Antropometric, biochemical and endothelial cardiovascular risk markers in subjects with metabolic syndrome: a control group comparison

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Abstract

Introduction and Aim: The metabolic dysfunctions and diseases clustered under the Metabolic Syndrome (MS) definition are all progressive and lead to increases morbidity and mortality due to cardiovascular causes. Endothelial dysfunction is an important feature of metabolic syndrome. Endothelium is a complex endocrine organ able to produce a large quantity of substances such as Endothelin-1 a powerful vasoconstrictor and Nitric Oxide (NO) a vasodilator related to oxidative stress. The aim of this study was to evaluate cardiovascular risk markers in subjects with metabolic syndrome and compare against a control group. Material and Methods: We set in 9 groups with normal laboratory values and without metabolic syndrome diagnosis (control group) and performed a complete clinical evaluation including anthropometry measurements and cholesterol, tryacilglycerides, C-HDL, C-LDL, glucose (INVELAB), insulin, endothelin-1 and NO (Commercial Cayman and Calbiochem ELISA kit). Results: lower NO levels where observed in MS subjects compared to control leading to vasoconstriction, vascular wall abnormalities and hypertension in addition to resistance to insulin, overweight and dyslipidaemia may lead to increased endothelial dysfunction and cardiovascular disease in this patients. Key Words: Metabolic syndrome, Cardiovascular Risk, Endotelina-1 and Nitric Oxido.
Introduction

The metabolic dysfunctions and diseases clustered under the Metabolic Syndrome (MS) definition are all progressive and lead to increased morbidity and mortality due to cardiovascular causes. MS is defined as the coexistence in the same individual of several metabolic abnormalities (obesity, dyslipidaemia, impaired glucose tolerance, insulin resistance and hypertension). Several mechanisms behind MS have not been completely elucidated nonetheless a sedentary lifestyle, diet, obesity and hypertension are modifiable risk factors that act together in its pathogenesis.

Endothelium is a complex endocrine organ able to produce a large quantity of substances such as Endothelin-1 (ET-1) a potent vasoconstrictor and Nitric Oxide (NO) a vasodilator related to oxidative stress. Endothelial dysfunction is an important feature of metabolic syndrome associated to distorted vascular remodelling, increased ET-1 production, and decreased NO production finally leading to hypertension. Thus angiotensin II levels increase in hypertension induce ET-1 synthesis and other mediators that could be used as early markers of cardiovascular disease risk and metabolic syndrome.

The main feature of MS is insulin resistance and hyperinsulinemia causing diminished glucose intake by myocytes and adipocytes, and hyperglycaemia until cellular apoptosis is observed in pancreatic β-cells due to long lasting hyperglycaemia and glucotoxicity. Hyperinsulinemia may lead to increased Na+ reabsorption by the kidney and induce androgen synthesis in the ovary leading to polycystic ovary syndrome risk. In the other hand hyperinsulinemia activates adrenergic system increasing vasoconstriction and hypertension, increased pro inflammatory cytokines (PCR, IL-1, IL-6) and pro thrombotic factors (fibrinogen, PAI-1).

Atherogenic Factors present in the Metabolic Syndrome

1. Atherogenic Dyslipidaemia: high VLDL and triacylglycerides, low C-HDL and small-dense C-LDL.
2. Hypertension: adrenergic vasoconstriction and increase in reninal Na+ reabsorption.
3. Central Obesity: insulin resistance.
4. Endothelial dysfunction and oxidative stress.
5. Increased vascular growth and proliferation due to hyperinsulinemia.
6. Impaired glucose tolerance or diabetes mellitus.
7. Pro inflammatory states: PCR, TNF-α, IL-1, IL-6, low adiponectin.
8. Pro thrombotic states: fibrinogen and PAI-1

Insulin resistance

Defects in the insulin action leading to compensatory hyperinsulinemia to maintain normal glycaemia is known as Insulin Resistance (IR). The main contributor to insulin resistance are high circulating free fatty acids (FFA) obtained from triacylglicerides (TAG) in adipose tissue due to AMPc-dependant Lipase or from TAG-enriched lipoproteins due to Lipoproteínlipase (LPL) action.

Once IR is established the increase in FFA from adipose tissue may interfere with glucose metabolism in insulin sensitive tissues, in muscular tissue interfere with protein kinases, in liver can induce insulin resistance. FFA induces hepatic gluconeogenesis and lipoprotein synthesis.

A large amount of metabolic active molecules are produced from adipose tissue in obese subjects including that cytokines related to an increase in insulin resistance such as TNF-α. Thus, resistance to leptin is given producing intracellular TAG accumulation and lower glucose intake in muscle and liver.

Metabolic syndrome patients show a decrease in adiponectin synthesis inversely correlated to body mass index and closely related to insulin resistance.

Impaired Glucose Tolerance

Defects in the insulin pathway lead to increased glucose production in liver. In pancreatic cells the resistance to insulin may be due to FFA that in proper conditions is able to insulin production but hypertryacilglyceridemia has shown to produce insulin synthesis decrease due to lipotoxicity.

Central Obesity

Obesity, a sedentary lifestyle and low calories consumption have been widely recognized as risk factor for diabetes but especially abdominal obesity andandroid distribution are the mayor components of metabolic syndrome according to the 2005 international diabetes federation criteria.

According to the World Health Organization overweight and obesity can be classified as follows:

<table>
<thead>
<tr>
<th>Body Mass Index (Kg/m2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Weight</td>
</tr>
<tr>
<td>&lt; 18.5</td>
</tr>
<tr>
<td>Normal Weight</td>
</tr>
<tr>
<td>18.5-24.9</td>
</tr>
<tr>
<td>Overweight</td>
</tr>
<tr>
<td>25-30</td>
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<tr>
<td>Obesity</td>
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<tr>
<td>&gt; 30</td>
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</table>

Atherogenic Dyslipidaemia

A distinctive feature of the MS is an increased FFA in portal circulation leading to increased synthesis of TAG-richVLDL and Apo-B. In the normal lipid metabolism FFA’s are liberated from adipocytes to the blood stream and then liver and muscle. In liver an important part is
oxidized and another is re-esterified to TAG nonetheless when this re-esterification process is saturated the TAG accumulation may lead to Fatty Liver Disease (FLD).

In normal conditions insulin may inhibit VLDL production and Lipoproteinlipase activity leading to diminished FFA production and IDL and LDL increase. TAG in VLDL are exchanged for cholesterol in HDL due to Cholesteryl ester transfer protein (CETP). Then, small HDL molecules are easily cleared from blood stream with cause a decrease of HDL and Apo-AI (both anti-atherogenic).(9,10)

Small and dense LDL molecules have a major role in atherosclerosis due to: are more likely to migrate through the endothelial basal membrane, are more antigenic, have higher affinity for glucosaminglycans are more susceptible to oxidation.(7)

From the clinical perspective the determination of Apo-B, HDL and TAG concentrations are the markers for dyslipidaemia in the MS.

**Hypertension**

Hypertension diagnosis according to the World Health Organization (WHO) is done when mean blood pressure (BP) taken from several well performed determinations is over 140/90mmHg and according the National Cholesterol Education Program (NCEP) criteria 130/85mmHg or more may be diagnostic for arterial hypertension. Although, when lower BP is detected under antihypertensive therapy this patient is also considered to have hypertension.

**Insulin Resistance and Hypertension**

Many factors have been involved in hypertension pathogenesis including genetics, environment, endocrine and metabolic variables such as hyperinsulinemia, hyperactivation of the Renin-Angiotensin-Aldosterone System (RAAS), activation of sympathetic nervous system, increased cardiac output and NO decreased synthesis.(7)

Thus, hyperinsulinemia may lead to hypertension due to augmented Na+ reabsorption in the proximal convoluted tubule, activation of sympathetic nervous system, and hyperplasia of smooth muscle cells in vascular walls. Also may lead to an increased response to Angiotensin II and catecholamines, increased Ca++ intracellular concentration and sustained myocite contraction leading to more vascular periphery resistance.(11)

Vasodilator functions have been demonstrated under the effect of insulin whereas the intracellular insulin pathway must have a normal activity thus insulin resistance may contribute con hypertensive states.(12)

The effects of insulin infusion in normotensive subjects rarely produce significant changes in blood pressure due to physiological mechanisms regulating BP. Nevertheless, in morbid states such as obesity this balance may be altered and insulin play an important role in BP changes. Significant associations have been observed between obesity and hypertension maybe due to adipocitokines production such as PAI and Leptin. The latter have shown to stimulate the activity of sympathetic nervous system and RAAS.(7,13,14)

By the other hand, insulin sensitivity can be estimated through several non invasive matematic models including QUICKY and the Homeostasis Model Assessment (HOMA) which after comparisons against the gold standard test, the hyperinsulinemic euglycemic clamp study a very accurate estimation of insulin sensitivity can be achieved.(15)

MS is a condition that groups into the same subject all these metabolic abnormalities significantly increasing coronary artery disease and diabetes risk.

**Objective**

The aim of this study was to evaluate the cardiovascular risk markers, anthropometric and biochemical variables and endothelial markers in subjects with metabolic syndrome and make comparisons against a control group with no metabolic syndrome diagnosis.

**Materials and methods**

Eight teen adult patients with ages from 31 to 53 years old were randomly selected (11 women and 7 men) from the Cardiovascular Prevention Program carried out in the Cardiopulmonary Function and Exercise Laboratory in the Medicine School Jose Maria Vargas, Central University of Venezuela. A complete medical history and clinical evaluation was carried out in all patients and those with thyroid disease, current pharmacologic treatment, bariatric surgery history and pregnancy were excluded. All patients agreed to participate in the study and signed a written informed consent. Data collection was done from January 2011 to November 2011. Metabolic syndrome diagnosis was done according to the NCEP criteria where 3 or more positive components were needed to make the diagnosis (table 1).

| Table N° 1. Metabolic Syndrome Criteria according to NCEP AT-PIII |
|---------------------------------|------------------|
| **Central Obesity** | WC ≥102 cm in men and ≥ 88 cm in women |
| **Triacylglycerols** | 150 mg/dL or more |
| **HDL** | < 40 mg/dL in men y < 50 mg/dL in women |
| **High Blood Pressure** | 130/85 mmHg or more |
| **Fasting Glycaemia** | 100 mg/dL or more |

Subjects were divided in two groups, 9 control and 9 metabolic syndrome subjects. A complete clinical evalu-
ation, blood pressure, anthropometry measurements and blood samples were taken. Each patient underwent a total and fractioned cholesterol, triacylglycerols, fasting glycaemia and insulin measurement, HOMA calculation\textsuperscript{(15)}, Nitric Oxide and Endotelin-1 determinations.

Blood samples were obtained from antecubital vein puncture after a 14 hours fasting using 2 Vacutainer tubes with and without EDTA which were processed through centrifugation (1000g for 20min) to obtain plasma and serum. Total Cholesterol, triacylglycerols, fasting glycaemia, insulin, HDL, VLDL, LDL determinations were done using the colorimetric method (INVELAB). Serum was frozen for future Nitric Oxide and Endothelin-1 measurement through the Elisa method using commercial Calbiochem and Cayman kits in a Microplaque Elisa Biotek Instrument, INC.lector.

Statistical Analysis
SPSS ver. 17 for Windows was used to the statistical analysis. Mean ± standard deviation was estimated for each continue variable and variance analysis was also carried out considering significant a p<0,05.

Results

| Table N° 2. Anthropometric, biochemical and endotelial variables in both groups |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Variable        | Control         | Metabolic Syndrome |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Cholesterol (mg/dL) | 164,11 ± 70,87 | 216,44 ± 51,07 * |
| Triacylglycerols (mg/dL) | 90,89 ± 54,88 | 212,75 ± 66,65* |
| HDL (mg/dL)     | 54,88 ± 30,10  | 38,00 ± 5,95*   |
| LDL (mg/dL)     | 88,25 ± 42,93  | 155,50 ± 102,67* |
| VLDL (mg/dL)    | 15,80 ± 8,99   | 90,45 ± 131,74* |
| Glicaemia (mg/dL)| 80,11 ± 30,28  | 70,00 ± 51,07*  |
| Insulin (mg/dL) | 2,68 ± 1,38    | 4,27 ± 0,73*    |
| HOMA            | 40,95 ± 12,56  | 43,95 ± 9,36    |
| Age             | 84,11 ± 32,69  | 116,44 ± 12,54* |
| Weight (Kg)     | 61,20 ± 26,37  | 100,22 ± 15,92* |
| Height (mts)    | 1,43 ± 0,54    | 1,70 ± 0,07     |
| BMI (Kg/m2)     | 26,61 ± 5,19   | 34,74 ± 5,89*   |
| Systolic Blood Pressure (mmHg) | 121,78 ± 19,01 | 131,33 ± 13,45* |
| Diastolic Blood Pressure (mmHg) | 79,67 ± 8,69   | 102,22 ± 34,20* |
| Nitric Oxide (µM) | 18,16 ± 2,23   | 16,80 ± 2,96*   |
| Endotelin-1 (pg/ml) | 2,20 ± 0,71    | 2,58 ± 0,81     |

Results are shown as mean ± SD
* (p < 0,05).

No differences were observed in mean age of the control compared to metabolic syndrome group nonetheless the later showed a higher mean age maybe due to higher central obesity prevalence in older subjects.

Significant differences were observed when comparisons in lipid profile were done showing in the MS group higher cholesterol, triacylglycerols, LDL and VLDL and lower HDL when compared to control group. Thus, glycaemia, insulin and insulin resistance were also higher en MS group.

Accordingly, weight and BMI were higher in MS group compared to controls (p<0,05) most of them being in the overweight and obesity I stratification according to WHO criteria. In this population triacylglycerols are stored in small insulin sensitive adipocytes but when the storage limit is reached FFA can accumulate within muscular tissues leading to insulin resistance.\textsuperscript{(19)} In addition, Systolic and Diastolic Blood Pressure and Insulin showed to be higher in the MS group.

In this study we observed significantly lower Nitric Oxide levels in MS subjects compared to controls and no differences were observed in Endotelin-1 levels.

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Conclusion

Excepting nitric oxide and HDL all parameters were observed to be elevated in MS individuals. All these determinations are considered as risk factors for Coronary Artery Disease Diabetes Mellitus and can be used in clinical practice as markers and risk stratification.

The patterns observed in the MS patients (low NO, high LDL, glycaemia, BP...) may lead to the pathophysiological mechanisms underlying endothelial dysfunction atherosclerosis and insulin resistance which explains the elevated morbidity and mortality due to cardiovascular causes patients with the metabolic syndrome.
References

18. Lares Mary, Pérez Elevina, Gestne Aure, Case Cynthia, Brito Sara, Ciarfella Ana, and Schroeder Mileibys. Main ingredient of the diet of the Warao tribe: moriche fruit, cassava, plantain, its possible influence on their anthropometric and biochemical values and positive effects on the prevention of metabolic syndrome. Food and Nutrition Sciences. 2011;2:5.