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BIOMARKERS OF INFLAMMATION AND GLYCEMIA BALANCE IN TYPE 2 DIABETIC MELLITUS OUTPATIENTS IN ABIDJAN (CÔTE D'IVOIRE)

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ABSTRACT

Background: The crucial role of inflammation in the pathogenesis of type 2 diabetes mellitus remains controversial. However, most studies suggest that the production of pro-inflammatory proteins in diabetes mellitus promotes insulin resistance and the development of type 2 diabetes. The aim of the study is to assess the evolution of markers of inflammation and glycaemia in diabetic type 2 during treatment of outpatients living in Abidjan. **Methods**: For this purpose, 90 subjects with type 2 diabetes untreated previously (35 to 69 years, both sexes) were recruited. According to their situation, they were equally classified into insulin dependent and non-insulin dependent and treated respectively with insulin and oral antidiabetic adrugs. Glycemia, HbA1c and CRP were determined in early and 3 months after the treatment using enzymatic methods. **Results**: Before treatment, there was a significant mean hyperglycemia ($p < 0.001$) in both treatment groups, with a higher value in insulin subjects (3.95 ±0.05 g/L). The concentration of CRP was also very high ($p < 0.001$) in insulin dependents (15.50 ±0.42 mg/L). Three months after treatments, the mean glycemia (1.10 ±0.02 g/L) in patients treated with insulin was much reduced against those treated with oral antidiabetics (1.50 ±0.02 g/L). However for these latter, the CRP (6.31 ±0.40 mg/L) was much reduced compared to insulin dependents subjects (10.50 ±0.02 mg/L). **Conclusion**: These findings support the role of inflammation in the pathogenesis of type 2 diabetes and suggests the CRP consideration in blood glucose balance order to better guide therapy combining anti-inflammatory drugs.

KEYWORDS: Type 2 diabetes, Glycemia, Inflammation, C-reactive protein.

INTRODUCTION

The rapid increase in the prevalence of Type 2 diabetes mellitus (T2DM) worldwide is a substantial public health problem; because over 80% of people with diabetes are type 2 (Badawi et al, 2010; Wang et al., 2013, Yeboua et al, 2016). In addition, recent studies report that type 2 diabetes is often accompanied by micro- or macrovascular complications in the long term, which may lead to morbidity and mortality (Fowler, 2011). Also, the crucial role of inflammation in the pathogenesis of T2DM remains controversial (Dehghan et al., 2007; Lee et al., 2009; Wang et al., 2013). However, most studies have suggested a relationship reported a link between high levels of pro-inflammatory proteins in diabetes mellitus, insulin resistance and the development of T2DM (Wang et al., 2013; Akash et al., 2013).

Previous studies have established a correlation between the abnormal production of pro-inflammatory cytokines, such as interleukin-1-beta (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor (TNF) in T2DM (Al-Shukaili et al., 2013; Akash et al., 2013). Other studies have reported an increase in acute phase proteins of inflammation such as C-reactive protein (CRP) in people with this disease (Doi et al., 2005; Marques-Vidal et al., 2013). The high synthesis of these pro-inflammatory proteins or inflammatory network in diabetic characterizes the early stages of T2DM and shows the progress of the disease (Badawi et al, 2010; Wang et al., 2013).

Currently available therapies for type 2 diabetes, although based on two options aimed to increase the secretion of insulin or decrease its resistance, do not prevent the disease progression (Esser et al, 2011; Donath et al., 2011). Thus, an understanding of the inflammatory network or study of biomarkers in the pathogenesis of T2DM is a potential public health importance. It has an interest for predicting and the occurrence of the disease outside the risk factors usually monitored, such as family history, clinical diagnostic standard profiles and assessment of lifestyle. Also

biomarkers of inflammation could help evaluate of new prevention strategies.

Previous meta-analyzes have evaluated a relationship between CRP levels and the risk of developing type 2 diabetes mellitus have reported conflicting results. One suggested a positive link between CRP levels and the risk of developing type 2 diabetes (Dehghan et al., 2007). However, other meta-analyzes have reported that CRP may not be an independent risk factor for the development of diabetes (Lee et al., 2009). These discordant results lead to carry out complementary work to properly identify the interaction between inflammation and the risk of developing type 2 diabetes mellitus.

The aim of this study is to determine biomarkers of inflammation and glycemia during treatment with insulin or oral antidiabetic drugs in type 2 diabetics outpatients in Abidjan.

MATERIAL AND METHODS

Material

Recruitment of subjects

Subject recruitment took place at Antidiabetic Center of Adjamé (Abidjan / Côte d'Ivoire), and was included in the study, the 2 type known diabetic subject, having a tracking file that can help confirm its status. In addition, the subject must be aged 35 to 69 years and should only be suspected of no other associated disease. A total ninety (90) monitored diabetic people were recruited. Moreover, forty five (45) without diabetes were randomly recruited in Abidjan as control groups. The consent of subject under study was obtained verbally.

Biological material

Whole blood, plasma and serum were used for the determination of biomarkers. Approximately 10 mL of whole blood were collected intravenously for each subject fasting, then distributed in three different tubes. Two of them contained particular anticoagulants, specifically sodium fluoride and ethylene diamine tetra-acetate (EDTA) to the respective glycemia and glycated hemoglobin (HA1c). The third tube (dry) was used to CRP assay; for this, the fluorinated and dry tubes were centrifuged and plasma or non-haemolysed serum was collected and stored systematically cold (-20 °C) for the determination of glycemia and CRP.

Methods

This is an experimental case-control study based on the determination of biochemical parameters including glycemia, glycated hemoglobin (HbA1c) and C-reactive protein (CRP). The assays were carried out at the laboratory of medical biochemistry, UFR medical sciences of Félix Houphouët-Boigny University in Abidjan (Côte d'Ivoire). Glycemia and glycated hemoglobin allowed monitoring glucose balance in diabetics patients. Being stable throughout the lifetime of the erythrocyte, the concentration of HbA1c reflects the average glucose levels in the blood of 4 to 6 weeks preceding the dosage.

The inflammatory process was monitored by the determination of CRP in serum of diabetic patients. CRP assesses the level of inflammation of blood vessels induced by the permanent status hyperglycemia in diabetics. The stability of this protein during long-term storage in the frozen blood and the availability of inexpensive tests, precise and standardized have helped its choice. (Pearson et al., 2003)

Glycemia assay

Glycemia was performed according to enzymatic method using as enzyme, glucose oxidase peroxidase adapted to a self-multiparameter analyzer.

Glycated hemoglobin assay

The determination of glycated hemoglobin (HbA1c) is performed using a self- multiparameter analyzer equipped with chromatography to cation exchange resin. The formation of HbA1c into erythrocytes is irreversible and progressive throughout their normal lifetime of three months (120 days).

C-reactive protein assay

C-reactive protein (CRP), glycoprotein synthesized by the liver cells, is a good blood marker for exploring acute inflammation (Pearson et al., 2003; Al-Shukaili et al., 2013). Its assay was performed by enzyme immunoassay ELISA according to instructions supplied by the Company's assay kit ABCAM®

Statistical analysis

The results were expressed in mean values accompanied by standard error of the mean (mean ±SEM). The graphical representations of data were performed from the Graph Pad Prism software. The statistical analysis was conducted using analysis of variance (ANOVA ONE WAY) and the t-test with Welch correction. Differences between means were considered significant at probability level of $p < 0.05$.

RESULTS

Characteristics of biomarkers studied in subjects early treatments

The early mean blood glucose (T0) in people with diabetes recruited to treatments was 3.95 ±0.05 g/L and 2.72 ±0.05 g/L respectively in insulin dependent people and non-insulin dependent. The comparison of these mean to the control group (0.91 ±0.02g/L) showed very significant results ($p < 0.001$). In addition, the assay of glycated hemoglobin also reported very high levels ($p < 0.001$) compared to the control group (Table I). Concerning the CRP, the initial values before the various treatments were 15.50 ±0.42 mg/L and 11.33 ±0.41 mg/L respectively in insulin dependent subjects and non-insulin dependent. These values are highly significant ($p < 0.001$) when they are compared with the control group.

Thus there is a hyperglycemia combined with a significant inflammatory condition in diabetic subjects.

Table I: Blood glucose and markers of inflammation in diabetic subjects and healthy controls at T0

Bio-markers	Control subjects n= 35	IDD subjects n= 45	NIDD subjects n= 45
Glycemia (g/L)	0.91 ±0.02	3.95 ±0.02***	2.72 ±0.05 ***
HbA_1c (%)	4.82 ±0.19	13.53 ±0.23***	9.80 ±0.10***
CRP (mg/L)	3.12 ±1.32	15.50 ±0.42***	11.33 ±0.41***

T0: starting treatment. IDD: insulin-dependent diabetics. NIDD: non-insulin dependent diabetics. (*): $p < 0.05$. (**): $p < 0.01$. (***) $p < 0.001$

Variation of glycemia during treatment

Three months after various treatments, mean glycemia in non-insulin independent persons (NIDD) receiving oral antidiabetic drugs as treatment was 1.50 ±0.02 g/L and in their counterparts who underwent insulin therapy (IDD), glycemia was 1.10 ±0.02 g/L (Figure 1). These results show a very significant reduction ($p < 0.01$) of blood glucose levels in subjects treated with insulin compared to those treated with oral antidiabetic drugs.

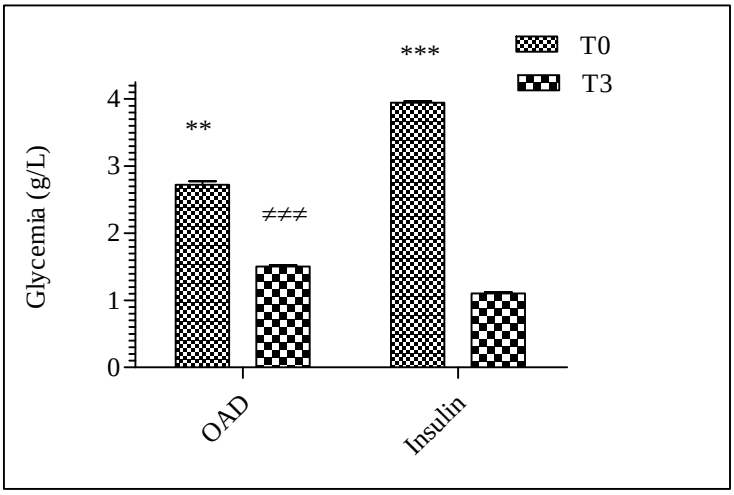

Figure 1: **Variation of glycemia in patients after 3 months of treatment.** *OAD: oral antidiabetic drugs; T0: starting treatment T3: 3 months after treatment. Comparison between treated group and untreated, (*): $p < 0.05$; (**): $p < 0.01$. (***): $p < 0.001$. Comparison between insulin and OAD groups (≠): $p < 0.05$; (≠≠≠): $p < 0.001$.*

Variation of glycated hemoglobin during treatment

The taking antidiabetic oral drugs or insulin pathway also decreased significantly ($p < 0.001$) the HbA1c level 3 months after treatment, but not significantly when the patients treated are compared between themselves (Figure 2).

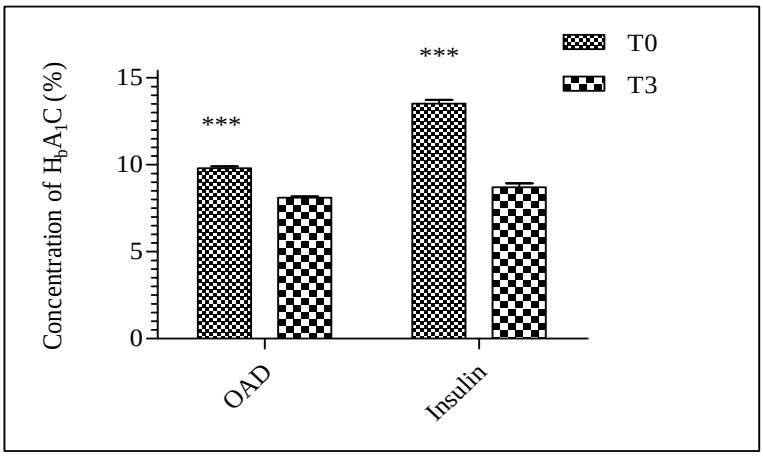

Figure 2: **Variation of HbA1c in patients after 3 months of treatment.** *OAD: oral antidiabetic drugs; T0: starting treatment T3: 3 months after treatment Comparison between treated group / untreated, (*): $p < 0.05$; (***): $p < 0.001$. Comparison between insulin / OAD groups (no significant): $p > 0.05$.*

Variation of inflammatory marker during treatment

For non-insulin dependent diabetic patients (NIDD) treated with oral antidiabetic drugs, the mean concentration of CRP was 6.31 ±0.40 mg/L. Through against, in insulin dependent persons (IDD) it was 10.50 ±0.02 mg/L (Figure 3) These results show that there was a reduction of the concentration of CRP in subjects, however the reduction was highly significant ($p < 0.01$) in subjects who received oral antidiabetic drugs compared with insulin dependent subjects.

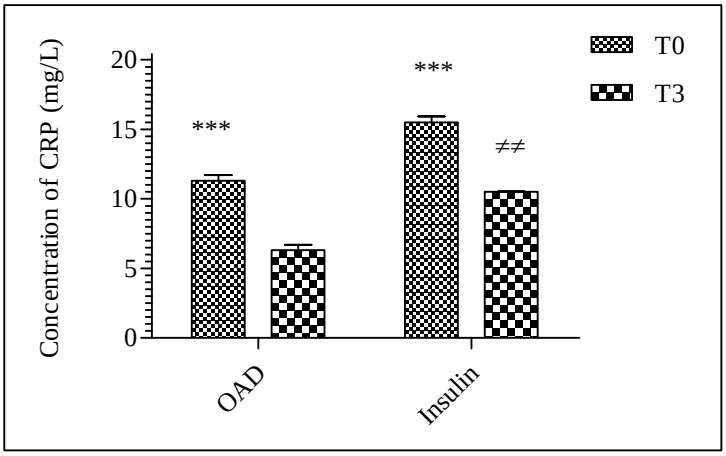

Figure 3: **Variation of CRP in patients after 3 months of treatment.** *OAD: oral antidiabetic drugs; T0: starting treatment T3: 3 months after treatment Comparison between treated group / untreated, (*): $p < 0.05$; (***): $p < 0.001$. Comparison between insulin / OAD groups (≠): $p < 0.05$; (≠≠): $p < 0.01$.*

Reduction of rates of biomarkers studied

Reducing the levels of different markers studied were significant ($p < 0.05$) three months after various treatments. Concerning the CRP of people receiving treatment, the percentage of reduction were 44.60% for those receiving oral antidiabetic drugs and 32.06% for insulin-dependent subjects (Figure 4). These results show that oral antidiabetic drugs better decrease inflammation associated with diabetes.

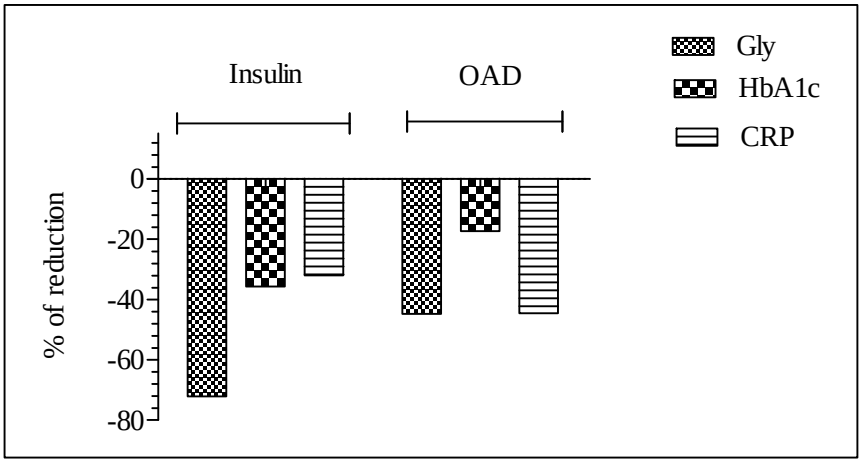

Figure 4: **Reduction of rates studied markers according to antidiabetic drugs used.** *OAD: oral antidiabetic drugs*: Gly: glycemia. HbA1c: glycated hemoglobin. CRP: C-reactive protein.

DISCUSSION

Many previous studies have already reported the existence of an inflammatory process in the development of type 2 diabetes (Badawi et al, 2010; Marques-Vidal el al., 2013; Odegaard et al, 2016). Indeed, the advance of diabetes mellitus during which blood glucose is generally around 1.26 g/L is accompanied by excessive production of pro-inflammatory proteins, including cytokines (IL-1, IL-6), acute inflammation phase proteins (CRP) and tumor necrosis factor (TNF).

This inflammatory condition installed by chronic hyperglycemia and an overabundance of adipose tissue is responsible for developing both a peripheral insulin resistance to insulin and a decrease of synthesis and secretion of insulin by the pancreas (Esser et al., 2010; Akash et al., 2013). Thus, significantly elevated levels of C-reactive protein (CRP) in untreated type 2 diabetes mellitus in before reports this study represent a risk of complications of the condition of these people. These physiopathological mechanisms also would explain

hyperglycemia observed in diabetic subjects before treatment.

Our results have reported respective hyperglycemia in type 2 diabetic persons recruited at the beginning of the experiment. This hyperglycemia confirmed by high HbA1c suggest that those recruited were not receiving treatment during the quarter preceding the recruitment. Three months after various treatments followed, there was a simultaneous decrease in blood glucose and hemoglobin, these findings show that treatment with insulin or oral diabetes reduces glycemia.

The study found also that the treatment of type 2 diabetes with oral medications does not normalize blood glucose, however, these oral antidiabetic drugs significantly reduce CRP levels. This reflects a reduction of the inflammatory process and allows to deduce that the oral diabetes based therapy might play two simultaneous roles; lower blood glucose levels and likewise installed the inflammatory response during diabetes.

These results are in agreement with data stating that the treatment of type 2 diabetes to mitigate the associated inflammatory condition help to reduce insulin resistance. The proof that the benefit of a therapy of type 2 diabetes based anti-inflammatory drugs was available in the literature (Fleischman et al., 2008; Esser et al., 2010).

A recent study using the salsate (a salicylate prodrug) reported an improvement in insulin sensitivity and reduce plasma levels of markers of inflammation, such as CRP 34% (Fleischman et al., 2008). Other studies have shown similar results (Larsen et al., 2007). The early management of diabetes is important for maintaining blood glucose control and avoided chronic hyperglycemia responsible for inflammation in diabetic condition that could lead to type 2 diabetes.

CONCLUSION

This study helps to note that insulin controls blood glucose better but does not ensure a significant reduction of the inflammatory process. Diabetes monitoring using glycemia control and HbA1c should thus be coupled to the CRP assay order to better guide therapy and prevent complications.

Competing interests

All authors declare that they have no competing interests.

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