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Mini Review Open Access

Subclinical Processes in the Development of Type Two Diabetes

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Abstract

The imbalance in metabolic homeostasis in type 2 diabetes (T2DM) is accompanied by cellular stress, altered expression and circulating concentration of heat shock proteins (HSP) and cytokines (pro and anti-inflammatory). Also T2DM subjects had altered redox state (oxidative stress) and erythrocytes parameters. This set of molecular, biochemical and immuno haematological alterations are usually subclinical events, but characterizes chemical and biological processes associated with the development of insulin resistance and the pathophysiology of T2DM. These process and markers of T2DM complications and development will be briefly outlined throughout this mini-review article. The establishment of new strategies for glycemic control required more attention and more studies about inflammatory related biomarkers in T2DM.

Keywords: Diabetes; Inflammation; Cytokines; Heat Shock Proteins; Oxidative stress; Erythrocyte parameters

Introduction

Diabetes Mellitus (DM) is a metabolic disorder with an enormous impact worldwide. Epidemiological data has shown that in 2010 there were 285 million individuals affected by diabetes in the world and is estimated around 440 million of diabetics for 2030 [1]. DM can be classified into two predominant types, type 1 DM (T1DM), defined by the destruction of pancreatic β- cells and the absence of endogenous insulin, and type 2 diabetes (T2DM), which is characterized by insulin resistance in periferal tissues, commonly associated with obesity. Both types are marked by hyperglycaemia and metabolic imbalances in cellular and systemic levels.

The imbalance in metabolic homeostasis in T2DM is accompanied by cellular stress, triggering changes in the expression and circulating concentration of heat shock proteins (HSP) and cytokines (pro and anti-inflammatory), and altered redox state (oxidative stress), and also erythrocytes parameters. This set of molecular, biochemical and immuno haematological alterations are usually subclinical events, but characterizes chemical and biological processes associated with the development of insulin resistance and the pathophysiology of T2DM. These process and markers of T2DM complications and development will be briefly outlined throughout this article.

Methods

We used a narrative (short) review of the literature. Our work is a narrative overview of the literature synthesizing the findings of literature retrieved from searches of the computerized databases PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) and Scielo (http://www.scielo.com). Also we used recent published books about the theme and the Brazilian government journals database named Periódicos-Capes (http://www.periodicos.capes.gov.br).

Cytokines and diabetes

Cytokines are molecules involved in many different cells and immunomodulation related functions. Initially the cytokines were associated with the response of an organism to infectious disease, but currently it is known that are also involved in the development and complications of non-infectious chronic diseases, such as diabetes [2].

Primarily, cytokines were studied as immune factors. Cytokines were listed first as derived from white blood factors, produced by immune cells that interact and modulate other immune cells. Currently, it is known that cytokines are released by different cell types, not only immune and interacting with several cell membrane receptors [2,3].

Interestingly, scientific advances were observed about the actions and interactions mediated by cytokines between the nervous and endocrine systems, related to immune status. This integrative approach can be named as neuroimmunomodulation, which is largely mediated by cytokines [4]. Moreover, from an evolutionary analysis on the production and effects of cytokines, We can consider that simple organisms have one type of mixed tissue, an “immune-metabolic” tissue, while more complex organisms such as the human body, at least macroscopically, have their immune functions located in different organs of the endocrine organs. However, in human body exists this immune-metabolic communication: many signal are release to the bloodstream and also within each tissue (as paracrine and autocrine function) by co-localization of metabolic and immune cells in the same tissue.

After many differences and inconsistencies about the nomenclature of cytokines, with the same molecule called as interleukins, lymphokines or monokines, actually is common the term cytokine. More generally, regardless of the source tissue or cell. Usually, these are classified according to their function and/or by its primary properties. In this way, the interleukins (so named and discovered by the action in the communication between leukocytes) IL-2 and IFN-γ are recognized as factors that orchestrate the clonal expansion of

cytotoxic T lymphocytes (Th1) and then in autoimmune diseases classified as cytokines Th1 type. Moreover, IL-4 may be classified as Th2, to increase antibody production in Th2 lymphocyte source.

The most of cytokines exerts immunomodulation by binding to specific receptors on the cell membrane, and thus, initiating intracellular signaling cascades that affect gene expression, mediated by transcription factors. Finally, the functional classification of cytokines can be split by the duality between pro and anti-inflammatory effects. IL-1β and TNF cytokines (Th1-related cytokines lymphocyte response) are classified as pro-inflammatory while the IL-10, IL-13 and TGF-β (cytokines associated with Th2-type immune response) has anti-inflammatory roles. In this scenario, autoimmune imbalance that may be causative of T1DM, can be directly associating with the increase of IL-2 and IFN-γ which in turn activates macrophage production of IL-1β and TNF.

On the other hand, in a related metabolic context, is clear that the adipose tissue, which is present in abundance in obese individuals, is largely responsible for the IL-6 and TNF cytokinessecretion, that can be called as adipokines. IL-6 and TNF levels are currently studied as sub-clinical biomarkers of subclinical damages caused by obesity in T2DM. These studies presented many comparisons, correlations and associations with classical cardiovascular risk factors and established inflammatory biomarkers. In this sense, there is a direct correlation between high levels of IL-6 and TNF in the circulation and high body mass index, high waist-hip ratio and increased central adiposity. The increase of the cytokines IL-6 and TNF is also correlated with decreased insulin sensitivity [5].

The association with these immune-inflammatory biomarkers and the development of T2DM can also be observed since healthy individuals have simultaneously increased levels of IL-6 and TNF and body mass index (BMI). This association between increased adiposity and increased pro-inflammatory cytokines persist even the data is adjusted for age or sex [6]. In diabetic individuals, pro-inflammatory cytokines are elevated and there is a clear unbalance between Th1 and Th2 cytokines that are associated with the type of clinical complications and comorbidities of T2DM subjects [7,8]. This “immune-metabolic” communication can also be observed in acute hypoglycemic episodes that triggers the IL-6 and HSPs release to the bloodstream [9].

As non-pharmacological strategy, exercise can be a strategy to prevent, control and even cure this immuno-inflammatory imbalance found in obesity related T2DM. The increasing in the systemic cytokine levels with anti-inflammatory properties, such as IL-10, plays a critical role in the control of insulin resistance in T2DM. In each exercise session, muscle activity produces and secretes IL-6 into the circulation. This increase in IL-6 is followed by the increase of IL-10 [10]. Chronically, the exercise (in both aerobic and strength type of exercise), provides immune modulating anti-inflammatory signaling, by increasing the IL-10 cytokine [11,12]. The increase in the anti/pro-inflammatory cytokine ratio may be dependent on the intensity of the exercise effort [13]. Thus the exercise prescription has a particular relevance for T2DM subjects [14].

HSP70 and diabetes

Obesity or fatty acids accumulation in the serum are associated with the biochemical process of an inflammatory state that predisposes the development of insulin resistance. Insulin resistance reduces glucose tolerance especially in adipocytes and muscle cells, tissues that are regulated by hormonal signaling by insulin. The impaired glucose uptake by peripheral tissues generates glucose accumulation in the circulation and consequently a hyperglycemic state. The metabolic stress and inflammation evoke the cellular stress response, which involves, among others mechanisms, increased expression of cellular proteins with significant cytoprotective functions: the heat shock proteins [15]. These are a family of proteins with different molecular weights. Here we highlighted the 70 kDa heat shock protein (HSP70) that are synthetized in larger amounts after challenges such as exercise and hyperthermia to promote the protection of the protein content of the many cells and tissues and also has anti-inflammatory action [16].

The inflammatory process is a central element of the triad "T2DM, obesity and HSP70." Unlike general inflammation, whose classic symptoms are pain, heat, redness, swelling and loss of function, the obesity related inflammation represents a chronic inflammatory state with attenuated resolution, or a inflammation development in a "silent" mode, but not less aggressive [17]. In condition of low fat mass (lean state), the M2 macrophages and regulatory T lymphocytes help counter inflammation in adipose tissue and maintain metabolic homeostasis. The cytokines IL-4 and IL-13 derived by eosinophils, promote the maintenance of fat M2 macrophages, and IL-10 secreted by regulatory T cells and macrophages M2, limited local inflammation. Moreover, obesity-related factors, including saturated free fatty acids, cholesterol, amyloid deposits and death of adipocytes, activating an “inflammasome” (intracellular multiprotein complex), which results in the secretion of IL -1β, which leads to the recruitment of macrophages to adipose tissue and promotes the alteration of phenotype of macrophages into M1 macrophages. These macrophages (M1) release pro-inflammatory cytokines, including IL-1β, causing infiltration of cells that perpetuate inflammation state by releasing interferon-γ (IFN-γ) and TNF. Thus, activities that stimulate M2 macrophages may be beneficial and important to reduce inflammation in adipose tissue and improve metabolic function [18].

High levels of circulating fatty acids also produces increased cellular content of lipid intermediates which, together with the action of circulating inflammatory cytokines cause activation of protein kinases, such as inhibiting kinase (IKK-β) NF-κB, protein kinase C (PKC) and the C-Jun-N-terminal kinase (JNK), causing impairment of insulin signaling cascade and the release/activation of the nuclear transcription factor NF-kB. NF-kB is responsible for the induction of pro-inflammatory cytokines expression, perpetuating the inflammatory cycle, indefinitely. In this context, it is highlighted the importance of the presence of HSP70 to limit or stop this cycle, restoring insulin signaling and improving glycemic control [19].

Decreased expression of HSP70 appears to be a primary factor leading to the development of T2DM [15,20]. Studies based on skeletal muscle biopsies from T2DM patients showed that the level of muscle HSP70 mRNA expression is reduced compared to healthy subjects. Also, the HSP70 mRNA levels in the skeletal muscle are correlated with many parameters of carbohydrate and lipid metabolism [21]. Corroborating these findings, studies in humans T2DM showed that the reduction in the skeletal muscle HSP70 expression is correlated with the degree of insulin resistance, it is negatively correlated with the glucose concentrations in the fasting state [22]. Other studies show that, in patients with T2DM, increased in the extracellular HSP70 concentration is associated with the duration of diabetes [23].

Induction of HSP70 expression reduces inflammation, improves insulin signaling, increases mitochondrial biogenesis, produces cytoprotection, with important effects on the metabolism, such as the

reduction of blood glucose and body fat, preserving pancreatic β cells function. The HSP70 has immuno-modulatory effects [24] in chronic diseases such T2DM, obesity and insulin resistance [25, 26]. Humans and experimental studies show the effect of HSP70 in the treatment or prevention of T2DM by induction during challenges that promote increased HSP70 expression, as exercise and thermal therapy (hot tub therapy). Hot tub therapy promoted a reduction of 18% in insulin administration (dose), prevented hypoglycaemia, and reduced fasting blood glucose and glycosylated haemoglobin concentration [27]. Also, studies with experimental models of obesity (high fat diet-induced obesity) showed that the thermal shock therapy (induced hyperthermia) improves glucose tolerance, restores the glucose transport stimulated by insulin, and increase insulin signaling in skeletal muscle, protecting it from development of insulin resistance. These effects were associated with increased expression of HSP70 and consequently inhibition of JNK [28].

The management of T2DM involves controlling/monitoring of complications (retinopathy, cardiovascular disease, nephropathy, neuropathy, e.g.), control of associated conditions (dyslipidemia, obesity, hypertension, coronary heart disease) and glycemic control. The control can be provided by changes in diet and lifestyle, exercise and/or by medication. In this way, hot tub therapy is now discussed and presented as a form of complementary and integrative therapeutic intervention option for obesity and/or insulin resistance, by inducing HSP70 expression and thus, modify the inflammatory status of T2DM subjects and consequently, improve the insulin resistance and contribute to the glycemic control.

Oxidative stress in diabetes

Studies have shown that in both types of DM, oxidative stress plays an important role in the disease development [29]. Oxidative stress is characterized by imbalance between the production of reactive oxidant species (ROS) and the activity of antioxidant defense systems, with a predominance of concentration of ROS [30].

In the human body reactive species (both oxygen and nitrogen species) are formed by different metabolic processes [31] and/or by different environmental factors [32]. These molecules are electronically unstable, have an extremely short half-life and are able to react with countless cellular components [33]. The main ROS are: the superoxide anion (O2-.), Hydrogen peroxide (H2O2) and the hydroxyl radical (OH.). Nitric oxide (NO.) and peroxynitrite (ONOO.) are reactive nitrogen species [34] that also are related to T2DM disorders.

To maintain oxidative balance, at least two antioxidant defense systems are required: the first composed by endogenous elements, such as antioxidant enzymes and antioxidant compounds, and the second formed by exogenous factors, obtained by the diet (vitamins, carotenoids and phenolic compounds). The enzymatic antioxidant defense converts the ROS in substances with less reactive potential. This is the case of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) enzymes. These enzymes are responsible for dismutate superoxide anion into hydrogen peroxide, hydrogen peroxide into water, reduced glutathione, and hydrogen peroxide into oxygen and water, respectively [35]. Classical exogenous antioxidants are vitamin E, vitamin C, glutathione, β-carotene, flavonoids and polyphenols. These substances primarily act by donating an electron to ROS making them less reactive and preventing the reaction with biomolecules (lipids, proteins, nucleic acids) from our cells, preventing the formation of cellular damage [36].

Several epidemiological and laboratory data (in vitro and in vivo studies) have shown that hypercaloric or high fat diet are associated with increased risk T2DM [37]. The main pathophysiological mechanism related to ROS is the development lipid oxidation reactions and DNA damage caused by oxidative stress [34]. Lipids are molecules vulnerable to oxidation, due to the membrane bilayer formation of our cells and by the structural constitution by unsaturated fatty acids [38]. The increased oxidative stress associated with lipid peroxidation in endothelial cells, can be a major cause of T2DM complications triggered by hyperglycaemia [29]. Also, the overproduction of mitochondrial superoxide in the endothelial cells results in activating pathways involved in the pathogenesis of diabetic complications, such as increased intracellular production of glycated products, leading to inflammation and endothelial dysfunction [39]. Since is common the metabolic syndrome in T2DM subjects, the endothelial dysfunction associated with modified LDL cholesterol accumulation (oxidized LDL, oxLDL) promotes an inflammatory vascular response that can directly contribute to increased DNA damage and subsequently the development of atherosclerosis [40].

Erythrocyte parameters and diabetes

T2DM, if not properly controlled, can progress, increasing the risk to develop complications such as dyslipidemia, nephropathy, macro vascular and microvascular complications, as well as the development of anemia [41,42]. Anemia has the potential to adversely affect the health T2DM subjects by different ways, from tiredness and a decreased work capacity to affecting social and sexual life quality [43].

The presence of anemia associated comorbidities in T2DM significantly contributes to increased morbidity and causes symptoms such as apathy, depletion, shortness of breath, dizziness, decreased appetite and cognitive function, accompanied by reduced exercise capacity. In anemic T2DM subject there is a reduced physical capacity and increased incidence of vascular diseases, which contributes to an increase in mortality in this population [44]. T2DM patients have 2 fold more chance than a normalglycemic population to develop anemia [45]. Additionally, anemia develops earlier in T2DM subjects with nephropathy, suggesting that the reduction in hemoglobin content can be a significant hematological parameters correlated to the development of other comorbid conditions associated with diabetes, such as renal failure [46,47].

As listed above, T2DM is an inflammatory related disease. Hyperglycaemia has direct association with pro-inflammatory cytokines such as IL-6 and TNF-α. Longer duration of the disease and/or loss of glycemic control are related to higher the inflammatory process [41,42]. The elevation of pro-inflammatory cytokines plays an essential role in kidney disease and anemia. The increase of IL-6 cause an anti-erythropoietic effect, since this cytokine changes the sensitivity of progenitors to erythropoietin (erythroid growth factor), and also promotes apoptosis of immature erythrocytes, causing a decrease in the number of circulating erythrocytes and thus reducing circulating haemoglobin [48-50].

Andrews and Arredondo [51] described the presence of anemia in T2DM and obese patients and the expression of genes related to inflammation and immune response. The results of this study show that diabetic patients with anemia exhibit increased expression of pro-inflammatory cytokines as compared to diabetic patients only (whose concentration is also elevated cytokines). In anemic subjects was confirmed the increase in IL-6 production, as well as the B cell activity, which reinforces the association between IL-6 and anti-erythropoietic

effect. Moreover, the T2DM and anemic patients had high levels of C-reactive protein and ferritin, however, had low iron contents, showing that ferritin increases were associated with chronic inflammatory process present in T2DM. Therefore anemia in T2DM has an adverse effect on quality of life and is associated with disease progression and the development of co-morbidities [51]. Together, these data suggest that treatment strategies that affect inflammatory profile in T2DM may represent anemia prevention and also future disorders.

Conclusion and Future Directions

T2DM is characterized by clinical and subclinical processes related to an imbalance in metabolic homeostasis. This mini-review we listed some multifactorial events, from cell to whole body complex systems. It is clear that T2DM represents a cellular stress condition that modifies the expression and circulating concentration of heat shock proteins and cytokines. These effects consolidating an unfavorable profile mediated by the pro-inflammatory and pro-oxidative imbalance and by undesired erythrocyte parameters. These set of changes characterize chemical and biological processes associated with the development of insulin resistance and the pathophysiology of T2DM, as well as the appearance of comorbidities. The establishment of new strategies for glycemic control required more attention and more studies about inflammatory biomarkers related to T2DM.

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