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RESEARCH ARTICLE

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EWAS in COPD by biomass-burning smoke exposure identifies low levels of endothelin-1 by hypermethylation of EDN1

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ABSTRACT

Background: Exposure to smoke from biomass combustion is a significant environmental risk factor for chronic obstructive pulmonary disease (COPD). Through epigenome-wide association studies (EWAS), changes in DNA methylation levels associated with pathological conditions can be identified. We aimed to determine the methylation patterns in genes involved in the development of COPD resulting from exposure to biomass-burning smoke (COPD-BBS).

Materials and methods: EWAS was conducted on induced sputum samples from 45 women with stable COPD (COPD-BBS) exposure and 45 women exposed to BBS but without the disease (BBES). Proteins whose genes showed significant differences in methylation and were soluble in the induced sputum supernatant were quantified.

Results: 205 CpG sites were found differentially hypomethylated, and 420 were hypermethylated. The top 50 show genes associated with lung remodeling (*FOXP1* $p = 0.002$, *FMOD* $p = 0.012$, *EDN1* $p = 0.044$), the immune system (*ALOX5* $p = 0.005$, *IL19* $p = 0.047$), mucus production (*MUC19* $p = 0.04$), and xenobiotic metabolism (*GSTO2* $p = 0.02$). Of the proteins evaluated, endothelin-1 was decreased in the reference group compared to patients ($p = 0.00054$).

Conclusions: Gene methylation changes are linked to lung remodeling, immune response, mucus production, and xenobiotic metabolism. Hypermethylation of the cg08450425 site (*EDN1*) is significant in women with COPD and associated with low endothelin-1 levels.

PLAIN LANGUAGE SUMMARY

DNA modifications not present in the sequence contribute to COPD in women exposed to biomass-burning smoke.

In both rural and urban areas, using biomass (firewood) for cooking and heating homes is quite common. Unfortunately, the smoke produced from burning firewood contains substances harmful to the respiratory system.

The primary population affected is women, leading some to develop lung disease. The aim of this study was to assess the changes that genetic material can exhibit and their impact on the production of proteins involved in the disease.

We found a significant effect on genes that play roles in inflammation and detoxification processes in the body. Notably, our findings highlight the influence on the endothelin gene, which encodes a protein that regulates inflammatory responses and manages high blood pressure in the pulmonary arteries.

These results will enable us to explore additional pathways that have not been previously analyzed to gain further insight into the disease.

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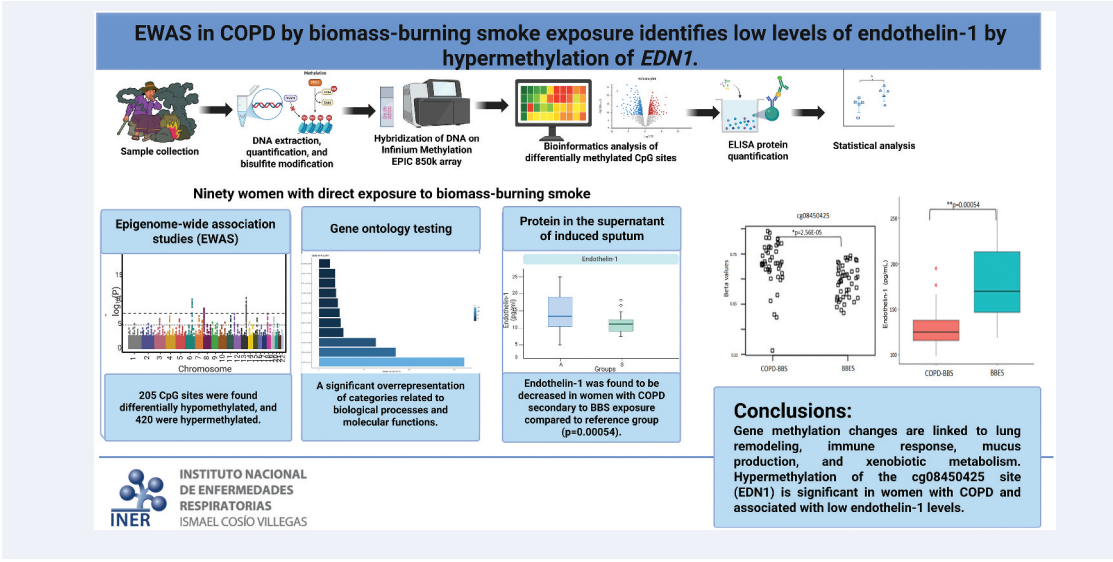

1. Introduction

Chronic obstructive pulmonary disease (COPD) is a complex and multifactorial pathology. While tobacco smoking is considered the leading risk factor for developing it, continued exposure to smoke from biomass combustion (burning wood, charcoal, crop waste, dung, etc.) is another critical risk factor [1,2]. Approximately 50% of the world's population and 90% of households in rural and suburban areas use it for various purposes [3]. In countries such as China, India, and sub-Saharan Africa, more than 80% of households use biomass as a fuel; in Latin America, the proportion ranges up to 75% [4]. The intensity of biomass exposure can be measured using the biomass exposure index (BEI), which is calculated by multiplying the duration of biomass combustion by the number of years and the hours per day. The minimum threshold of BEI of 60 is associated with a high risk of developing chronic bronchitis [5]. The development of COPD has been associated with genetics [6,7], environmental factors [8], and their interactions. Genome-wide association studies (GWAS) and genome-wide interaction studies have identified genetic variants in genes associated with susceptibility to COPD, decreased lung function, and increased frequency of exacerbations [8,9]. Through epigenome-wide association studies (EWAS), changes in DNA methylation levels associated with tobacco smoking have been identified, with the most replicated findings located in the *AHRR*. The protein encoded by this gene participates in the aryl hydrocarbon receptor-signaling cascade, which mediates dioxin toxicity and regulates cell growth and differentiation [10]. DNA methylation is a tissue-specific epigenetic modification that plays a crucial role in regulating gene expression, exhibiting a high level of cell-type specificity. These mechanisms are reversible, making them potential biomarkers for the diagnosis and prognosis of diseases [11]. The epigenetic causes of COPD secondary to exposure to smoke from biomass burning have yet to be identified. This study aimed to determine methylation patterns in genes involved in the pathophysiology of COPD secondary to biomass-burning smoke exposure through an EWAS and its effect on protein concentration in induced sputum.

2. Materials and methods

2.1. Study population

Ninety women with direct exposure to biomass-burning smoke (BBS, ≥100 hours per year for ≥10 years; self-report data) and never-smokers were included and divided into two groups. The EWAS was performed in the patient group, which comprised 45 women with stable COPD (COPD-BBS), while the reference group consisted of 45 women exposed to BBS but without the disease (BBES). To assess whether changes in methylation affected protein levels, we analyzed a subgroup of 20 participants from each study group. The participants were recruited from COPD early detection campaigns in rural villages in the Oaxaca highlands and suburban areas in the Tlalpan mayoralty, Mexico City, where they used biomass for cooking and heating [12,13]. The COPD Clinic of the Tobacco Smoking and COPD Research Department of the Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas (INER) organized these campaigns. After a detailed explanation of the protocol's aims, subjects were invited to participate voluntarily. Upon acceptance, they signed the informed consent form corresponding to the study. The protocol was previously approved by the Institutional Committees of Research, Biosecurity, and Ethics in Research of the INER (protocol code B18–17), and it complied with the Declaration of Helsinki (2013).

2.2. Collection of induced sputum

Subjects were asked to wash their mouth with water. Nebulization was conducted for 5 minutes, with a 5-minute rest period between each session. This procedure was performed a maximum of 3 times according to the protocol established by the European Respiratory Society (ERS). Samples of induced sputum were collected using nebulization with a 7% sterile saline solution. [14]. Finally, sputum was collected into sterile containers and transported to the laboratory for analysis.

As previously described, the sputum was broken up in sterile isotonic saline solution using mechanical procedures [15]. In a biosafety cabinet, saliva was eliminated using

Article highlights

- The methylation status in women exposed to biomass-burning smoke is relevant in COPD.
- Genes affected are involved in lung remodeling, the immune system, and mucus production.
- The hypermethylation of the site in EDN1 shows a low concentration of endothelin-1.

a transfer pipette. In a 1:1 ratio, sterile saline solution (0.9%) was added to the sputum using a sterile syringe and 20-gauge needle to disaggregate the sputum (up and down a minimum of 10 times); subsequently, a 22-gauge needle was used until complete disintegration. The resulting sample was filtered (Falcon 70 µm Cell Strainer) and centrifuged at 3000 rpm at 20°C for 10 min. The supernatant of the induced sputum was stored at −80°C for the quantification of soluble proteins by enzyme-linked immunosorbent assay (ELISA) and the precipitate of cells that were destined for human DNA extraction; this was obtained following the protocol of the commercial kit sputum DNA Collection, Preservation, and Isolation kit (Norgen Corporation, Thorold, ON, Canada).

2.3. DNA quantification and bisulfite modification

The purity and concentration of DNA were evaluated through an ultraviolet light absorption spectrophotometer (NanoDrop™ 2000, Thermo Fisher Scientific, Massachusetts, USA) at 260 nm wavelength. Protein contamination was determined at 280 nm, and a sample was considered free of contaminants when the 260/280 ratio was between 1.7 and 2.0. Samples were adjusted to 50 ng/µL. The DNA integrity of the samples was assessed by electrophoresis. 500 ng of genomic DNA from each sample was converted with sodium bisulfite, following the EZ DNA Methylation kit protocol (Zymo Research Corporation, Orange, CA, USA).

2.4. Evaluation of differentially methylated CpG sites

We utilized the Infinium Methylation EPIC 850k array (Illumina, San Diego, CA, USA) according to the manufacturer's recommended protocol. Each CpG site's score was represented by β-values, according to its fluorescence intensity, ranging from 0 (unmethylated) to 1 (methylated). We imported the intensity data files obtained from the microarray, performed quality control, removed unhybridized probes, and conducted data normalization and analysis of differentially methylated site scores in Base-R RStudio v.3.5.0 [16]. We utilized the Bioconductor workflow for methylation analysis of the 450K array, which we adapted for the 850K microarray. This pipeline encompasses quality control, filtering, normalization, data exploration, and statistical testing for probe-wise differential methylation analysis. The minfi package was employed to calculate p-values for intensity detection and during the quantile normalization process [17]. IlluminaHumanMethylationEPICkanno.ilmn12hg19 and IlluminaHumanMethylationEPICmanifest provide the Illumina manifest as an R object that can be easily loaded into the environment. This manifest contains all the annotation information for each of the CpG probes on the EPIC 850K array, which is useful for determining the genomic context of differentially methylated probes [18,19]. MissMethyl, minfiData, and DMRcate, which are designed explicitly for methylation analysis, were also utilized in the workflow [17,20]. The differential methylation analysis between the comparison groups was conducted using the M-value matrix in the limma package [21]. We consider a probe to be differentially methylated if the absolute value of the difference between robust β-values medians in samples from each group exceeds 0.2 [22].

2.5. Gene ontology testing

To better understand the biological processes in which differentially methylated CpG sites may be involved, a Gene Ontology (GO) or KEGG pathway analysis was performed using the gometh function of the missMethyl package [23] in RStudio 4 [16]. We used over-representation analysis, and significant clusters were selected based on a p-value of < 0.05.

2.6. Protein in the supernatant of induced sputum

Biologically relevant genes were selected that could be involved in the pathogenesis of COPD and whose protein product could be measured in the induced sputum supernatant (extracellular protein). We selected three CpG sites in genes with statistically significant differences in methylation levels. Evaluation of proteins was performed using the ELISA technique in the induced sputum supernatant; 20 samples of each group were analyzed in triplicate, and a standard curve was performed in duplicate for each test. The human fibromodulin (FMOD) ELISA kit (Elabscience, catalog No. E-EL-H2098, Houston, Texas, USA) had a detection range of 0.31–20 ng/mL and a sensitivity of 0.19 ng/mL. The human endothelin antibody ELISA kit (MyBioSource, catalog MBS025621, San Diego, CA, USA) had a detection range of 6.25–200 pg/mL and a sensitivity of 1 pg/mL. Finally, the human phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase ELISA kit (MyBioSource, catalog MBS761600, San Diego, CA, USA) had a detection range of 0.156–10.0 ng/mL, and the sensitivity is $<$ 0.094 ng/mL.

2.7. Sample-size estimation of EWAS

The sample size was calculated using the G*Power program version 20 [24], with a statistical power of 95%, a significance level of 0.05 (bilateral test), and a 1:1 case-to-control ratio. Under these criteria, the study required 45 patients and a reference group of 45 subjects.

2.8. Statistical analysis

Demographic variables and protein concentrations in induced sputum supernatant were analyzed in RStudio version 3.5.0 [16]. Parametric tests were used for variables with normal distribution, and non-parametric tests were used for variables with non-normal distribution. Cell DNA methylation levels in induced sputum samples from women with COPD and

Table 1. Demographic and clinical characteristics of women exposed to biomass-burning smoke.

Variable	COPD-BBS ($n = 45$)	BBES ($n = 45$)	p
Age (years)	70 (51–85)	63 (47–82)	< 0.001**
BMI (kg/m^2)	28.5 (18.4–43.1)	27.6 (14.8–43.1)	NS
BEI (hours/year)	398.6 (±185.3)	226.8 (±125.5)	< 0.001*
Exposure to BBS (years)	56 (40–64)	50 (41–54)	NS
FEV1%)£	74 (42–89)	107 (53–154)	< 0.001**
FVC (%)£	89 (76–104)	99 (91–113)	< 0.001**
FEV1/FVC%£	62 (36–69)	82 (70–99)	< 0.001**

COPD-BBS: Women with COPD secondary to exposure to biomass-burning smoke. BBES: Women exposed to biomass-burning smoke but without COPD. BMI: Body mass index. BEI; biomass-burning smoke exposure index. BBS: Biomass-burning smoke. FEV1: Forced expiratory volume in the first second. FVC; Forced Vital Capacity. *p-value by Student's t-test or ** Mann-Whitney U-test. NS; non-significative. £Post-bronchodilator values.

exposure to BBS without COPD were assessed using Bayes' t-test, followed by a multiple-test correction to obtain corrected p-values. This statistical method reduces the estimated sample variance of the ordinary t-statistic to a pooled estimate, leading to greater power and more stable inference in studies, regardless of sample size [25]. The Manhattan plot was created using the qqman package [26], and the ggplot2 package version 2.2.1 was used for the volcano plot [27]. The data presented in the study are deposited in the GEO online repository, accession number GSE268635 https://www.ncbi.nlm.nih.gov/geo/subs/.

3. Results

We included women who were exposed to smoke from biomass burning. COPD patients were older and had higher BEI in comparison with exposed women without COPD; however, years exposed to BBS did not show statistical differences (Table 1).

We analyzed 853,307 CpG sites in 90 samples of induced sputum. After quality control (p-value < 0.002) (Supplementary Figure S1), removal of non-hybridized probes, and normalization of the data using the quantile function (Supplementary Figure S2), we obtained 824,219 probes that met the quality control criteria. The differential methylation analysis revealed 625 CpG sites between the study groups, with statistically significant differences in p-values adjusted for multiple testing using the false discovery rate (FDR) (adjusted p-value ≤0.05) [28] (Supplementary Figure S3). Of these CpG sites, 205 were hypomethylated (log fold change, FC ≤-0.2) and 420 hypermethylated (log FC ≥ 0.2) (Figure 1). We present the top twenty CpG sites with log fold change (FC) ≤ −0.2 or ≥ 0.2, exhibiting differential methylation ($p < 0.05$) and located in genes relevant to COPD pathogenesis (Table 2).

The selected CpG sites for protein quantification were cg26508775 (log FC = 0.518), cg08450425 (log FC = 0.254), and cg09676860 (log FC = 0.302), each site in *FMOD, EDN1*, and *PTEN*, respectively, and with differences statistically significant by multiple tests ($p = 0.012$, $p = 0.044$, and $p = 0.044$,

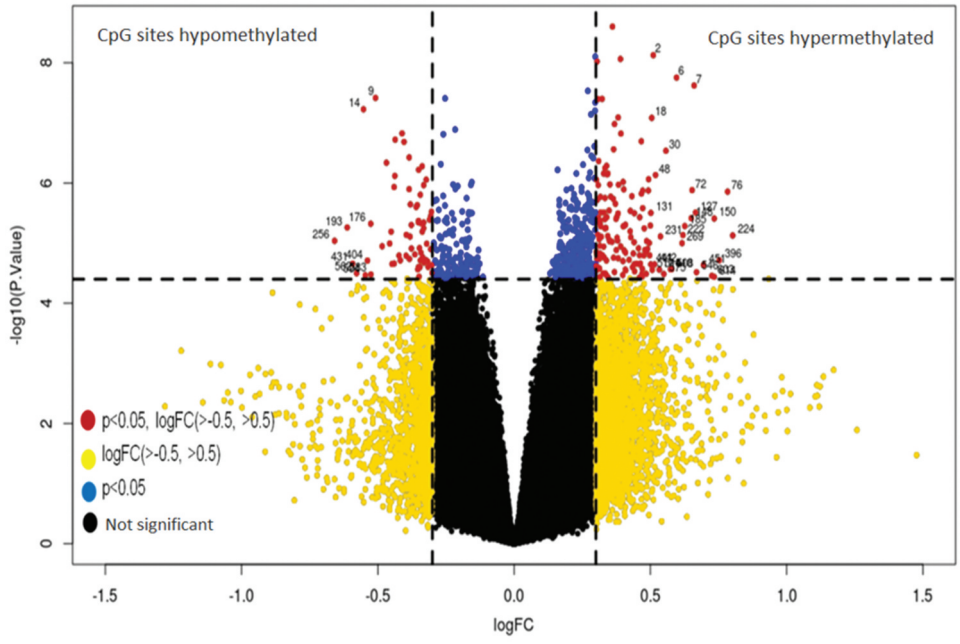

Figure 1. Volcano plot showing the differential methylation analysis between patients with COPD by BBS and the subjects of the reference group. The x-axis shows log Fold change (logFC), and the y-axis shows the – log10 p-value of each CpG site. Red represents hypermethylation and hypomethylation sites.

Table 2. Top twenty CpG sites with differential methylation and relevance in COPD-BBS pathogenesis.

ID	chr	Name	Relation to Island	Gene name	Log FC	p-adjusted
cg25533943	1	cg00002646	Open Sea	*S100A5*	−0.396	0.037
cg03118206	2	cg03175652	N-Shelf	*MEIS1*	−0.382	0.027
cg20469639		cg09965849	Open Sea	*LTBP1*	0.375	0.033
cg12042864	3	cg16770720	Open Sea	*FOXP1*	0.308	0.002
cg23237765		cg14564722			0.348	0.029
cg17588904	6	cg04286337	N-Shore	*TNXB*	0.381	0.003
cg15294150	8	cg07482692	N-Shore	*TRAPPC9*	0.304	0.016
cg12214908	10	cg08229320	S-Shore	*ALOX5*	0.392	0.005
cg16455757		cg27293118	Island	*GSTO2*	0.311	0.029
cg23642677		cg22575127	Open Sea	*MGMT*	−0.342	0.035
cg09676860		cg13528847	Island	*PTEN*	0.302	0.044
cg06099276	11	cg06828386	Open Sea	*DLG2*	−0.411	0.005
cg16135716		cg06008433	N-Shore	*ZNF143*	0.616	0.030
cg04318602	12	cg01940139	Open Sea	*KLRD1*	−0.322	0.013
cg16206871		cg06875318	Island	*ORAI1*	0.734	0.021
cg16219031		cg00292004	S-Shore	*NCOR2*	0.3590	0.030
cg17131553		cg12438536	Open Sea	*RBM19*	−0.484	0.031
cg24396691	17	cg08388822	Island	*ZSWIM7*	0.373	0.021
cg18252968		cg26987720	Open Sea	*BAIAP2*	−0.376	0.033
cg06288154	21	cg12269905	Open Sea	*TTC3*	0.351	0.014

Chr: Chromosome; log FC: log fold change; N-Shore: upstream, 5′ end; S-Shore: downstream, 3′ end; p: adjusted p-value for multiple tests.

respectively). Therefore, soluble proteins quantified in the induced sputum supernatant were fibromodulin, endothelin-1, phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase, and dual-specificity protein phosphatase (PTEN).

We did not find statistically significant differences in the levels of fibromodulin and PTEN (Supplementary Figure 4a and 4b); however, endothelin-1 was found to be decreased in women with COPD secondary to BBS exposure 125.05 pg/ml (98.32–194.94) compared with the reference group 169.33 pg/ml (118.33–264.70, $p = 0.00054$) (Figure 2).

The results of the enrichment tests revealed a significant over-representation of categories related to biological processes and molecular functions (Supplementary Table S1). Among the molecular function category, we highlight, among other functions, the structural constituent of ribosome (GO:0003735), ATPase binding (GO:0051117), and promoter-specific chromatin binding (GO:1990841). Regarding biological processes, highlight the negative regulation of cellular component organization (GO:0051129), regulation of protein-containing complex assembly (GO:0043254), and epithelial cell development (GO:0002064) (Figure 3).

4. Discussion

This study is the first EWAS of COPD in women exposed to biomass-burning smoke. The relevance of this study is that the pathogenesis of BBS-induced COPD is distinct from cigarette smoke-induced COPD [29,30]. Ninety percent of rural households rely on biomass fuel for cooking, which primarily affects women [31]. We included women exposed to BBS (≥100 hours per year, for ≥10 years) with and without COPD. The patients were older compared to women without the disease (70 *vs*. 63 years); for women over 30 who were predominantly undertaking domestic duties in rural areas, the

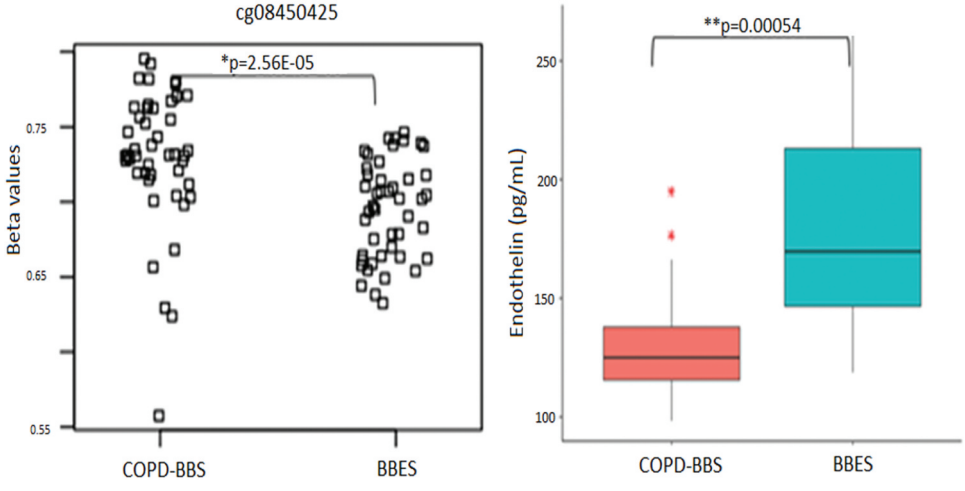

Figure 2. (a) Methylation differential in cg08450425 (*EDN1*) between patients with COPD by BBS and the reference group. (b) Protein level of endothelin-1 in the supernatant of induced sputum. COPD-BBS: women with COPD secondary to exposure to biomass-burning smoke. BBES: women exposed to biomass-burning smoke but without COPD.

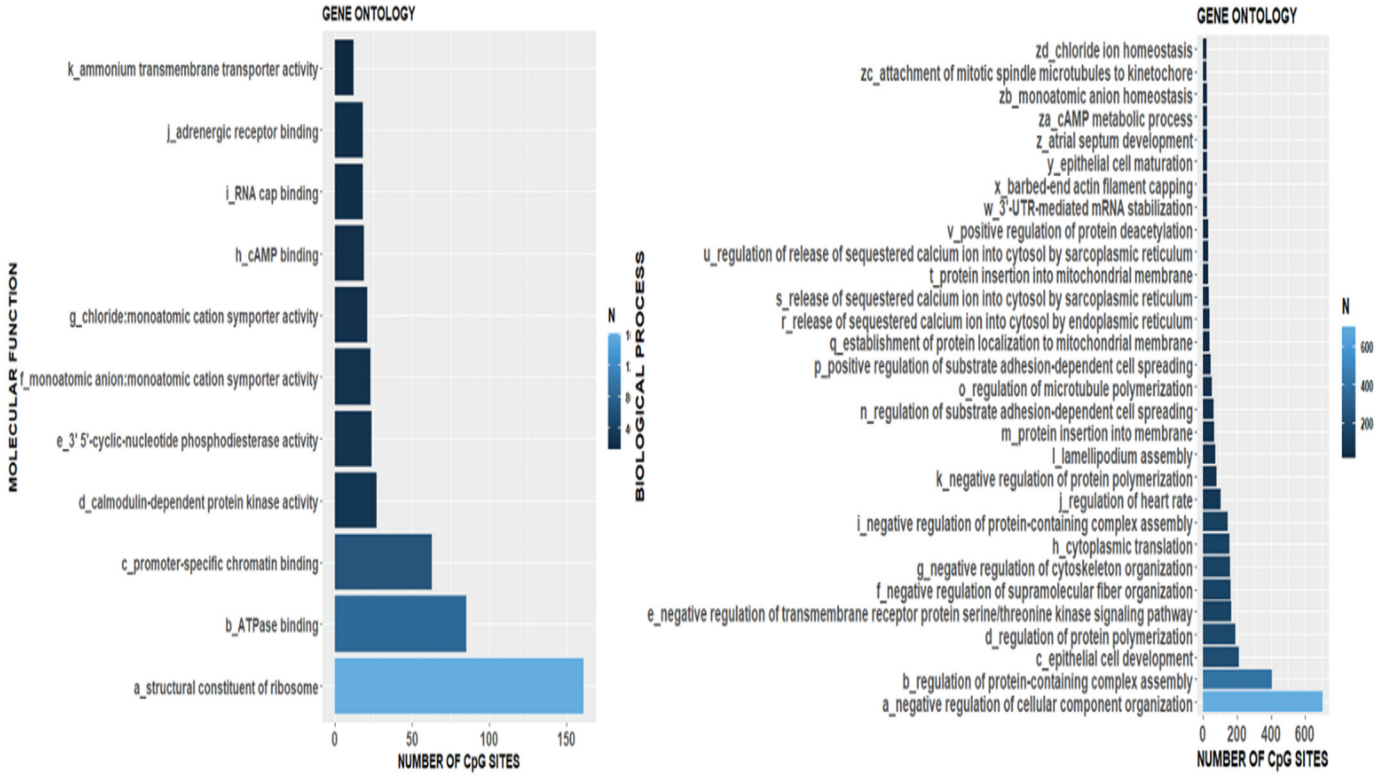

Figure 3. (a) Gene ontology testing enrichment analysis of molecular function for differential methylation analysis; (b) and biological process. The x-axis shows the number of CpG sites.

relative risk for COPD was estimated as either 3.2 (95% CI 2.3–4.8) [32]. Groups have not shown significant differences in years of exposure to BBS; however, a difference exists in the biomass exposure index. Our population is similar to other populations analyzed, such as those with prolonged exposure to biomass combustion smoke (more than 100 hours per year, for at least ten years) [33].

We identified 205 hypomethylated and 420 hypermethylated CpG sites. Among the lung's most associated and relevant sites and the extracellular protein product, we evaluated fibromodulin, endothelin-1, and PTEN in the induced sputum supernatant.

FMOD encodes for fibromodulin, a member of the family of small leucine-rich proteoglycans. It participates in mechanisms such as the assembly of collagen fibers in the extracellular matrix, modulates TGF-β activity, stimulates endothelial cell growth, and participates in the inflammatory response through the classical complement pathway [34]. An *in vitro* assay showed that fibromodulin in serum significantly increased C3b deposition and decreased survival of *Moraxella catarrhalis*, which is clinically important in COPD exacerbations [35,36]. We found that the CpG site (cg26508775) of the *FMOD* is hypermethylated in women with COPD compared to the control group. However, hypermethylation of cg26508775 does not affect fibromodulin levels in induced sputum supernatant from women with COPD.

We identified a CpG site (cg08450425) that is hypermethylated in *END1*, which encodes endothelin-1. The induced sputum supernatant of women with COPD showed a low level of endothelin-1, contrary to what was reported in the sputum of COPD tobacco smokers, where endothelin-1 levels increased during exacerbation [37]. We evaluated women with COPD by exposure to BBS and with cg08450425 hypermethylated, suggesting this epigenetic mechanism is affecting endothelin-1 levels in stable patients. This difference between COPD by tobacco smoking and COPD by exposure to BBS suggests different mechanisms involved in the pathogenesis of these phenotypes. In COPD caused by tobacco smoking, the amplification of inflammation is maintained, especially in small airways, despite quitting smoking. High levels of endothelin-1 in bronchoalveolar lavage (BAL) are implicated in COPD pathogenesis through their involvement in the inflammatory cycle [38]. Instead, in women with COPD by biomass-burning smoke, levels of endothelin-1 depend on methylation of cg08450425, which is low.

Endothelin-1 is a bronchoconstrictor peptide expressed in bronchial epithelium, pulmonary endothelium, and alveolar macrophages, with pro-inflammatory effects in the airways. It acts as both a chemoattractant and an upregulator of other inflammatory mediators [37]. Higher endothelin-1 serum levels were associated with lower FEV1 among African Americans [39]. It is also a crucial mediator for establishing pulmonary artery hypertension (PAH); patients with advanced COPD frequently present with pulmonary artery hypertension [40]. High levels of endothelin-1 are a biomarker of endothelial dysfunction [41]. However, further studies are needed to clarify the relevance of endothelin-1 in COPD associated with biomass-burning smoke.

We demonstrated hypermethylation in *PTEN*; however, protein determination in the supernatant of induced sputum from women with COPD by BBS did not show significant differences. Reports in COPD patients smoking show low levels of PTEN, contributing to the activation of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway and the release of pro-inflammatory mediators [42]. *PTEN* was found to be hypermethylated in women with COPD due to BBS. The silencing of this could result in processes that alter the cell cycle, promoting the presence of cancer cells or accelerated cellular aging, as well as an increased risk of lung cancer in COPD [42].

Hypermethylation in the *FOXP1* can decrease its expression in COPD patients; we identified two sites of CpG hypermethylated in *FOXP1*. Low levels of FoxP1 protein increased IL-6 levels and secretion of matrix metalloproteinases in the supernatant of cell culture in the presence of cigarette smoke extract. *FOXP1* is known to bind to CSF1R promoter elements and is involved in regulating monocyte differentiation and macrophage functions [43]. Hypermethylation of this gene may contribute to the expression of the protein and prevent the regeneration of epithelial cells in the lungs of women with COPD caused by wood smoke.

Another CpG site highlighted in COPD due to BBS exposure is located in *GSTO2* (cg16455757), a gene associated with the metabolism of xenobiotics. This site was found to be hypermethylated in women with COPD, which may prevent the gene from being expressed, thereby reducing the efficiency of xenobiotic metabolism. GSTO2 is involved in the biotransformation of arsenic and may exhibit modest expression in bronchial epithelial cells. GSTO2 could contribute to maintaining cellular redox balance [44,45]. Gene ontology analysis revealed significance in molecular function, including the structural constituent of ribosomes, ATPase binding, and promoter-specific chromatin binding, among others. The significant biological processes were negative regulation of cellular component organization, regulation of protein-containing complex assembly, epithelial cell development, and other biological processes. Regarding the structural constituents of the ribosome, COPD smokers exhibited high activity of the ribosome pathway and increased expression of ribosomal housekeeping genes (also known as hub genes) [46].

At the biological processes, in those related to the negative regulation of cellular component organization, previous reports in fibroblasts from patients with COPD smoking showed endoplasmic reticulum condensation, changes in the size of the Golgi Complex, and the distribution of lysosomes, suggesting changes in the trafficking of proteins through the cells of COPD patients [47].

The biological processes related to epithelial cell development are relevant in COPD. Cells are directly exposed to the harmful components in biomass smoke; this constant exposure leads to an exaggerated production of reactive oxygen species and inflammatory mediators, followed by alteration of the epithelial barrier and innate immune response. The evaluation of cilia size from endobronchial biopsies shows that smokers' cilia are shorter than non-smokers [48]. Interestingly, exposure to cigarette smoke increased membrane permeability in human bronchial epithelial cells of smokers with normal lung function when compared to nonsmokers ($p < 0.001$). As well as decreased levels of intracellular glutathione in cells of COPD patients compared to non-smokers ($p < 0.048$) and smokers with normal pulmonary function ($p = 0.015$) [49].

Chronic bronchitis in COPD, a typical phenotype associated with exposure to BBS, is characterized by changes in the epithelium that contribute to chronic mucus secretion and an increase in secretory cells, leading to increased mucus production and exacerbating symptoms, recurrent respiratory infections, and accelerated deterioration of lung function [50].

The validation of methylated sites in genes that encode exclusively soluble proteins is a limitation of our study; exploring other pathways and validating methylated sites in genes relevant to COPD is necessary. Our results generate hypotheses about biological processes and molecular functions critical to COPD pathogenesis. We showed the average methylation levels corresponding to the proportion of different cell types in induced sputum from COPD patients. Ideally, methylation should be measured in the cells most relevant to COPD due to exposure to biomass burning; however, induced sputum contains a mixture of different cells characteristic of the lung, reflecting the epigenetic changes occurring in that organ.

5. Conclusion

Women with COPD resulting from biomass-burning smoke exposure show 205 hypomethylated and 420 hypermethylated CpG sites in induced sputum. The induced sputum of women with COPD exhibits hypermethylation in cg08450425 (*EDN1*) and low levels of endothelin-1.

Author contributions

Conceptualization: Ramcés Falfán-Valencia, Gloria Pérez-Rubio Methodology:Salvador García-Carmona, Gloria Pérez-Rubio Software: Salvador García-Carmona, Juan C Fernández-López Validation: Alejandra Ramírez-Venegas, Ingrid Fricke-Galindo Formal analysis: Fernando Morales-González, Salvador García-Carmona, Juan C Fernández-López Investigation: María E Ramírez-Díaz, Filiberto Cruz-Vicente, María L Martínez-Gómez Resources: Rafael Hernández-Zenteno, Ingrid Fricke-Galindo Data curation: Alejandra Ramírez-Venegas, Raúl Sansores Writing – original draft preparation: Salvador García-Carmona, Gloria Pérez-Rubio Writing – review and editing Juan C Fernández-LópezL, Alejandra Ramírez-Venegas Visualization: Fernando Morales-González, María E Ramírez-Díaz, Filiberto Cruz-Vicente, María L Martínez-Gómez, Rafael Hernández-Zenteno Supervision: Ingrid Fricke-Galindo, Raúl Sansores Project administration: Gloria Pérez-Rubio acquisition: Ramcés Falfán-Valencia

Disclosure statement

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.
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Ethical Declaration

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Data availability statement

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