RELATIONSHIP OF TRIGLYCERIDES/HIGH DENSITY LIPOPROTEIN-CHOLESTEROL INDEX WITH ANTIOXIDANT DEFENSE AND OUTSTANDING ASSOCIATION WITH HIGH SENSITIVE C-REACTIVE PROTEIN IN MEXICAN SUBJECTS

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ABSTRACT

Background: The TriGlycerides/High-Density Lipoprotein-Cholesterol (TG/HDL-C) ratio is a new index that has been proposed to estimate CardioVascular Risk (CVR). However, the relationship between this index with the antioxidant defense and inflammation is unknown. The aim of the study was to determine the relationship between the TG/HDL-C index with antioxidant defense and inflammation marker in Mexican subjects.

Materials and Methods: In the study, Mexican subjects participated who were anthropometrically, biochemically, and clinically characterized. The TG/HDL-C index was calculated by dividing TG levels by HDL-C levels; a cut-off point >3 was considered CVR. The study subjects were divided into three groups: 1) subjects without CVR; 2) subjects with CVR, and 3) subjects with Type 2 Diabetes (T2D). In the subjects, we evaluated antioxidant defense by determine SuperOxide Dismutase (SOD) and Glutathione Peroxidase (GPx) activities, and high sensitive C-Reactive Protein (hsCRP) as inflammation marker.

Result: A total of 1,491 subjects participated in the study: 553 without CVR; 674 with CVR, and 264 with T2D. It was found that antioxidant defense was diminished in subjects with CVR and with T2D, and that hsCRP levels were increased, in contrast with subjects without CVR (p <0.05). An association between the TG/HDL-C index and hsCRP levels was observed (p <0.01), as well as a correlation with SOD, GPx, and hsCRP levels (p <0.01).

Conclusion: the TG/HDL-C index is related to the inflammatory process through the hsCRP marker and with low antioxidant defense through the correlation found with the activity of enzymes SOD and GPx.

Keywords: TG/HDL-C index, superoxide dismutase, glutathione peroxidase, high sensitive C reactive protein.
1.0 Introduction

The Triglycerides/High-Density Lipoprotein-Cholesterol (TG/HDL-C) ratio is a new index that has been proposed to estimate CardioVascular Risk (CVR). This index has been utilized in different types of populations, such as in subjects at high risk for coronary disease (Da Luz, Favarato, Faria-Neto Junior, Lemos, & Chagas, 2008), in subjects with Type 2 Diabetes (T2D) (Boizel et al., 2000), and in patients with coronary artery disease (Frohlich & Dobiášová, 2003); in all of these studies, the TG/HDL-C index was an independent predictor of CardioVascular Disease (CVD). In a study conducted in Mexican subjects with obesity, we found that the TG/HDL-C index was higher in those with overweight and obese compared with healthy subjects (Baez-Duarte et al., 2012). Recently, it was reported that this index was associated with low insulin sensitivity and metabolic syndrome in apparently healthy subjects, suggesting this index as a reference criterion-of-risk for low insulin sensitivity and metabolic syndrome (Baez-Duarte et al., 2017).

Metabolic syndrome is a set of metabolic disorders that represent risk factors for CVD, atherosclerosis, and T2D. With the metabolic syndrome driving the twin global epidemics of T2D and CVD, there is an overwhelming moral, medical, and economic imperative to identify individuals with metabolic syndrome early (Alberti, Zimmet, & Shaw, 2006; Moreira, Cipullo, Ciorlia, Cesarino, & Vilela-Martin, 2014).

Different studies have reported a decrease in antioxidant enzyme activity, SuperOxide Dismutase (SOD), and Glutathione Peroxidase (GPx) in subjects with metabolic syndrome (Baez-Duarte et al., 2016; Sabir et al., 2016) and, on the other hand, an increase in the levels of inflammation markers, among which high sensitive C-Reactive Protein (hsCRP) stands out (Barbalho et al., 2016; Cardoso-Saldana et al., 2007; Moreto et al., 2015). However, the relationship between the activity of these antioxidant enzymes and hsCRP levels in subjects with and without CVR determined by the TG/HDL-C index is unknown.

Taking this information into account, and the fact that there is an imperious need for a sole, simple diagnostic tool for clinical practice that could be used relatively easily in any country by any physician to identify patients at a considerable increased risk of developing CVD and/or T2D (Alberti et al., 2006), we assessed the relationship between the TG/HDL-C index with antioxidant defense and inflammation marker in Mexican subjects, to detect individuals-at-risk at an early stage of metabolic alterations related with T2D and CVD.

2.0 Materials and Methods

2.1 Subjects and setting

A total of 1,491 Mexican subjects (from Central Mexico) participated in the study. Subjects with an incomplete clinical history or who had ongoing chronic inflammatory (arthritis, rhinitis, and trauma), endocrine (hyperthyroidism, and hypothyroidism), or any chronic disease (except hypertension, hyperlipidemia and T2D) were excluded from the study. Use of medications, alternative treatments, smoking, and alcoholism were also considered exclusion criteria.
criteria. The subjects of the present study were Mexican mestizos who resided in Puebla City, Mexico.

The study was approved by the Scientific Research and Ethics Committee of the Puebla University Hospital and signed informed consent was obtained from all individual participants included in the study.

2.2 Clinical characterization

Body Mass Index (BMI) was determined utilizing an electronic digital scale (Model HBF-514C Sensor Full Body; Omron). Waist Circumference (WC) was measured at the midpoint between the highest point of the iliac crest and the lowest point of the costal margin at the mid-axillary line, employing a non-stretching anthropometric measuring tape.

2.3 Biochemical characterization

Following an overnight fast (10-12 h) by the study participants, blood samples were obtained by venipuncture. Fasting glucose, fasting insulin, Total Cholesterol (TC), TG, HDL-C, and Glucose levels of the Oral Tolerance Test (GlcOTT), determined 2 h after a 75-g glucose load, were measured using the periodic endpoint method according to the conventional laboratory protocols of the Puebla University Hospital, and were determined employing the Architect System (Architect I 2000 SR; Abbott Laboratories).

Subjects were diagnosed with T2D according to the cut-off point established in Official Mexican Norm NOM-015SSA2-2010 (Mexican Ministry of Health, 2010). Subjects with T2D with more than 5 years of evolution of the disease and/or with T2D complications were excluded from the study.

The TG/HDL-C index was calculated by dividing the concentration of TG by HDL-C. The cut-off point for CVR was TG/HDL-C >3.0 (Boizel et al., 2000; González-Chávez, Simental-Mendía, & Elizondo-Argueta, 2011). The study subjects were divided into three groups: 1) subjects without CVR; 2) subjects with CVR, and 3) subjects with T2D.

2.4 Antioxidant defense determination

2.4.1 GPx activity analysis

For determination of GPx activity, blood collection was carried out in tubes with EDTA, and then extraction of the plasma fraction was performed following the manufacturer’s instructions. Determination of GPx plasma activity was performed using the GPx Assay kit (Cayman Chemical Company) following the manufacturer’s instructions. The equipment used was the FilterMaxTM F5 Multi-Mode Microplate Reader.

2.4.2 SOD activity analysis

For determination of SOD activity, blood collection was carried out in tubes with EDTA, and then extraction of the plasma fraction was performed following the manufacturer’s instructions. Determination of SOD plasma activity was conducted using the SOD Assay kit.
(Cayman Chemical Company) following the manufacturer’s instructions. The equipment used was the FilterMaxTM F5 Multi-Mode Microplate Reader.

2.4.3 Inflammation Marker hsCRP analysis

For determination of hsCRP levels, blood collection was carried out in tubes with EDTA, and then extraction of the plasma fraction was performed following the manufacturer’s instructions. Determination of hsCRP levels was conducted using the CardioPhase hsCRP kit (Siemens Healthcare Diagnostics) following the manufacturer’s instructions. The equipment utilized was the BN ProSpec System (Siemens Healthcare Diagnosis).

In order to deepen the analysis of the relationship between the TG/HDL index and the hsCRP, the population was divided according to hsCRP levels: 1) group with hsCRP <1 mg/dL; 2) group with hsCRP 1-3 mg/dL; 3) group with hsCRT >3–10 mg/dL, and 4) group with hsCRP >10 mg/dL, according to the classification reported by Pfützner et al. (Pfützner et al., 2006).

2.4.4 Statistical analysis

The Kurtosis normality of residuals test was employed to determine normality of data distribution. Continuous variables with normality and equal variances were analyzed employing the One-way Analysis Of Variance (ANOVA) test. Nonparametric continuous variables were analyzed utilizing the Kruskal-Wallis one-way ANOVA on ranks test and, to establish the association between the variables, multinomial logistic regression was utilized when dividing the population into two groups: subjects without CVR, and subjects with CVR (subjects with an TG/HDL index of >3.0 and subjects with T2D). Correlation analysis was conducted using the Spearman rank test. Data were analyzed with NCSS ver. 11 statistical software (2016), (NCSS, LLC, Kaysville, UT, USA, ncss.com/software/ncss). Differences between groups were considered significant at p ≤0.05.

3.0 Result

The study involved 1,491 subjects, of whom 64.9% were women and 35.1%, men. These subjects were divided into three groups: 1) group without CVR (TG/HDL-C index <3.0; n = 553; 423 women and 130 men); 2) group with CVR (TG/HDL-C index >3.0; n = 674; 388 women and 286 men), and 3) group with T2D (n = 264; 156 women and 108 men); no significant differences were found among the study groups with respect to gender (p >0.05).

In Table 1, it can be observed that the group of subjects with T2D was the group who present greatest age in comparison with the other two groups (p ≤0.05), and that the group without CVR presented lower values in terms of the anthropometric and biochemical variables compared with the other two groups (p ≤0.05).
Table 1. Age, anthropometric, and biochemical variables of the study groups

<table>
<thead>
<tr>
<th>Study groups</th>
<th>Group without CVR n = 553</th>
<th>Group with CVR n = 674</th>
<th>Group with T2D n = 264</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>40.0 ± 13.3a</td>
<td>40.8 ± 12.2a</td>
<td>53.6 ± 11.5b</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.7 ± 5.1a</td>
<td>28.8 ± 4.8b</td>
<td>29.6 ± 5.1b</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>89.2 ± 12.8a</td>
<td>95.9 ± 11.3b</td>
<td>99.7 ± 10.8c</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>91.4 ± 9.8a</td>
<td>95.2 ± 10.1a</td>
<td>158.6 ± 65.6b</td>
</tr>
<tr>
<td>Fasting insulin (µU/ml)</td>
<td>8.3 ± 6.1a</td>
<td>12.0 ± 8.5b</td>
<td>14.4 ± 11.5c</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>185.8 ± 37.3a</td>
<td>193.0 ± 44.4b</td>
<td>189.3 ± 47.3ab</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>51.9 ± 12.5a</td>
<td>36.5 ± 10.3b</td>
<td>34.2 ± 10.8c</td>
</tr>
<tr>
<td>TG (mg/mL)</td>
<td>95.6 ± 30.3a</td>
<td>212.7 ± 104.8b</td>
<td>235.3 ± 116.4c</td>
</tr>
<tr>
<td>TG/HDL-C index</td>
<td>1.9 ± 0.6a</td>
<td>6.5 ± 4.7b</td>
<td>8.1 ± 7.4c</td>
</tr>
</tbody>
</table>

Results were expressed as means ± Standard Deviation (SD). P ≤0.05 Kruskal-Wallis one-way Analysis Of VAriance ANOVA on ranks/Kruskal-Wallis multiple comparisons. Different letters (a, b, c) in a row indicate a significant difference (p ≤0.05). CVR: CardioVascular Risk; BMI: Body Mass Index; WC: Waist Circumference; TC: Total Cholesterol; HDL-C: High-Density Lipoprotein Cholesterol; TG: TriGlycerides.

Antioxidant defense was found to be reduced in the groups with CVR and T2D in comparison with subjects without CVR, observing a decrease in the activity of GPx and SOD enzymes. On the other hand, hsCRP levels were elevated in the group with T2D (p ≤0.05) (Table 2). By dividing the study population according to hsCRP levels, it was found that, as the hsCRP levels increase, the TG/HDL-C index increases (p <0.01) and that SOD activity decreases (p <0.01) (Table 3).

Table 2. Antioxidant defense and inflammation marker of the study groups

<table>
<thead>
<tr>
<th>Study groups</th>
<th>Group without CVR n = 553</th>
<th>Group with CVR n = 674</th>
<th>Group with T2D n = 264</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPx activity (nmol/min/mL)</td>
<td>65.3 ± 11.1a</td>
<td>59.6 ± 11.8b</td>
<td>55.8 ± 16.3b</td>
</tr>
<tr>
<td>SOD activity (U/mL)</td>
<td>0.203 ± 0.109a</td>
<td>0.179 ± 0.123b</td>
<td>0.143 ± 0.139c</td>
</tr>
<tr>
<td>hsCRP (mg/dL)</td>
<td>0.45 ± 1.2a</td>
<td>1.4 ± 3.6a</td>
<td>3.7 ± 6.2b</td>
</tr>
</tbody>
</table>

Results were expressed as means ± Standard Deviation (SD). P ≤0.05 Kruskal-Wallis one-way ANalysis Of VAriance ANOVA on ranks/Kruskal-Wallis multiple comparisons. Different letters (a, b, c) in a row indicate a significant difference (p ≤0.05). CVR: CardioVascular Risk; GPx: Glutathione Peroxidase; SOD: SuperOxide Dismutase; hsCRP: high sensitive C-Reactive Protein.
Table 3. TG/HDL index and antioxidant defense in the groups according to hsCRP levels

<table>
<thead>
<tr>
<th>Groups according to hsCRP levels</th>
<th>Group with &lt; 1 mg/dL n = 62.2%</th>
<th>Group with 1-3 mg/dL n = 27.8%</th>
<th>Group with &gt;3–10 mg/dL n = 6%</th>
<th>Group with &gt;10 mg/dL n = 4%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>46.5 ± 11.3</td>
<td>45.7 ± 12.8</td>
<td>50.1 ± 12.2</td>
<td>51.4 ± 14.4</td>
</tr>
<tr>
<td>Gender (%)</td>
<td>F/M (34.8 /27.4)</td>
<td>18.1/9.7</td>
<td>3.7/2.3</td>
<td>3.0/1.0</td>
</tr>
<tr>
<td>TG/HDL-C index</td>
<td>3.4 ± 2.4a</td>
<td>4.9 ± 4.7b</td>
<td>7.8 ± 5.2c</td>
<td>5.8 ± 2.8abc</td>
</tr>
<tr>
<td>GPx activity (nmol/min/mL)</td>
<td>62.5 ± 3.9</td>
<td>59.3 ± 11.3</td>
<td>54.1 ± 12.3</td>
<td>55.7 ± 14.6</td>
</tr>
<tr>
<td>SOD activity (U/mL)</td>
<td>0.200 ± 0.160a</td>
<td>± 0.181 ± 0.109a</td>
<td>0.126 ± 0.049b</td>
<td>0.099 ± 0.053c</td>
</tr>
<tr>
<td>hsCRP (mg/dL)</td>
<td>0.13 ± 0.1a</td>
<td>0.85 ± 0.6b</td>
<td>6.7 ± 1.7c</td>
<td>16.5 ± 5.2a</td>
</tr>
</tbody>
</table>

Results were expressed as means ± Standard Deviation (SD), except for gender (percentage). $P \leq 0.05$, Kruskal-Wallis one-way ANalysis Of VAriance (ANOVA) on ranks/Kruskal-Wallis multiple comparisons. $P \leq 0.05$, Chi square test. Different letters (a, b, c, d) in a row indicate a significant difference ($p \leq 0.05$). F, Feminine gender; M, Masculine gender; TG/HDL-C, TriGlycerides/High Density Lipoprotein Cholesterol; GPx: Glutathione Peroxidase; SOD: SuperOxide Dismutase; hsCRP: high sensitive C-Reactive Protein.

When evaluating the relationship between the TG/HDL-C index and antioxidant defense and the inflammation marker, it was found that the index correlated negatively and significantly with the activity of GPx and SOD, even after adjusting for gender, age, and WC (Table 4). In addition, an association was found between the TG/HDL-C index and the hsCRP levels, with an Odds Ratio (OR) = 2.06; (95% Confidence Level [CL]: 1.27-2.06); $p <0.01$, adjusted for age, gender, and WC.

Table 4. Antioxidant defense and inflammation marker correlation with the TG/HDL-C index in study subjects

<table>
<thead>
<tr>
<th>TG/HDL index</th>
<th>Variable</th>
<th>Rho</th>
<th>$P$</th>
<th>Rho*</th>
<th>$P*$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GPx activity</td>
<td>-0.298</td>
<td>&lt;0.01</td>
<td>-0.310</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>SOD activity</td>
<td>-0.338</td>
<td>&lt;0.01</td>
<td>-0.360</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>hsCRP</td>
<td>0.293</td>
<td>0.01</td>
<td>0.315</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

4.0 Discussion

This study supplies evidence for the relationship between the TG/HDL-C index and antioxidant defense and the inflammation marker in Mexican subjects. To our knowledge, our results demonstrate for the first time, a strong association between the TG/HDL-C index and hsCRP in Mexican subjects. In addition, it was observed that the index positively correlated with hsCRP and negatively with both SOD and GPx activities; these antioxidant enzyme activities were decreased in subjects with CVR and with T2D in comparison with subjects without CVR. This study is a continuation of previous work (Baez-Duarte et al., 2017), in which we suggested this index as an adequate reference criterion of risk for low insulin sensitivity and metabolic syndrome and an index that might facilitate the detection of metabolic changes in early stages, avoiding further complications. In accordance with the aforementioned, with this work the usefulness of this index is strengthened.

In agreement with our results, Yang et al. (Yang, Gerber, & You, 2017) found an association between the TG/HDL-C index and hsCRP in a study conducted in Korean adults not taking medication for hypertension, dyslipidemia, or diabetes. The population was divided according to insulin levels into five groups, and the authors observed that as insulin levels increased, levels of hsCRP, TG, HDL-C, and the TG/HDL-C index increased.

With respect to the observed correlation between the TG/HDL-C index and hsCRP levels, similar results have been found by others. Vieira et al. found a positive correlation between the TG/ HDL-C index and hsCRP only in women with coronary disease. These authors suggested that the TG/HDL-C index and hsCRP may be used as indicators of an increase in CVR among women with coronary artery disease (Vieira, Carvalho, Aras Júnior, Couto, & Couto, 2011). In this study, 36 women and 24 men participated; thus, we suggested that sample size may have influenced not finding a correlation in the male gender. In contrast, we found a positive correlation in general population even after adjusting for age, gender, and WC. Likewise, in a study conducted in subjects with and without hyperglycemia undergoing coronary arteriography, it was reported that patients with higher glycemia exhibited increased values of TG, TC, and TG/HDL index, and low values of HDL-C. In addition, a significant correlation was reported between hsCRP and the TG/HDL index (Barbalho et al., 2016). Furthermore, in adolescents with obesity evaluated at the Children’s Hospital of Wisconsin who were Caucasian, Mexican-Americans, and African-American, a correlation was reported between the TG/HDL index and hsCRP, and this correlation remained significant after dividing the subjects according to gender (Alemzadeh & Kichler, 2014).

On the other hand, in a study conducted in Mexican subjects from Guerrero State, divided into the following four groups, that is 1) subjects with diabetes, 2) subjects with obesity, 3) subjects with diabetes and obesity, and 4) healthy subjects, it was found that hsCRP and TG levels were higher in the groups of subjects with diabetes and/or obesity compared to healthy subjects. A positive correlation was also found between hsCRP and TG levels. It was suggested that high hsCRP levels are related with obesity and central distribution of body fat leading to a higher CVR (Flores-Alfaro, Parra-Rojas, Salgado-Bernabé, Chávez-Maldonado, & Salazar-Martínez, 2008). In Mexican adolescents from Mexico City, classified according to lipid-lipoprotein abnormalities, those with HDLC ≤35 mg/dL and TG ≥150 mg/dL were the more metabolically altered in respect to individuals with only HDL-C ≤35 mg/dL or TG ≥150 mg/dL levels and presented the highest TG/HDL-C index. Also, lowest hsCRP levels were
found in the normolipidemic subjects. The authors concluded that the adolescents with reduced HDL-C levels and/or high TG also have an abnormal pattern of HDL subclass distribution and chemical compositional changes that, in adults, are associated with a greater CVR (Medina-Urrutia et al., 2008).

There are not many studies that evaluate the relationship between the TG/HDL-C index and antioxidant defense, specifically with GPx and SOD activities; therefore, studies related to antioxidant defense in general and this index were presented. A study conducted on 78 men from Estonia, who were divided into two groups, including 53 former athletes and 25 sedentary controls (age range, 39-59 years), showed that subjects who were former athletes had a lower mean overweight, a better spectrum of atherogenesis indicators (TG/HDL-C index, TC, HDL-C, TG), and a lower systemic and cellular oxidative stress status, as well as lower hsCRP levels, than the control group (Phil et al., 2003).

In a study conducted on Mexican subjects from Puebla City, it was found that GPx3 serum levels correlated with the TG/HDL-C index and that its genetic polymorphisms are associated with this index (Baez-Duarte et al., 2014). In other study with subjects from the same locality, Baez-Duarte et al. (Baez-Duarte et al., 2016) found that subjects with metabolic syndrome had lower SOD and GPx activities than those without metabolic syndrome, and that GPx activity correlated with TG and HDL-C, and SOD activity with HDL-C, although the TG/HDL-C index was not determined.

Specifically in subjects with T2D, different studies have reported the evaluation of the behavior of antioxidant defense. Among these studies, the study conducted by Surapon et al. (Surapon, Suwipar, & Orathai, 2016) in which 292 subjects with T2D with chronic kidney disease participated, it was reported that hsCRP levels were increased and total antioxidant capacity decreased in subjects with T2D. In addition, it was found that the TG/HDL-C index and hsCRP increased and total antioxidant capacity decreased as glomerular filtration decreased. A significant correlation between the TG/HDL-C index and total antioxidant capacity and hsCRP was found.

In a study conducted in 80 patients with T2D and 79 apparently healthy controls, the authors found that GPx activity and HDL-C were significantly lower in patients with T2D, while CRP and TG were significantly higher in comparison with control subjects. No differences were observed in GPx activity or in CRP between patients with T2D with or without chronic complications (Gawlik et al., 2016). These data agree with the findings reported by our investigation group, since subjects with CVR and with T2D presented lowest GPx activity and the group with T2D exhibited highest hsCRP level.

5.0 Conclusion and recommendation

In conclusion, the TG/HDL-C index is related to the inflammatory process through the hsCRP marker and with low antioxidant defense by the correlation found with the activity of enzymes SOD and GPx. It is suggested that this index may be suitable for the selection of subjects requiring earlier and aggressive treatment of lipid abnormalities, the inflammation process, and low antioxidant defense.
Acknowledgement

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Declaration

Author(s) declare that there is no conflict of interest and that if this manuscript is accepted for publication, we grant the copyright to International Journal of Public Health and Clinical Sciences.

Authors contribution

Author 1: conceived of the presented idea, Author 2: contributed to the results discussion, Author 3: contributed to the final manuscript, Author 4: discussed the results and contributed to the final manuscript, and Author 5: knowledge on cardiovascular risk.

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Medina-Urrutia, A., Juarez-Rojas, J. G., Martinez-Alvarado, R., Jorge-Galarza, E., Posadas-


