ROLE OF ALA-D ACTIVITY ON THE ASSOCIATION BETWEEN BLOOD LEAD LEVELS AND CHILDREN'S INTELLECTUAL FUNCTION

Presented by:
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PLAN

• Introduction

• Methodology

• Results and discussion

• Conclusion
Lead is a toxic metal; it causes damages in almost all essential systems.  

- Especially blood tissue, Central Nervous System (PbB <5µg/dL)
  - Loss of Intellectual quotient (IQ), concentration, attention, …
- Human activities (industrial and artisanal) are the main source of exposure.
ALA-D enzyme (δ-aminolevulinic acid dehydrogenase)

Figura 1: Lead Interruption of Heme Biosynthesis.
Any mutation on the genomic sequence of ALA-D can affect the affinity of that enzyme for lead and therefore blood lead level (PbB):

- **SNP G177C**: Phenotypes ALA-D 1 and ALA-D 2 (associated to high ↑PbB);
- Role of ALA-D2? **Risk factor** for brain damage or **Neuroprotective function**?

ZHENG et al., 2001; CHEN et al., 2008; TAHA et al, 2015; SOBIN et al., 2014
Our research focus was the secondary exposure to lead of the children in the community of the Village of Maragogipinho (district of Aratuípe):

Figura 2 : Maragogipinho (District of Aratuípe; State of Bahia, Brasil)
**General Objective:**

To investigate the role of ALA-D activity on the association between blood lead levels and children's intellectual function.

**Specific Objectives:**

1. To estimate the environmental exposure of children to lead;
2. To determine the genotype and activity of the ALA-D enzyme;
3. To evaluate the intellectual function of children;
4. Investigate the role of the ALA-D enzyme.
**Study Population and Design:** Cross-sectional design; 7 elementary schools divided into 3 exposure groups based on the **Lead Dust Content**:

- **Control Group/ Basal Exposure (CG):** 3 schools in the urban area.
- **Low Exposure (LEx):** 2 schools at the entrance of the Village
- **Moderate Exposure (MEx):** 2 schools in the Village near the potteries workshop

**Figure 3:** Location of the groups in the municipality of Aratuípe.
• Ethical Issues and the Recruitment of Children
  • Approved by the Ethics Committee of the Federal University of Bahia (N° 1,762,493).

• Inclusion criteria
  5.5 to 13 years; male and female; One year of living in the village; parental consent and children’s agreements

• Exclusion criteria
  - Children diagnosed with neurological, psychiatric, or hearing problems.
  - Without biological samples and/or intellectual assessment test
• Socio-demographic and Anthropometry Data

• Environmental contamination: Lead Dust content expressed as loading rates; µg Pb/m²/30 days)

WHO, 2009; SISVAN, 2008; ABEP, 2011; RODRIGUES et al. 2017
**Biomarkers of:**

- **Lead exposure**: Blood lead level (PbB)
- **Lead toxicity effect**: ALA-D activity
- **Susceptibility to lead**: Mutation of ALA-D enzyme gene

Menezes-Filho et al., 2012; Nascimento et al. 2016; RODRIGUES et al. 2017; Eleftheriadis et al. 2010
• Evaluation of nonverbal intelligence (Raven's Progressive Matrices):

CEPA, 2008; NASCIMENTO et al. 2015; MACKINTOSH; BENNETT, 2005
Data Analysis

- SPSS software version 23 for Windows (p <0.05).

- Descriptive statistical analysis

- Distributions of variables: Kolmogorov-Smirnov Test

- Chi-square test or T-test: difference according to degree of exposure

- Analyze continuous variables obtained: Mann Whitney test or the T test.

- Spearman correlation; multiple linear regression (MLR).
• General Characteristics of Study Population

- 05/05/2017 to 14/04/2018

598 children (7 schools selected)
177 children included
143 (samples and Raven’s test)
- CG: 69
- LEx: 34
- MEx: 40
• Female represent 53%;
• Majority is black (94%);
• Almost 80% of families have income lower than the brazilian minimum salary;
• 10% of mothers and 18% of fathers are potter;
• And despite the low socioeconomic status, children presented a good nutritional status according to World Health Organization Standard
The current reference from US EPA (2001): $<431 \, \mu g/m^2$

Pb accumulation over TIME ($\mu g/m^2$ instead of $\mu g/m^2/30$ days)

4.224 $\mu g/m^2/year$

17 to 31 $\mu g / m^2 / 30$ days: Australia (Sydney), Germany
Surprisingly, results are more similar to those found in USA and developed countries of Europe.

(MEYER et al. 1999; GULSON et al., 2006; GULSON et al., 2014; FERRON et al., 2012; RODRIGUES et al., 2017; ZHENYAN et al. 2017; NASCIMENTO et al. 2015; WONG et al., 2003; AZCONA-CRUZ et al., 2000; Estrada-Sánchez et al., 2017; WHO EUROPE, 2009, McClure et al., 2016)
Table 2: Biological parameters and environmental exposure assessment according to the exposure groups.

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Total</th>
<th>GC</th>
<th>LEx</th>
<th>MEx</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALAD activity (U/L)</td>
<td>Med (int)</td>
<td>Med (int)</td>
<td>Med (int)</td>
<td>Med (int)</td>
</tr>
<tr>
<td></td>
<td>71* (31 – 113)&lt;sup&gt;MT&lt;/sup&gt;</td>
<td>86 (31 -113)</td>
<td>55 (31 -77)</td>
<td>54 (37 – 80)</td>
</tr>
</tbody>
</table>

Frequency of SNP ALA-D G177C (Mutation):

- ALA-D 1/1 : 97.9 %
- ALA-D 1/2 or 2/2 : 2.1 % (2 black and 1 white children);
- ALAD 2 : 0 a 4% (rare in black people)
  - but 11 to 20% in white and Asiatic population

(MEYER et al. 1999; GULSON et al., 2006; GULSON et al., 2014; FERRON et al., 2012; NASCIMENTO et al. 2015; AZCONA-CRUZ et al., 2000; ASTRIN et al., 1987; SOUSA et al., 1991; SHEN et al., 2001; MONTENEGRO et al., 2006)
Bivariate analysis and MLR model for ALAD activity

• Correlation ($r = -0.587; p=0.001$) between Pb exposure (LogPbB) and effect biomarkers (ALAD enzyme activity).

<table>
<thead>
<tr>
<th>Model</th>
<th>Beta</th>
<th>Std. Error</th>
<th>T-Stat</th>
<th>CI (95%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>78.58</td>
<td>1.86</td>
<td>42.20</td>
<td>74.90 to 82.26</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Exposure groups</td>
<td>-11.21</td>
<td>166</td>
<td>-6.76</td>
<td>-14.49 to -7.93</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LogPbB</td>
<td>-7.55</td>
<td>2.17</td>
<td>-3.48</td>
<td>-11.84 to -3.27</td>
<td>0.001</td>
</tr>
<tr>
<td>Child age</td>
<td>0.11</td>
<td>-</td>
<td>1.86</td>
<td>-</td>
<td>0.065</td>
</tr>
</tbody>
</table>

Models’ statistics: $n = 148$, $r^2 = 0.487$, $F = 69.774$, $p < 0.001$

A 10-fold increase in blood lead level concentration is associated with a decrease of 7.6 U/L of the ALAD activity, independently of the exposure groups and child’s age.
Table 4: Performance in the intellectual assessment test and HOME score according to the degree of exposure to Pb.

<table>
<thead>
<tr>
<th>RAW SCORE</th>
<th>Total Mean ± SD</th>
<th>CG Mean ± SD</th>
<th>LEx Mean ± SD</th>
<th>MEx Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children</td>
<td>19.3 (5.6)</td>
<td>19.5 (5.4)</td>
<td>16.8 (5.7)</td>
<td>21.1 (4.8)</td>
</tr>
<tr>
<td>Ratio Sc./Age</td>
<td>2.1 (0.6)</td>
<td>2.0 (0.5)</td>
<td>2.1 (0.7)</td>
<td>2.2 (0.6)</td>
</tr>
<tr>
<td>Mothers</td>
<td>25 (4 - 51)</td>
<td>22 (4 - 51)</td>
<td>27 (11 - 45)</td>
<td>25 (9 - 46)</td>
</tr>
</tbody>
</table>

* Student Test T p<0.05; # Mann-Whitney;
• Environmental exposure to lead and IQ of Children

Table 5: Summary of the multiple linear regression models with Child Raw score as a dependent variable

<table>
<thead>
<tr>
<th></th>
<th>Beta</th>
<th>IC 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Child Raw Score</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>2.773</td>
<td>-1.856 to 7.401</td>
</tr>
<tr>
<td>Age (Years)</td>
<td>1.297</td>
<td>0.877 to 1.716</td>
</tr>
<tr>
<td><strong>Mother’s Raw score</strong></td>
<td>0.158</td>
<td>0.083 to 0.234</td>
</tr>
</tbody>
</table>

• Raven’s less sensitive as it measures only nonverbal intelligence

• Raven test application conditions and variables

SOLON O., 2008; MENEZES-FILHO et al., 2010; JAROTIMI; IJADUNOLA, 2007; NYARADI et al., 2013
• ALA-D and intellectual function

**ALAD activity:**

• Neuroprotective role

• ALA-D ↓ while IQ ↑

• ALA-D ↓ while PbB ↑ to protect the CNS

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<table>
<thead>
<tr>
<th>Mother's score</th>
<th>Raw Sco.</th>
<th>Perc. Sco.</th>
<th>Sc./Age</th>
<th>Mother's score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rho</td>
<td>0.235**</td>
<td>0.265**</td>
<td>0.302**</td>
<td>1.000</td>
</tr>
<tr>
<td>P</td>
<td>0.008</td>
<td>0.005</td>
<td>0.001</td>
<td>.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PbS</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Rho</td>
<td>-0.032</td>
<td>0.003</td>
<td>0.038</td>
<td>-0.063</td>
</tr>
<tr>
<td>P</td>
<td>0.708</td>
<td>0.975</td>
<td>0.652</td>
<td>0.486</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Home Esc tot</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Rho</td>
<td>0.235*</td>
<td>0.102</td>
<td>0.140</td>
<td>0.445**</td>
</tr>
<tr>
<td>P</td>
<td>0.009</td>
<td>0.285</td>
<td>0.124</td>
<td>0.000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ativ. ALAD</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Rho</td>
<td>-0.048</td>
<td>-0.185</td>
<td>-0.205*</td>
<td>-0.047</td>
</tr>
<tr>
<td>P</td>
<td>0.588</td>
<td>0.055</td>
<td>0.021</td>
<td>0.625</td>
</tr>
</tbody>
</table>

*SOLON O., 2008; MENEZES-FILHO et al., 2010; JAROTIMI; IJADUNOLA, 2007; NYARADI et al., 2013*
*There was evidence of environmental exposure to lead, but the degree of contamination is low: as Pb from pottery workshop is not volatile (Lead oxide, heavy particles).

*Hematological toxicity confirmed even at low blood lead levels.

*Association was not observed between environmental exposure to Pb and non-verbal intelligence; and it was not possible to evaluate the genetic influence as ALAD2 frequency is rare in black population.

*ALAD enzyme has a neuroprotective role in condition of high lead blood level:

\[
PbB \uparrow = ALA-D \downarrow \text{ & Pb free } \downarrow \rightarrow \text{ less damage in the brain}
\]
Agradecimientos

Gracias