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Environmental Exposure Associated with Oxidative Stress Biomarkers in Children and Adolescents Residents in Brazilian Western Amazon

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Abstract

Oxidative stress is a biological process that occurs in response to an imbalance between prooxidant and antioxidant substances and has been described in the pathophysiology of more than 200 clinical disorders. In this study, we evaluate the relationship between genetic, demographic, social, environmental, and health factors and redox imbalance biomarkers in a group of children and adolescents environmentally exposed to atmospheric pollutants and mercury in the Brazilian Western Amazon. This is a cross-sectional study of the relationship between demographic, genetic, and socioenvironmental factors and serum concentrations of redox imbalance biomarkers (thiol groups, malondialdehyde, and glutathione S-transferase [GST]) in children and adolescents living in the municipality of Porto Velho, Rondônia. The investigated factors were hierarchically organized into groups of variables and their relationship with redox imbalance biomarkers was estimated by a multiple linear regression model. Children and adolescents with asthma, with C-reactive protein values, with the polymorphic variant GSTP1, and exposed to indoor air pollution presented lower thiol serum concentrations when compared to those categorized in their respective reference groups. GST activity and malondialdehyde serum concentrations were positively related to weekly fish consumption and exposure to $PM_{2.5}$. This study showed that enzymatic GST activity and malondialdehyde serum concentrations are positively associated with envi-

ronmental factors, especially air pollution (β = 8.64 U/L for GST and β = 0.244 μmol/L for MDA in high exposure group; p-value < 0.01); while serum thiol concentrations presented an inversely proportional relationship with markers of general health status, such asthma (median: 0.45 mmol/L vs. 0.48 mmol/L; p-value < 0.05), acute inflammation (β = −0.25 mmol/L; p-value < 0.01), and positively with genetic factor (β = 0.12 mmol/L for Val/Val; p-value < 0.05).

Keywords

Redox Imbalance, Glutathione S-Transferase, Thiol Groups, Malondialdehyde, Brazilian Amazon

1. Introduction

Redox imbalance is defined as the difference between the prooxidant and the antioxidant systems, with a predominance of the oxidants. This imbalance is established by the overproduction of reactive species—molecules containing one or more unpaired electrons in their orbital and highly prone to react with atoms of other molecules—and/or by failures or an inefficient performance of the antioxidant system [1] [2] [3].

The reactive species are produced naturally or by biological dysfunction, and under normal physiological conditions, the reactive species are maintained in equilibrium due in large part to the neutralizing capacity of the enzymatic antioxidant defense system. This biological process can be influenced by a range of modifiable and non-modifiable factors, such as environmental, demographic, ethnic, cultural, clinical, and nutritional factors [4]. For instance, the redox imbalance condition has been reported as a result of numerous environmental exposures, such as air pollutants [5] [6]; chemical compounds present in water, soil, and food; smoking [7]; ultraviolet rays [8]; intense practice of physical exercise [9]; stress [10]; and poor diet [11] [12].

In environmental health, children may be vulnerable to this imbalance because they present naturally low concentrations of antioxidants, from conception to childhood [13] [14]. This natural deficit tends to increase the risk of permanent and irreversible damage associated with early environmental exposure, since some of these antioxidants, like glutathione, participate in the pollutant detoxification process [15]. In this context, the effects of environmental exposure in children and adolescents living in the Brazilian Amazon region are particularly challenging for the scientific community, since this region presents a complex scenario of interactions among environmental, social, cultural, spatial, temporal, and economic factors. The region features poor socioeconomic indicators and a history of exposure to atmospheric pollutants from biomass burning and methylmercury as an environmental byproduct of the mining activities in the region. Recently, an *in vitro* study carried out in the Brazilian Amazon showed an increase in the level of reactive oxygen species in human lung cells

exposed to particulate matter smaller than 10 μm (PM_{10}) from the biomass burning [16]. We evaluated the associations between genetic, demographic, social, environmental, and health factors and redox imbalance biomarkers in a group of children and adolescents environmentally exposed to atmospheric pollutants and mercury in the Brazilian Western Amazon.

2. Materials and Methods

2.1. Study Area

The municipality of Porto Velho, Rondônia, is located in the western region of the Brazilian Amazon, at latitude 8˚45'00" south and longitude 63˚58'00" west. About 90% of the population lives in the urban area and 23% of the 428,527 inhabitants are children and adolescents aged 5 to 17 years old [17]. The municipality is a rural population that lives in community nuclei consisting of 20 to 30 homes along the banks of the Madeira River, which is one of the most important tributaries of the Amazon.

The tropical climate is typical of the Brazilian Amazon region, hot and humid, with average temperature of 26˚C and relative humidity of 80% [18]. It has well defined rain and dry cycles between November and April and July and October, respectively. During the seasonal drought, the maximum temperature reaches 39˚C on average and the relative humidity varies between 52% and 75%. In addition to high temperatures, during this period the municipality is vulnerable to intense fires and the smoke from fires in the neighboring countries such as Bolivia.

2.2. Study Design and Population

A cross-sectional study was conducted on the relationship between demographic, genetic, socioenvironmental, and health factors and serum concentrations of redox imbalance biomarkers in children and adolescents living in the municipality of Porto Velho during the drought period (July and August 2012). The study population consisted of 200 children and adolescents aged 5 to 17 who had resided in the Environmental Cuniã Reserve (a rural/isolated area), in the Belmont community (located in the periurban area of the city), or in the Nacional neighborhood (located in the urban area). The criteria to be included in this study were: at least 1 year living in the community, being between 5 and 17 years old, not being pregnant, freely given consent, and have parent's authorization. Legal guardians of the children and adolescents signed the informed consent term (ICT), consenting their participation in the study. This study was approved by the Ethics Committee of the National School of Public Health (FIOCRUZ) N˚ 936.350/CEP/ENSP/2014.

2.3. Theoretical-Explanatory Model

Serum concentrations of redox imbalance biomarkers were regarded as outcomes, and demographic, genetic, socioenvironmental, and health factors as

predictors. The latter factors were organized hierarchically in three groups: Group I) non-modifiable biological factors such as age, sex, and polymorphic variants in glutathione S-transferase (GST) enzymes; Group II) factors related to history and general health, such as asthma, pulmonary obstruction, respiratory signs and symptoms, blood glucose, iron deficiency anemia, serum iron, inhalant-specific immunoglobulin E, acute inflammation (reactive protein C), eosinophil counts, anthropometric evaluation, obesity, and weekly fish consumption; Group III) socioeconomic characteristics (income [in 2012 minimum wage was US$ 300], schooling, and local of residence) and environmental exposures (outdoor and indoor air pollution [defined by the use of a wood stove or in-house flour production], smoking, temperature, humidity, and mercury) (Figure 1).

2.4. Experimental Approaches

2.4.1. Blood Sampling

All blood samples were collected after at least 8 hours of fasting with 10 mL syringes with 25 × 7 disposable BD® needles and transferred to their respective EDTA collection tubes (vacutainers) for collection of whole blood and without gel anticoagulant separators for further immunological assays. The collection was carried out under the responsibility of the laboratory belonging to the 9 de Julho Hospital-Ceaclin (Porto Velho, Rondônia). The samples destined for the redox imbalance biomarker measurements were distributed in Eppendorf-type microtubes and transported on dry ice to the CESTEH Toxicology Laboratory (ENSP/FIOCRUZ) in Rio de Janeiro, where the analyses were performed. All samples were kept in an ultrafreezer at −80˚ in both the CEACLIN and CESTEH laboratories and were thawed only at the time of analysis.

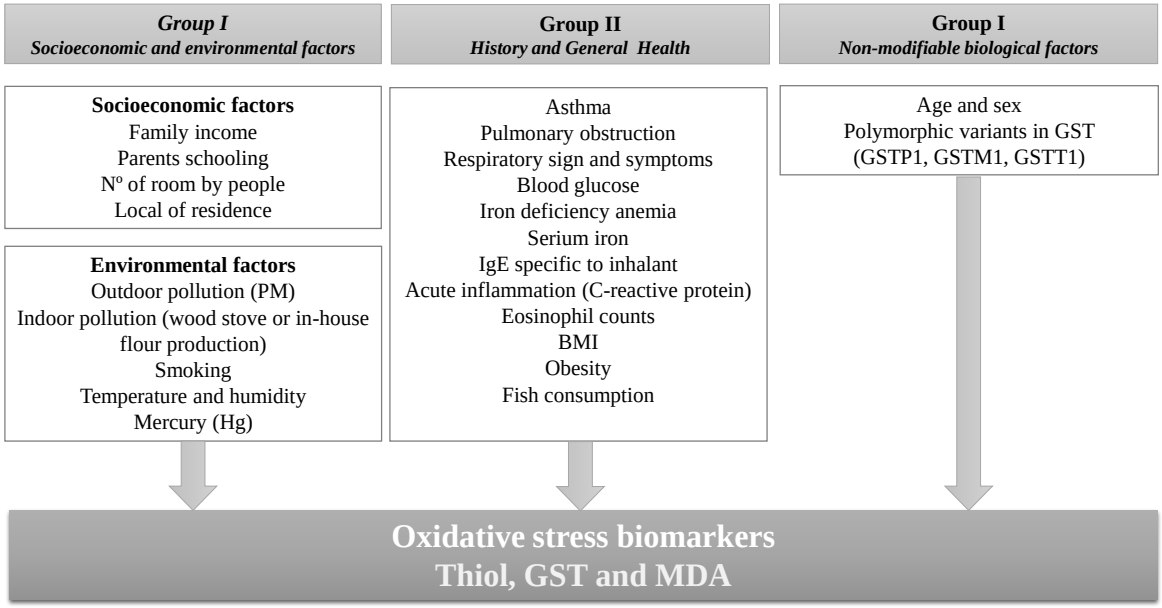

Figure 1. Graphical representation of the theoretical-explanatory model.

2.4.2. Laboratory Test

The complete blood count, ferritin, glucose, C-reactive protein, and multiple IgE for inhalants determinations were also performed by the 9 de July Hospital laboratory-Ceaclin.

2.4.3. Redox Imbalance Biomarkers

Three redox imbalance biomarkers were measured: thiol groups (a component of the antioxidant system), GST activity, and malondialdehyde (MDA). The thiol groups were measured by the methods originally described by Ellman [19] and modified by Hu [20]; the enzymatic activity of GST was determined by the method reported by Habig *et al*. [21] and adapted by Habdous *et al*. [22]; and MDA determinations were performed using the Cayman Chemical TBARS Assay Kit. All analyses were performed using UV-Vis spectrophotometry as the instrumental technique on Jasco V-530 (Kyoto, Japan) and Shimadzu UV-1601 (Kyoto, Japan) spectrophotometers.

2.4.4. Mercury in Blood

Because of the potential for human contamination by methylmercury (MeHg) in this region via fish consumption and also because this is an environmental exposure directly associated with redox imbalance, mercury (Hg) was analyzed in the blood of the evaluated children and adolescents by the Chemistry Laboratory of the Pontifical Catholic University (PUC-Rio) using the ICP-MS method [23]. Exposure to MeHg was also assessed by using as a cut-off point fish consumption intake higher or lower than 3 times a week.

2.4.5. Polymorphic Variants of GST Enzymes

The polymorphic variants GSTM1 and GSTT1 were identified by amplification by the multiplex polymerase chain reaction (PCR) using simultaneous amplification of beta-globin as a positive control and visualization of the results on agarose gels [24] [25]. Genotyping of the polymorphic variant GSTP1 was performed by PCR-restriction fragment length polymorphism (RFLP) and the results revealed by agarose gel electrophoresis. Subsequently, the amplified fragment (176 bp) was subjected to digestion with the restriction enzyme ALW261 [26] [27].

2.4.6. Air Quality Monitoring

Air pollution concentrations were measured at the monitoring station of the Institute of Physics of the University of São Paulo (USP), positioned at the Porto Velho city park. $PM_{2.5}$ data were collected using a fine and coarse particle sampler (AFG) fitted with polycarbonate filters 47 mm in diameter with 8 μm and 4 μm pores. The 5-day moving average of $PM_{2.5}$ concentrations were categorized into quartiles. Temperature and relative humidity were provided by the Center for Weather Forecasting and Climate Studies (CPTEC).

2.4.7. Evaluation of Pulmonary Function

Pulmonary function was assessed by spirometry, performed with a Fleish pneu-

motachograph (KOKO). From this examination, the forced expiratory volume parameter in the first second was used to define children with pulmonary obstruction.

2.4.8. Epidemiological Survey

A validated semi-structured questionnaire was given to the parents or guardians. The instrument consisted of specific modules to investigate child/adolescent's general health, eating habits, environmental exposures, household, and socioeconomic condition. Asthma status was defined according to the International Study of Asthma and Allergies in Childhood [28].

2.4.9. Anthropometric Evaluation

Weight was measured by means of a calibrated digital scale G-TECH Glass PRO model (Glicomed, Rio de Janeiro, Brazil) and height by means of a portable stadiometer made up of anodized aluminum and a wooden column. Obesity was defined according to the growth curves of World Health Organization (WHO). These data were compiled and analyzed in the WHO Anthro 2005 program (http://www.who.int/growthref/tools/en/).

2.3. Statistical Analyses

Mean, median, standard deviation, and interquartile range of the redox imbalance biomarkers were calculated. Mann-Whitney and Kruskal Wallis tests were used to compare the concentrations of the thiol groups. ANOVA and Student t test were used to compare the means of GST activity and MDA. Pearson's correlation coefficients were calculated for the numerical variables, which were log-transformed when the variable did not show normal distribution.

The relationship between redox imbalance biomarkers (thiol groups, GST activity, and MDA) and demographic, genetic, social, environmental, and health factors was estimated by multiple linear regression models. The inclusion of variables in the multivariate model was performed according to the hierarchically organized blocks of variables with p-value < 0.10 in the bivariate analysis. Equivalent models were compared using the Akaike Information Criterion (AIC), adjusted R2, and residuals analysis. All statistical analyses were carried out with the R software package, version 3.3.1 (https://www.r-project.org/).

3. Results

Of the total of 200 children and adolescents, 53% were between 5 and 11 years old and 59% were female. Regarding the redox imbalance biomarkers, thiol groups and MDA were able to be determined in 197 children and adolescents, while GST activity was determined in 194 individuals.

Mean serum thiol group concentrations, GST, and MDA enzyme activity were 0.48 mmol/L (SD 0.09), 19.8 U/L (SD 7.5), and 1.50 μmol/L (SD 0.328), respectively. The means, standard deviation, median and interquartile ranges of the thiol group according to the variable groups are presented in **Table 1**. In relation

Table 1. Serum thiol concentrations according to the variables in group I, II and III.

Variables		N	Median (P25% - P75%)	Mean (Standard Deviation)	p-value*
Thiol (mmol/L)		197	0.47 (0.43 - 0.51)	0.484 (±0.109)	
Group II					
Age	5 - 11 years old	104	0.46 (0.44 - 0.51)	0.47 (±0.110)	0.089
	12 - 17 years old	93	0.49 (0.44 - 0.50)	0.49 (±0.109)	
Sex	Male	80	0.46 (0.44 - 0.50)	0.48 (±0.122)	0.302
	Female	117	0.48 (0.44 - 0.51)	0.48 (±0.100)	
GSTM1	Null	52	0.47 (0.42 - 0.50)	0.47 (±0.129)	0.184
	Positive	97	0.49 (0.44 - 0.52)	0.50 (±0.118)	
GSTT1	Null	9	0.44 (0.44 - 0.51)	0.47 (±0.072)	0.636
	Positive	139	0.48 (0.44 - 0.51)	0.49 (±0.125)	
GSTP1	Ile/Ile	45	0.48 (0.44 - 0.51)	0.48 (±0.061)	0.073
	Ile/Val	92	0.48 (0.44 - 0.50)	0.49 (±0.145)	
	Val/Val	14	0.52 (0.48 - 0.54)	0.53 (±0.122)	
Group II					
Iron deficiency anemia	Yes	37	0.47 (0.44 - 0.50)	0.47 (±0.059)	0.642
	No	160	0.48 (0.44 - 0.51)	0.49 (±0.118)	
IgE specific to inhalant	Reactive	119	0.47 (0.43 - 0.50)	0.48 (±0.122)	0.263
	Non-reactive	78	0.48 (0.44 - 0.51)	0.48 (±0.087)	
C-reactive protein	<6 mg/L	188	0.48 (0.44 - 0.51)	0.48 (±0.095)	0.357
	≥6 mg/L	09	0.43 (0.35 - 0.49)	0.50 (±0.289)	
Asthma	Yes	36	0.45 (0.43 - 0.49)	0.45 (±0.042)	**0.029**
	No	152	0.48 (0.44 - 0.51)	0.48 (±0.114)	
Pulmonary obstruction	Yes	29	0.49 (0.44 - 0.53)	0.51 (±0.148)	0.140
	No	163	0.47 (0.44 - 0.51)	0.47 (±0.095)	
Respiratory symptoms	Yes	101	0.47 (0.44 - 0.51)	0.48 (±0.075)	0.688
	No	93	0.48 (0.44 - 0.51)	0.49 (±0.126)	
Fish consumption	<3 times/week	143	0.48 (0.44 - 0.51)	0.49 (±0.122)	0.514
	≥3 times/week	51	0.46 (0.44 - 0.51)	0.47 (±0.058)	
BMI	Normal	161	0.47 (0.43 - 0.51)	0.48 (±0.116)	0.595
	Overweight	26	0.48 (0.44 - 0.51)	0.48 (±0.045)	
	Obesity	08	0.48 (0.32 - 0.51)	0.42 (±0.115)	
Group III					
Family income	<1 minimum wage	43	0.49 (0.44 - 0.51)	0.54 (±0.189)	**0.047**
	1 - 2 minimum wage	68	0.46 (0.44 - 0.50)	0.46 (±0.058)	
	≥3 minimum wage	76	0.47 (0.44 - 0.51)	0.48 (±0.064)	
Parents schooling	Pre and elementary school	79	0.48 (0.44 - 0.51)	0.51 (±0.153)	0.520
	Middle school	64	0.47 (0.45 - 0.50)	0.47 (±0.057)	
	High school	50	0.49 (0.46 - 0.53)	0.48 (±0.059)	

Continued

People/room	<2 people	157	0.47 (0.43 - 0.52)	0.49 (±0.121)	0.795
	≥2 people	39	0.48 (0.44 - 0.50)	0.48 (±0.045)	
Local of residence	Rural/riverside	52	0.46 (0.43 - 0.50)	0.46 (±0.046)	0.073
	Periurban/Urban	145	0.48 (0.44 - 0.51)	0.49 (±0.124)	
Smoking in house	Yes	55	0.49 (0.44 - 0.52)	0.48 (±0.094)	0.315
	No	137	0.47 (0.44 - 0.50)	0.48 (±0.117)	
Burning of trash	Yes	124	0.47 (0.43 - 0.51)	0.48 (±0.123)	0.167
	No	70	0.49 (0.44 - 0.51)	0.48 (±0.082)	
***Indoor air pollution*[a]**	Yes	51	0.46 (0.43 - 0.50)	0.48 (±0.128)	0.252
	No	143	0.48 (0.44 - 0.51)	0.48 (±0.104)	
Oudoor $PM_{2.5}$[b]	Very low	56	0.50 (0.49 - 0.52)	0.49 (±0.047)	**0.053**
	Low	60	0.49 (0.39 - 0.57)	0.50 (±0.183)	
	Moderate	29	0.48 (0.42 - 0.51)	0.46 (±0.053)	
	High	52	0.46 (0.43 - 0.50)	0.48 (±0.043)	

*Mann-Whitney and Kruskal Wallis Test. [a]Indoor air pollution defined by the use of a wood stove or in-house flour production. [b]$PM_{2.5}$ exposure categories defined from the moving average of 4 days prior to blood collection.

to Group I, no statistically significant differences were observed in serum thiol concentrations for age, sex, and GST polymorphisms. For Group II variables, children and adolescents with asthma presented lower thiol serum concentrations when compared to children without asthma (0.45 mmol/L [SD 0.042] vs. 0.48 mmol/L [SD 0.114]; p-value < 0.05). Among the Group III variables, thiol concentrations were different among the middle-income strata families (p-value < 0.05).

Figure 2 summarizes GST activity values according to the studied blocks of variables. In group I, contrary to what was expected, GST activity, on average, was higher among patients with the polymorphic GSTM1 variant (22.8 U/L [SD 7.64] vs. 20.0 U/L [SD 7.65]; p-value < 0.05). In Group II variables, the means of GST activity were higher among children and adolescents with iron deficiency anemia (22.0 U/L [SD 8.22] vs. 19.3 U/L [SD 7.20], p-value = 0.053) and those who consume fish more than 3 times a week (25.4 U/L [SD 6.31] vs. 17.8 U/L [SD 6.78], p-value < 0.001). For Group III variables, on average, higher GST activity was observed among those with lower family income (p-value < 0.001), lower education (p-value < 0.001), residents in the rural/riverside region (p-value < 0.001), those exposed to indoor (households with wood stoves and flour production) (p-value < 0.001) and outdoor (moderate and high exposure to $PM_{2.5}$) air pollution (p-value < 0.001).

In relation to the lipid peroxidation product, MDA means were higher in children than in adolescents (1.50 μmol/L [SD 0.350] vs. 1.39 μmol/L ± [SD 0.300], p-value < 0.05). In Group II variables, the "reagent" strata of the variable IgE hx2 and consumption of fish greater than 3 times a week presented, on average, higher serum MDA concentrations when compared to their reference

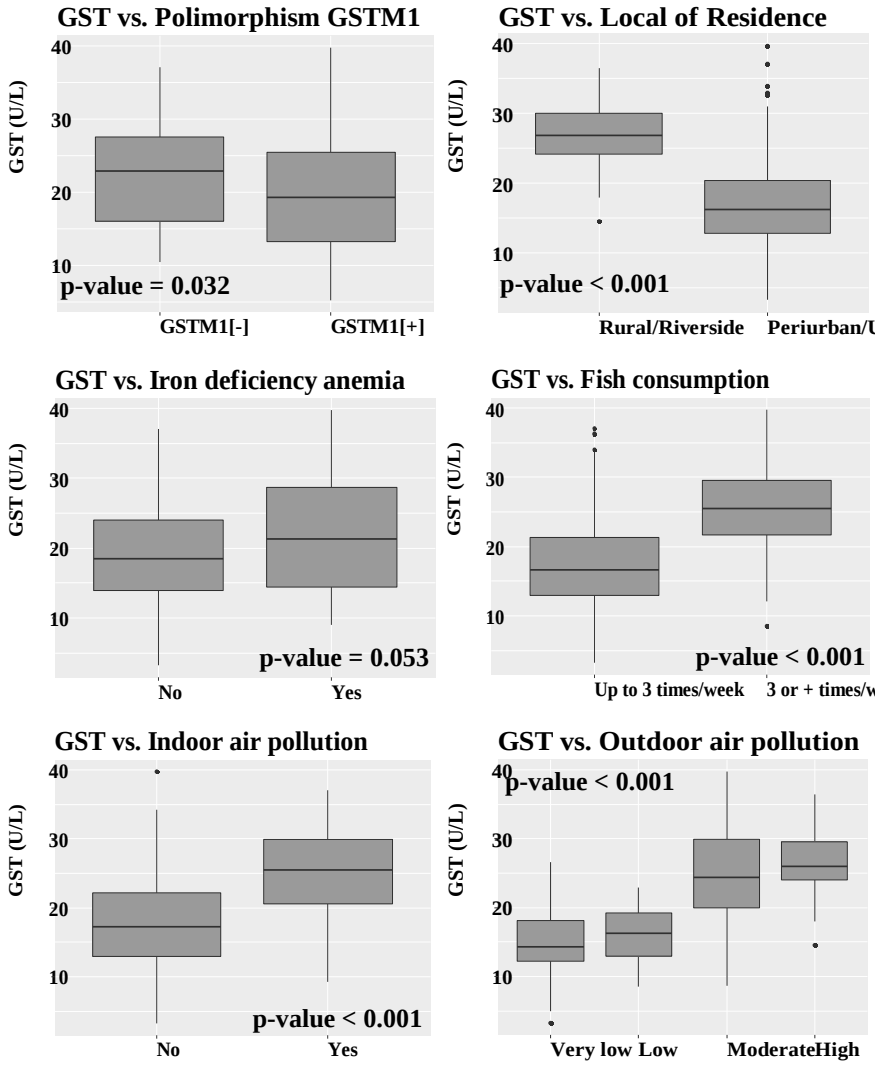

Figure 2. GST activity values according to polymorphism in GSTM1, local of residence, iron deficiency anemia, fish consumption, indoor and outdoor air pollution. [a]Student t test and ANOVA one-way. [b]$PM_{2.5}$ exposure categories defined from the moving average of 4 days prior to blood collection.

groups. When analyzing Group III variables, higher MDA averages were observed among children and adolescents living in the riverside area and parents with more than 5 years of study. The MDA averages were also higher among children and adolescents who reported having some source of indoor pollution in their homes and those outdoor groups exposed to higher $PM_{2.5}$ concentrations (Figure 3).

The Pearson correlation between the redox imbalance biomarkers was calculated for continuous and discrete variables and presented in Table 2. Serum thiol concentrations were positively correlated with haemoglobin ($r = 0.172$; p-value < 0.05) and with serum iron ($r = 0.206$, p-value < 0.05). GST activity was positively correlated with serum MDA concentrations ($r = 0.312$, p-value < 0.05), iron ($r = 0.300$, p-value < 0.05), and blood Hg ($r = 0.398$; p-value < 0.05).

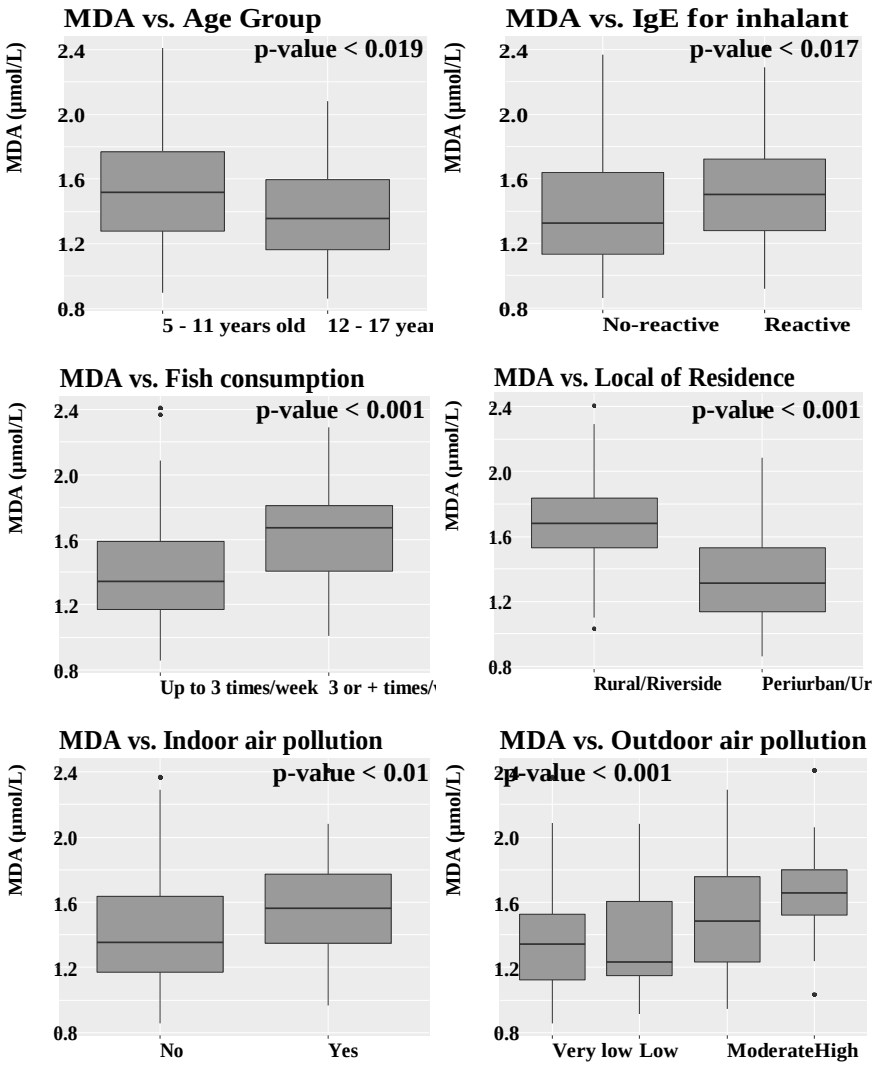

Figure 3. Malondialdehyde values according to age, IgE specific to inhalant, fish consumption, local of residence and indoor and outdoor air pollution. [a]Student t test and ANOVA one-way. [b]$PM_{2.5}$ exposure categories defined from the moving average of 4 days prior to blood collection.

On the other hand, GST activity was negatively correlated with BMI ($r = -0.162$, p-value < 0.05) and serum glucose ($r = -0.266$, p-value < 0.050). Regarding MDA concentrations, a positive correlation was observed with eosinophil count ($r = 0.178$, p-value < 0.05) and blood Hg ($r = 0.228$, p-value < 0.05), while a negative correlation to age was found ($r = -0.143$; p-value < 0.05).

The multiple linear regression model constructed to evaluate the thiol concentrations (ln-transformed) that presented the best fit and adjusted R^2 was composed of the following variables: C-reactive protein, polymorphic GSTP1 variant, haemoglobin, serum iron, family income, indoor pollution, and $PM_{2.5}$ exposure groups. For the model with GST activity as a response variable, in addition to thiol concentrations, fish consumption variables and $PM_{2.5}$ exposure groups were also associated with this biomarker (adjusted $R^2 = 55\%$). Regarding

Table 2. Pearson correlation of thiol serum concentrations, GST activity and malondialdehyde according to variables groups.

	Thiol*	GST	MDA	Age*	BMI*	Eosinophil*	IgE*	Haemoglobin	Iron	Ferritin*	Glucose	Hg*
Thiol*	1.000	0.124	−0.040	0.080	−0.057	−0.028	−0.069	**0.172**	**0.206**	−0.025	0.076	0.108
GST		1.000	**0.312**	−0.108	**−0.162**	0.131	0.103	−0.047	**0.300**	0.004	**−0.277**	**0.398**
MDA			1.000	**−0.143**	−0.056	**0.178**	0.104	0.072	0.116	0.047	−0.022	**0.228**
Age*				1.000	**0.504**	**−0.188**	0.010	**0.384**	0.078	0.017	**0.164**	−0.069
BMI*					1.000	**−0.155**	0.035	**0.232**	−0.003	0.063	0.123	−0.032
Eosinophil*						1.000	**0.205**	−0.016	0.041	**0.145**	−0.043	**0.205**
IgE*							1.000	**0.205**	0.082	**0.143**	−0.050	0.110
Haemoglobin								1.000	**0.162**	**0.187**	**0.226**	0.031
Iron									1.000	**0.197**	−0.070	0.137
Ferritin*										1.000	0.069	−0.131
Glucose											1.000	**−0.147**
Hg*												1.000

*Ln-transformed; values highlighted in bold: p-value < 0.05; MDA: malondialdehyde; BMI: body mass index; IgE: immunoglobulin E specific for inhalant; Hg: levels of mercury in the blood.

MDA, a negative correlation with age and positive correlation with IgE, pulmonary obstruction, higher fish consumption, and $PM_{2.5}$ exposure groups (adjusted R^2 = 27%) were observed (Table 3).

The relationship between the redox imbalance biomarkers and the $PM_{2.5}$ quartiles is displayed in Figure 4. Without adjustment, the $PM_{2.5}$ exposure groups contributed to 49% and 14% of biomarker variance for GST activity and MDA content, respectively. As observed, the multiple linear regression coefficient increases, especially in the GST activity and MDA content models, according to the $PM_{2.5}$ exposure groups, even after adjusting for demographic, genetic, social, and health variables.

4. Discussion

This is the first study carried out in the Brazilian Amazon regarding the relationship between acute environmental $PM_{2.5}$ exposure and Hg and effects on oxidative stress biomarkers in children. The imbalance between prooxidant and antioxidant species can be influenced by a range of intrinsic and extrinsic factors [4] [29] and the results presented herein demonstrated a significant relation of environmental factors, such as exposure to $PM_{2.5}$ and Hg, to serum MDA concentrations and increased GST enzyme activity in children and adolescents of the Brazilian Amazon.

During the last 20 years, the Brazilian Amazon has undergone drastic changes in the pattern of soil use, with the conversion of forests into pasture areas that represent a long-term source of pollutant emissions through biomass burning [30]. Cardozo and contributors [31] have shown that, even with the reduction of deforestation in certain areas like Rondônia, fire is still used to maintain and/or create new areas for agriculture.

Table 3. Multiple linear regression model according to oxidative stress biomarkers.

	Models	Coefficients	95% CI	p-value
Thiol*	Intercept	−1.233	−1.659; −0.907	**<0.001**
	PCR (>6 mg/L)	−0.252	−0.440; −0.064	**0.009**
	GSTP1 (Ile/Val)	−0.037	−0.088; 0.013	0.156
	GSTP1 (Val/Val)	0.112	0.022; 0.202	**0.015**
	Haemoglobin	0.046	0.016; 0.076	**0.003**
	Serum iron	0.002	0.0001; 0.004	0.058
	1 - 2 minimum wage	−0.114	−0.178; −0.049	**<0.001**
	≥ 3 minimum wage	−0.114	−0.179; −0.044	**<0.001**
	$PM_{2.5}$ (2˚ quartile/Low)	−0.127	−0.206; −0.048	**0.002**
	$PM_{2.5}$ (3˚ quartile/Moderate)	−0.064	−0.137; −0.007	0.083
	$PM_{2.5}$ (4˚ quartile/High)	−0.080	−0.169; −0.008	0.080
	Indoor air pollution (yes)	−0.086	−0.148; −0.025	**0.006**
	R^2 multiple			**24% (<0.001)**
GST **	Intercept	19.25	9.31 a 29.2	**<0.001**
	Age (adolescents)	−1.637	−3.29 a 0.016	0.054
	Sex (female)	−1.241	−2.83 a 0.35	0.129
	Thiol	10.76	3.88 a 17.6	**0.002**
	Blood glucose	−0.109	−0.22 a −0.001	**0.050**
	Fish cons. (3 or + times/week)	2.549	0.42 a 4.67	**0.020**
	$PM_{2.5}$ (2˚ quartile/Low)	3.127	0.78 a 5.47	**0.009**
	$PM_{2.5}$ (3˚ quartile/Moderate)	8.637	6.49 a 10.8	**<0.001**
	$PM_{2.5}$ (4˚ quartile/High)	11.0	8.31 a 13.7	**<0.001**
	R^2 multiple			**55% (<0.001)**
MDA**	Intercept	1.211	1.104 a 1.317	**<0.000**
	Age (adolescents)	−0.084	−0.177 a 0.008	0.075
	IgE (reactive)	0.149	0.059 a 0.240	**0.001**
	Pulmonary obstruction (yes)	0.134	0.014 a 0.254	**0.030**
	Fish cons. (3 or + times/week)	0.172	0.056 a 0.287	**0.004**
	$PM_{2.5}$ (2˚ quartile/Low)	0.100	−0.028 a 0.229	0.128
	$PM_{2.5}$ (3˚ quartile/Moderate)	0.126	0.013 a 0.242	**0.034**
	$PM_{2.5}$ (4˚ quartile/High)	0.244	0.093 a 0.997	**0.002**
	R^2 multiple			**27% (<0.001)**

*Thiol Ln-transformed and the model excluded the *outliers* (thiol > 1.0 mmol/L). **The results were similar excluding the *outliers*.

The effects of air pollution on human health have been widely reported in epidemiological studies [32] [33] [34]. In the Brazilian Amazon, several studies have reported the effects of pollution on human health, especially in vulnerable groups such as children [35] [36] [37] [38]. In a time series study on the effects of exposure to atmospheric pollutants conducted in the subequatorial region of this area, percentage increases of 6% (CI 95%: 1.4 - 10.8) in lag 3 and 5.1%

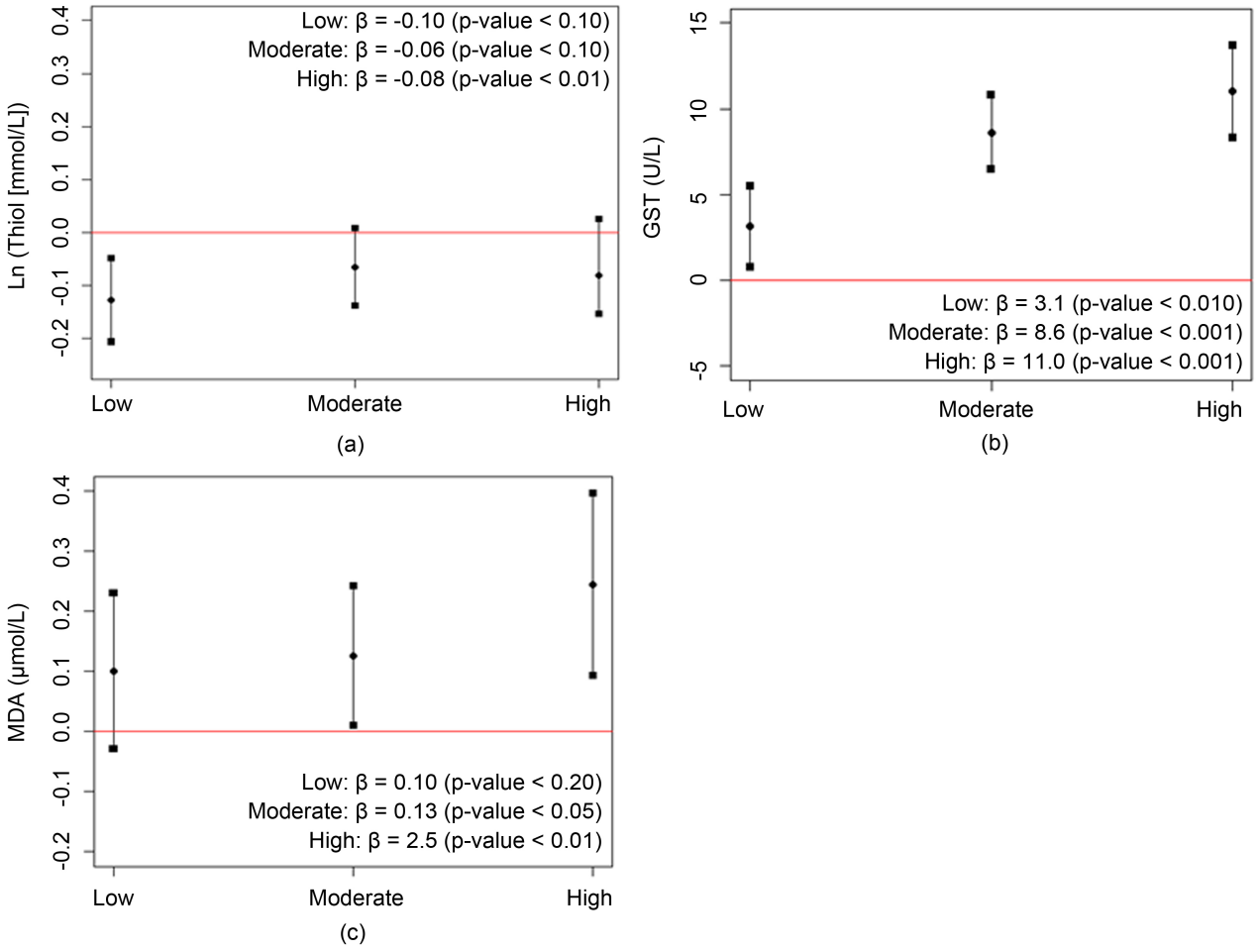

Figure 4. Multiple linear regression coefficient of the groups of exposure to $PM_{2.5}$, according to the thiol group (a), GST activity (b) and malondialdehyde (c).

(IC95: 0.6 - 9.8) in lag 4 in hospitalizations for respiratory diseases in children under 5 years of age were observed at each increase of 10 µg/m^3 during the 2005 drought [35].

In addition to epidemiological studies, exposure to particulate matter (PM) from burnings in the Amazon region was evaluated regarding the ability of PM to cause genotoxic and mutagenic damage [16] [39] [40]. Oliveira and contributors [16] have shown that exposure to PM in the Brazilian Amazon can alter the regulation of the cell cycle and that responses to cellular damage, especially in DNA, induce death by apoptosis in human lung cells. In this context, redox imbalance can be included as one of the main mechanisms of action of these pollutants in the human organism [5] [41]. Atmospheric pollutants may directly induce the formation of reactive species due to their chemical composition [41] and their reactions in the human body, or indirectly, through their proinflammatory effects [42] [43]. However, the increase in redox imbalance markers is not yet clearly established, since the production of reactive species can be influenced by a range of other factors, such as health status, physical activity, and

diet.

Several redox imbalance biomarkers have been studied and associated with air pollution, such as those linked with the enzymatic antioxidant system, like the enzymatic activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GST-Px), as well non-enzymatic activities of α-tocopherol and reduced glutathione [15]. Among the biomarkers that reflect oxidative damage in lipids, proteins, and DNA, the most studied are isoprostanes, oxidized LDL, malondialdehyde (MDA), carbonylated protein, 8-hydroxydexiguanosine (8-OHdG) and 8-oxo-2'-deoxyguanosine (8-oxodi) [15].

In children, the relationship between air pollution and redox imbalance biomarkers has been demonstrated in several studies [44] [45] [46] [47], corroborating the results of the present study that indicate increases in MDA serum concentrations and GST enzymatic activity in children and adolescents exposed to higher $PM_{2.5}$ exposures. In a quasi-experimental intervention study conducted with 36 schoolchildren before, during, and after the Beijing Olympics (2008), MDA concentrations in urine were associated with increased $PM_{2.5}$ and black carbon [47]. In addition, this biomarker was identified in samples of condensed exhaled air, and its relationship with PM was later evidenced in asthmatic children [44] [48]. However, the use of this biomarker to assess the effects of air pollution should be conducted with caution, adjusting potential confounding factors such as diet and medication use [15].

The association between air pollution and activity of GST enzymes in children was particularly explored in gene-environment interactions through the presence of polymorphic variants in these enzymes. The presence of these polymorphisms may result in a deficient state of detoxification and, consequently, increase the risk of adverse effects of air pollution [49] [50] [51]. GST polymorphisms in the Mu (glutathione S-transferase M1/GSTM1), theta (glutathione S-transferase T1/GSTT1), and pi (glutathione S-transferase P1/GSTP1) class genes have been the most studied [51] and their occurrence was reported as a modification factor for the effects of pollution in asthmatic children and children presenting allergic processes [52].

Children and adolescents with the GSTP1 polymorphic variant, Val/Val genotype, also had higher thiol concentrations when compared to those with the Ile/Ile genotype. These results are expected, as children with the Val/Val genotype may show less GST enzyme activity and, consequently, a decrease in the detoxification process of xenobiotics and the conjugation of GSH—the most abundant thiol in the intracellular environment [52]. In fact, GST activity was dependent on thiol concentrations, further strengthening the study findings.

Indoor air pollution—characterized in this study as the presence of flour houses in the residence and/or use of wood stoves—was an important factor in reducing thiol concentrations. Exposure to indoor pollution has recently been characterized as chronic, and continuous exposure to high concentrations of pollutants contribute to a recurrent state of redox imbalance and acute inflam-

mation [15]. In a study of 13-year-old children living in traditional rural communities in Nigeria, indoor $PM_{2.5}$ concentrations reached a median value of 1575 $\mu m/m^3$ with a consequent decrease in antioxidant defenses and inflammation of the airways and increased MDA concentrations that reached mean values of 5.44 μmol/L (SD ± 1.88) in blood [46].

Fish consumption was another factor related to redox imbalance biomarkers and specifically to increases in GST activity and MDA concentrations. Although a negative relationship is expected because of the antioxidant properties of fish, fish consumption may indicate environmental exposure to methylmercury (MeHg). MeHg is the most toxic form of Hg and, due to its physical and chemical properties, can be incorporated into aquatic organisms and accumulate in those at the top of the trophic chain [53] [54]. Thus, the effects of MeHg are of concern in areas where the main source of food is fish, as in the riverside regions along the Madeira River.

The neurotoxicity attributed to exposure to MeHg was associated to changes in the redox grouping like thiols and selenols, promoting a depletion of GSH and enzymes acting in the antioxidant system, thus enabling ROS actions [55]. In the Brazilian Amazon, in a study carried out in traditional communities, Grotto and contributors [56] found an inverse relationship between exposure to Hg (measured in hair and blood) and biomarkers acting on the antioxidant system, including GSH. In the present study, concentrations of Hg in blood were tested, but the results showed a collinearity of this variable with fish consumption that presented better fit and contribution to the models.

In relation to the antioxidant defense system, the thiol group analysis provided a proxy for GSH concentrations, considered a traditional biomarker of antioxidant system performance and redox imbalance [57] [58] [59]. On average, thiol serum concentrations in children and adolescents was 0.48 mmol/L (± 0.09), similar to values found in healthy children and adolescents in the studies developed by Carratelli *et al*. [60] and Aycicek *et al*. [61], which presented thiol concentrations of 0.46 mmol/L (±0.054) and 0.42 mmol/L (±0.03), respectively.

Variables associated with the health of children and adolescents were related to serum thiol concentrations that were, on average, lower among those with asthma and C-reactive protein ≥ 6 mg/L. High levels of antioxidants are found in the respiratory tract, but in asthmatics this condition may be altered with the depletion of antioxidant factors such as GSH [62] [63]. In addition, reduced GSH values may increase the inflammatory response and therefore raise the concentrations of inflammatory biomarkers such as C-reactive protein, as presented by Sauerwein *et al*. [64] when studying children with Kwashiokor prosthetic-caloric malnutrition. Among the evaluated socio-environmental variables, in addition to exposure to indoor pollution, family income was negatively related to thiol concentrations. This is possibly due to the fact that 75% of children with C-reactive protein levels higher than 6 mg/L reported a family income greater than 1 minimum wage.

In addition to the role played in the pathophysiological mechanism of various diseases, redox imbalance was also related to metabolic alterations due to innumerable environmental and occupational exposures. Therefore, its biomarkers are considered nonspecific, since they do not indicate a biological effect resulting from a particular exposure, but reflect the total and integrated effect of a biomarker set [65]. Although exposure to atmospheric pollution and mercury were related to enzymatic GST activity and to MDA concentrations, the synergistic effect between the two exposures was not explored. However, the potential use of redox imbalance biomarkers as biomarkers of exposure has been explored. Obolenskaya and contributors [66] suggested that the determination of the 7-ethoxycoumarin O-desethylase (ECOD) enzymatic activity in the placenta of 143 pregnant women could serve as a biological marker of redox imbalance in areas with high radioactive and chemical exposure—implying a biomarker of exposure function. The linear relationship suggested here in this study between the $PM_{2.5}$ exposure groups and GST enzyme activity and MDA concentrations, despite indicating this possibility, must necessarily be investigated in other studies.

Regarding exposure to $PM_{2.5}$, the distance between the monitoring station and the rural/riverine area and the imputation of 20% of the measures corresponds to a limitation and fragility of this study. However, other variables such as litter burning and indoor pollution contributed to characterizing the exposure to air pollution in this region. In addition, meteorological variables, such as shallow and non-precipitating convective systems typically found in the Amazon basin, favor large scale transport of gases and particles at long distances [67] [68].

Overall, this study showed that increased GST activity and MDA concentrations were especially related to exposure to $PM_{2.5}$ and mercury (by fish consumption), whereas thiol groups were lower in children who had acute inflammation and GSTP1 polymorphism. Finally, as the role of redox imbalance and the potential factors involved in its occurrence become more clearly defined, there will also be greater safety in the use of biomarkers to predict effects on the body or to indicate a particular environmental exposure.

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