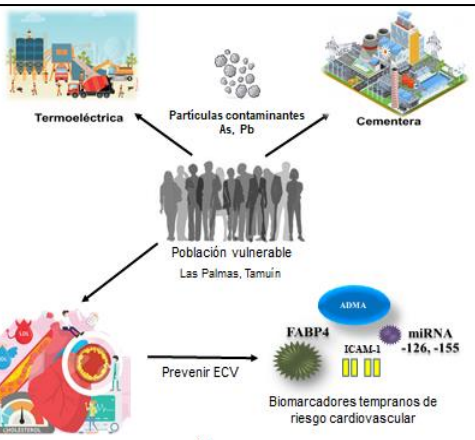
Partículas contaminantes As, Pb

Secretaría de Salud, SLP, 2022

8

JUSTIFICACIÓN

9



10

HIPÓTESIS

La exposición a arsénico y plomo, en población adulta, está asociado al incremento de las concentraciones de biomarcadores tempranos de riesgo cardiovascular

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OBJETIVO GENERAL

Evaluar la concentración de biomarcadores de riesgo cardiovascular en población adulta expuesta a arsénico (As) y plomo (Pb)

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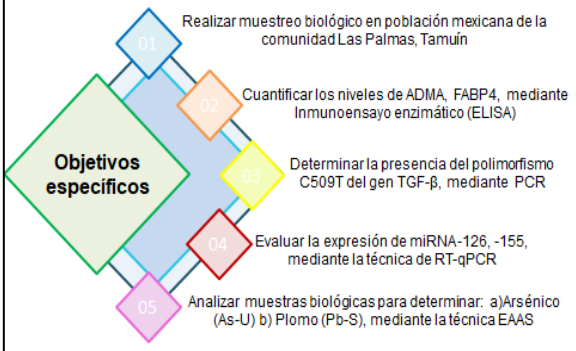
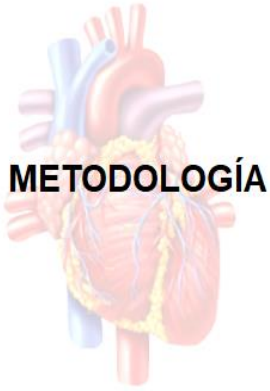


13



METODOLOGÍA

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Población de estudio

Las Palmas

La localidad está situado en el Municipio de Tamuín. Población 1592 habitantes.

- Grado de marginación y rezago social: medio

Centrales termoeléctricas con mayor volumen de emisiones de sustancias contaminantes

Termoeléctrica	Emisión de contaminantes (toneladas)
Lerma Campeche, Campeche (CFE)	3.8
Felipe Carrillo, Puerto Valladolid, Yucatán (CFE)	2.3
Monterrey, San Nicolás de los Garza, Nuevo León (CFE)	1.0
Salamayuca I, Ciudad Juárez, Chihuahua (CFE)	0.6
Termoeléctrica del Golfo, S de RL de CV Tamuín, San Luis Potosí	0.3

Figura 1. La ubicación geográfica del Ejido "Las Palmas", municipio de Tamuín, San Luis Potosí, México

Zona agrícola-industrial

Termoeléctricas del Golfo y Peñoles AES Corporation

Cementera Cemex. Planta Tamuín

Zona de alto grado de exposición

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Reunión informativa dirigida a la población

- Objetivo del proyecto
- Procedimiento
- Beneficios y riesgos potenciales

Consentimiento informado

SALUD
SECRETARÍA DE SALUD

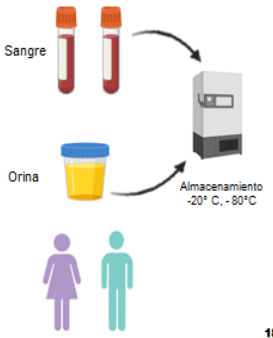
Protocolo fue sometido al Comité de Bioética Estatal Secretaria de Salud

Criterios de selección de la población de estudio

Muestreo		
Criterios inclusión	**Criterios de no inclusión**	**Criterios de eliminación**
Que vivan en la comunidad	Tabaquismo	Cantidad de muestra insuficiente
Firma del consentimiento informado	Tratamiento Farmacológico	Abandono voluntario
Edad mayor de 18 años		
Ambos sexos		

Muestreo

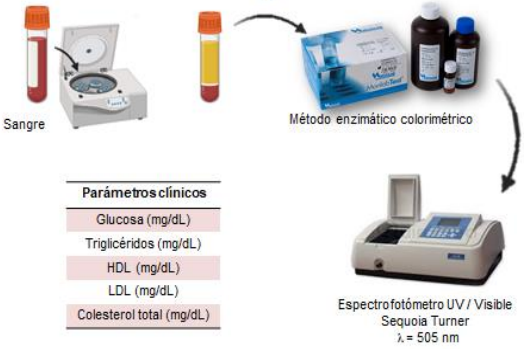
Obtención de muestras



Medidas antropométricas
Peso (gr)
Talla (cm)
IMC
Circunferencia abdominal
Circunferencia cadera
Índice cintura/cadera
% Grasa
Grasa visceral

Balanza bioimpedancia

Determinación de los parámetros clínicos



Parámetros clínicos
Glucosa (mg/dL)
Triglicéridos (mg/dL)
HDL (mg/dL)
LDL (mg/dL)
Colesterol total (mg/dL)

Evaluación de los índices aterogénicos

Índice de riesgo de Castelli

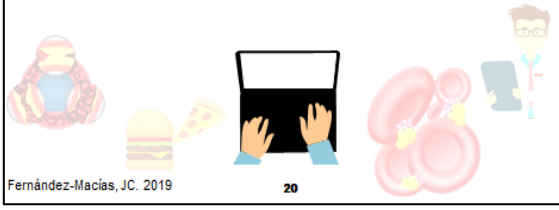
$$CRI = \frac{TC}{HDL - C}$$

CRI = índice de riesgo de Castelli
TC (mg / dL) = nivel de colesterol total
HDL-C = colesterol de lipoproteínas alta densidad

Índice aterogénico de plasma

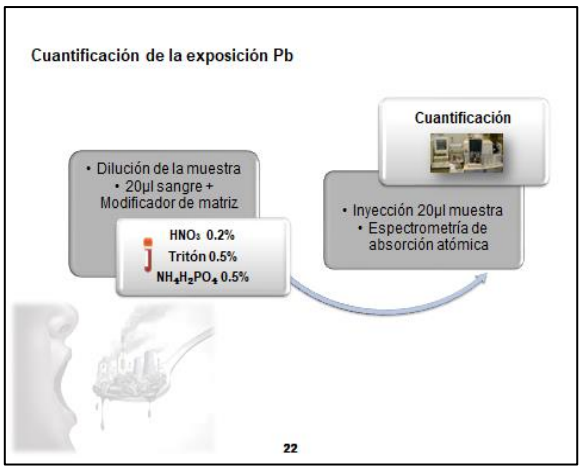
$$AIP = \log\left(\frac{TG}{HDL - C}\right)$$

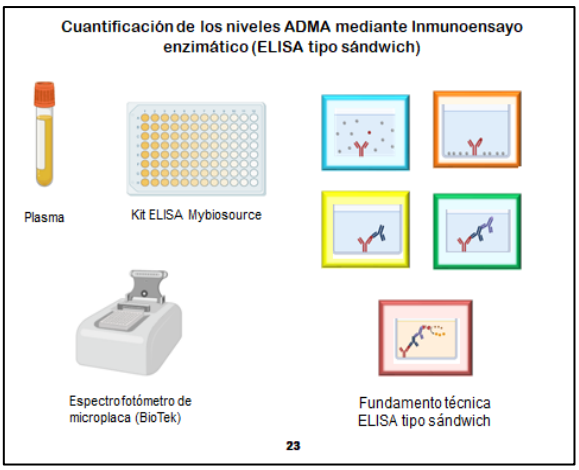
AIP = índice aterogénico de plasma
TG=concentraciones de triglicéridos

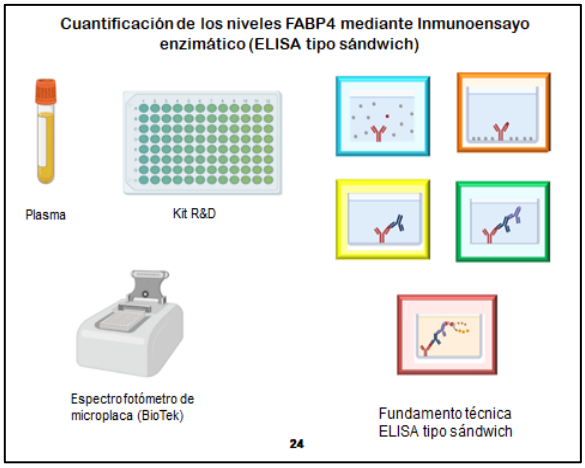


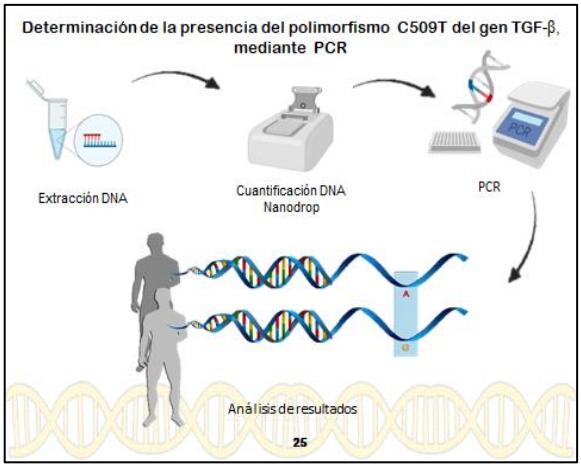
Fernández-Macías, JC. 2019

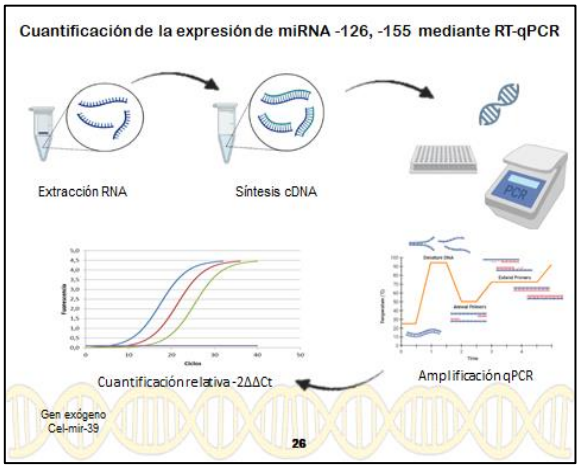
Cuantificación de la exposición As


Cuantificación de la exposición Pb
Cuantificación
• Dilución de la muestra
• 20µl sangre +
Modificador de matriz
HNO3 0.2%
Tritón 0.5%
NH4H2PO4 0.5%
• Inyección 20µl muestra
• Espectrometría de
absorción atómica
22


Cuantificación de los niveles ADMA mediante Inmunoensayo enzimático (ELISA tipo sándwich)
Plasma
Kit ELISA Mybiosource
Espectrofotómetro de microplaca (BioTek)
Fundamento técnica ELISA tipo sándwich
23


Cuantificación de los niveles FABP4 mediante Inmunoensayo enzimático (ELISA tipo sándwich)
Plasma
Kit R&D
Espectrofotómetro de microplaca (BioTek)
Fundamento técnica ELISA tipo sándwich
24


Determinación de la presencia del polimorfismo C509T del gen TGF-β, mediante PCR
Extracción DNA
Cuantificación DNA Nanodrop
PCR
Análisis de resultados
25


Cuantificación de la expresión de miRNA -126, -155 mediante RT-qPCR
Extracción RNA
Síntesis cDNA
PCR
Cuantificación relativa-2ΔΔCt
Amplificación qPCR
Gen exógeno Cel-mir-39
26

RESULTADOS

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Muestreo

Descripción de la población	
Muestra total	53 adultos
Edad promedio	50 años

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Medidas antropométricas

Variable	Media (DE)	Rango	% > Valor de referencia a
Peso (kg)	68.9 (5.3)	43 – 95	-
Talla (cm)	156.9 (16.6)	142 – 183	-
IMC (Normal)	22.4 (1.8)	19.3 – 24.9	71.4
Sobrepeso	27.5 (1.4)	25.0 – 29.8	42.8
Obesidad	34.7 (3.2)	30.2 - 35.3	28.6
Circunferencia abdominal (cm)	95.8 (10.3)	73 – 125	57.0
Circunferencia cadera (cm)	102.9 (12.6)	83 – 127	-
Índice cintura/cadera	0.93 (0.06)	0.76 – 1.03	75.0
% Grasa	32.3 (7.4)	18.3 - 47.5	53.6
Grasa visceral (kg)	9.5 (4.8)	2 – 20	48.2

Tabla 1. Descripción de las características de la población

Valor de referencia a
IMC: Normal (18.5-24.9) Sobrepeso (>/=25) Obesidad (>/=30) (OMS, 2018)
Circunferencia abdominal: >80cm mujeres >94cm (OMS, 2020)
Índice cintura/cadera: Factor de Riesgo (>/=0.90) (OMS,

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Parámetros clínicos

Variable	Media (DE)	Rango	% > Valor de referencia a
Glucosa	89.2 (37.22)	58.3 – 222.1	34.2
Triglicéridos	125.6 (70.04)	25.3 – 390.2	48.2
Colesterol HDL	43.2 (36.99)	17.7 – 201.4	75.0
Colesterol LDL	94.3 (14.01)	19.3 – 193.6	51.8
Colesterol total	162.6 (43.62)	87.0 – 251.0	19.7

Tabla 2. Descripción de los parámetros clínicos

Valor de referencia a

Glucosa: 100 mg/dL (ADA, 2021)
Triglicéridos: 150 mg/dl (Ili et al. 2001)
Colesterol HDL: 45 mg/dL (Ili et al. 2001)
Colesterol LDL: 100 mg/dL (Ili et al. 2001)
Colesterol total: 200 mg/dL (Ili et al. 2001)

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Riesgo de enfermedades cardiovasculares

Solo el 28% de la población presenta un peso normal de acuerdo a la OMS.

Más del 50% de la población presenta circunferencia de cintura y exceso de grasa visceral

20 – 51% población presenta un parámetro clínico alterado, asociado a un mayor riesgo a ECV

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Índices aterogénicos

Índice de riesgo de Castelli

$$CRI = \frac{TC}{HDL - C}$$

Índice de riesgo de Castelli

	Promedio	Rango
Mujeres	5.5	1.2 -11.7
Hombres	4.9	2.1 - 8.7

Valores <5 para los hombres
< 4.5 para las mujeres
Riesgo cardiovascular normal-bajo

Índice de riesgo de Castelli

	n	%
Sin riesgo	26	48.2
Riesgo	27	51.8

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Índice aterogénico de plasma

$$AIP = \log\left(\frac{TG}{HDL - C}\right)$$

Índice aterogénico de plasma		
	Promedio	Rango
Mujeres	5.7	1.7 - 14.1
Hombres	4.1	1.2 – 8.9

Valores <5 para hombres
< 4 para mujeres
Riesgo cardiovascular normal-bajo

Índice aterogénico de plasma		
	n	%
Sin riesgo	24	42.8
Riesgo	32	57.1

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Discusión

Tanto el índice de Castelli y el índice aterogénico de plasma son considerados como predictores de riesgo cardiovascular

Diversos estudios, entre los que se encuentran
Croda, T. et. al.2014
Álvarez ,A., et. al. 2015
De la Torre-Cisneros, K., et. al. 2019

Han utilizado estos índices, para evaluar la predisposición a desarrollar ECV

En nuestro estudio
Más del 50% de la población, de acuerdo a los índices presenta un mayor riesgo a desarrollar ECV

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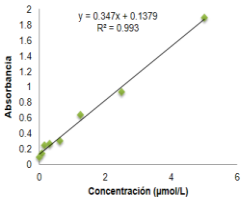
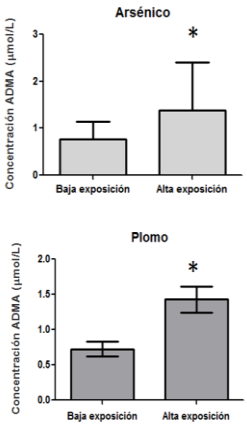
Cuantificación de los niveles ADMA





Prueba kolmogorov smirnov
Prueba U de Mann-Whitney
Prueba t student
*p<0.05

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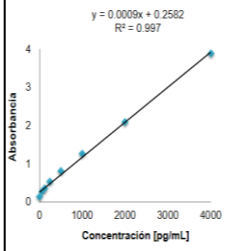
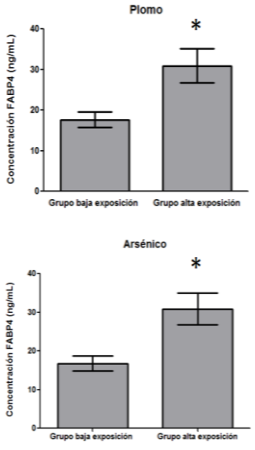
Cuantificación de los niveles FABP4





Prueba kolmogorov smirnov
Prueba U de Mann-Whitney
*p<0.05

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Correlación de parámetros bioquímicos con niveles séricos de ADMA y FABP4

Marcador molecular	Variables	r	95% CI	p
ADMA	Edad	0.14	-0.16 - 0.42	0.342
	Triglicéridos	0.28	-0.0098 - 0.52	0.048*
	Colesterol HDL	0.099	-0.2015 - 0.38	0.5051
	Colesterol total	0.276	-0.0124 - 0.52	0.0606
	CRI	-0.272	-0.52 - 0.0167	0.0645
	AIP	0.097	0.026 - 0.549	0.033*
FABP4	Edad	0.1870	-0.096 - 0.442	0.1934
	Triglicéridos	0.2832	-0.008 - 0.53	0.056
	Colesterol HDL	0.1890	-0.104 - 0.458	0.2032
	Colesterol total	0.2614	-0.028 - 0.51	0.0759
	CRI	0.05283	-0.053 - 0.33	0.079
	AIP	0.1570	-0.014 - 0.425	0.039*

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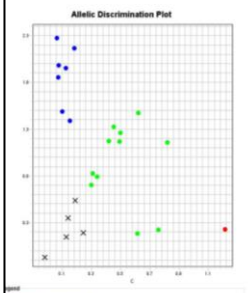
Frecuencias genotípicas y alélicas del polimorfismo TGF-β (C509T)



Genotipo	n	%
CC	7	14
CT	23	46
TT	20	40

Alelo	F
C	0.37
T	0.63

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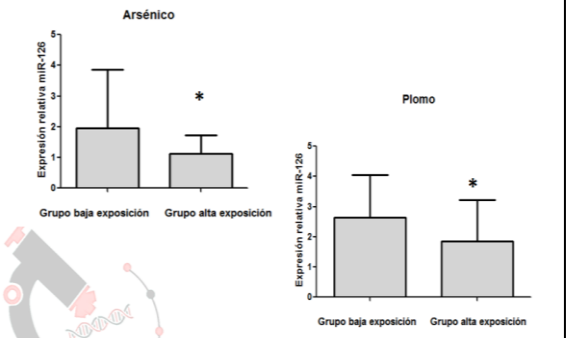
Cuantificación de la expresión de miRNA -126 mediante RT-qPCR



Prueba kolmogorov smirnov
Prueba t student
*p<0.05

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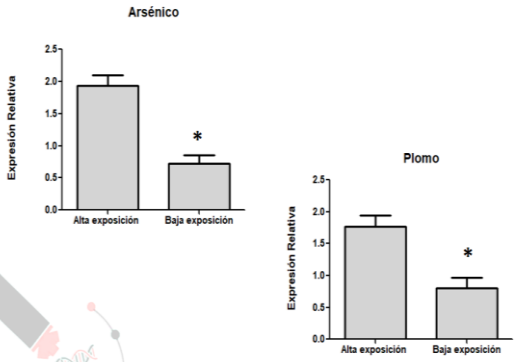
Cuantificación de la expresión de miRNA -155 mediante RT-qPCR



Prueba kolmogorov smirnov
*p<0.05

40

Correlación de parámetros bioquímicos con niveles de expresión relativa de miR-126, miR-155

Marcador molecular	Variables	r	95% CI	p
miR-126	Edad	-0.01689	-0.301 - 0.270	0.9073
	Triglicéridos	-0.008067	-0.29 - 0.278	0.95
	Colesterol HDL	0.02116	-0.26 - 0.305	0.8840
	Colesterol total	-0.2016	-0.461 - 0.089	0.1602
	CRI	0.03769	-0.24 - 0.315	0.7971
	AIP	-0.03117	-0.31 - 0.257	0.8299
miR-155	Edad	-0.19	-0.44 - 0.093	0.1852
	Triglicéridos	0.3471	0.097 - 0.576	0.031*
	Colesterol HDL	0.02774	-0.26 - 0.312	0.8484
	Colesterol total	-0.07160	-0.35- 0.219	0.621
	CRI	0.0965	-0.17 - 0.385	0.225
	AIP	0.2021	-0.0019 - 0.52	0.045*

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Validación del método espectrofotométrico de As en orina

Curva de calibración	
Rango de concentración	1– 40 ppb
Límite de detección	0.5 ppb
Límite de cuantificación	2.0 ppb
Exactitud	92.7 %
Repetibilidad	91.0 %

Control de calidad, As-U ClinChek® nivel I y II

EAA (PERKIN ELMER 3110), con generación de hidruros (Analyst 100-FIAAS), celda de cuarzo (900°C) y gas acarreador argón.

Muestra: orina

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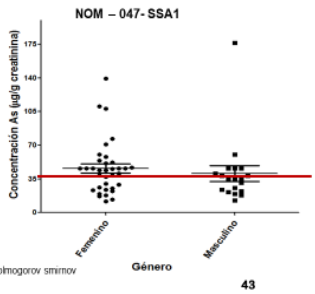
Cuantificación de la exposición As

Género	No. Personas	Media +/- DE	Rango
Femenino	35	45.8 +/- 20.6	11.68 - 139.4
Masculino	19	40.5 +/- 15.7	17.91 - 176.6



Prueba kolmogorov smirnov

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Validación del método espectrofotométrico de Pb en sangre

Curva de calibración	
Rango de concentración	0.5- 20 ppb
Límite de detección	1.8 ppb
Límite de cuantificación	6.1 ppb
Exactitud	95.0 %
Repetibilidad	90.0 %

Control de calidad, Pb-S
Sangre liofilizada ClinChek® nivel II

EAA (PERKIN ELMER 3110), con generación de hidruros (Analyst 100-FIAAS), celda de cuarzo (900°C) y gas acarreador argón.

Muestra: sangre

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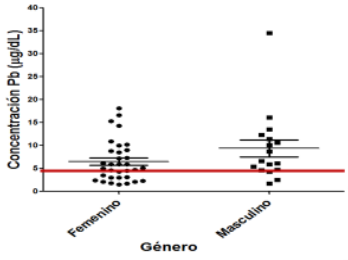
Cuantificación de la exposición a Pb

Género	No. Personas	Media +/- DE	Rango
Femenino	33	6.5 +/- 2.1	1.5- 18.1
Masculino	17	9.4+/-6.3	1.7 – 34.5

NOM – 047- SSA1



Prueba kolmogorov smirnov

Estancia de investigación
Laboratorio de Género, salud y ambiente
Facultad de Medicina, UASLP

Cromatografía de gases – masas
Cuantificación de DAP´s en orina

Cromatografía de líquidos– masas
Cuantificación de DAP´s en orina

Cronograma de actividades

Actividad / Fecha	Sep/ Feb´20	Mzo/ Ago´20	Sep/ Feb ´21	Mzo/ Ago ´21	Sep/ Feb ´22	Mzo/ Ago´22	Sep/ Feb ´23	Mzo/ Ago ´23
Revisión bibliográfica								
Someter el protocolo en el Comité de Ética								
Muestreo								
Evaluación de parámetros bioquímicos								
Determinación de índices aterogénicos								
Cuantificación de la exposición a As y Pb								
Cuantificación de biomarcadores de riesgo cardiovascular (ICAM-1, ADMA, FABP4)								
Evaluación de la expresión del polimorfismo C509T								
Cuantificación de la expresión miRNA-126 miRNA-155								
Redacción de tesis y artículo								
Defensa de tesis								