Immunohistochemical Expression of Growth Hormone Secretagogue Receptor (GHS-R) of Adipose Tissue Macrophages in Obese Bariatric Patients

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Our study aims to evaluate the immunohistochemical expression of CD68 and GHS-R in subcutaneous and visceral adipose tissue in normal and obese individuals. Adipocyte diameter, macrophage infiltration, and GHS-R expression in AT are associated with obesity inflammation. Obesity is a pro-inflammatory condition in which hypertrophied adipocytes and adipose tissue-resident immune cells (primarily macrophages) both contribute to a state of chronic low-grade systemic inflammation [1,2]. Excessive lipid load causes adipocyte stress, which in turn accounts for many adverse effects of obesity, particularly insulin resistance and glucose intolerance either directly by affecting the insulin signaling pathway or indirectly via stimulation of inflammatory pathways [3]. There are considerable evidence that obese adipose tissue is markedly infiltrated by adipose tissue macrophages (ATMs); these cells appear to be at the center of obesity-related inflammation [4-6]. Once recruited, the macrophages secrete additional chemokines, which contribute to insulin resistance [7, 8]. Ghrelin (GHRL), a gut peptide consisting of 28 amino acids, is the endogenous ligand of a G protein coupled receptor (GPCR), growth hormone secretagogue receptor 1a (GHS-R1a). The GHS-R1a mRNA is expressed in regions of the CNS, as well as in various peripheral organs including the stomach, intestine, pancreas, thyroid, adrenal gland, kidney, heart and blood vessels [9]. This ubiquitous expression of GHS-R1a mRNA suggests that GHRL/GHS-R1a system has multiple functions, including anti-inflammatory effect [10, 11]. The expression of GHS-R1a mRNA is low in adipose tissue and the concentrations of plasmatic ghrelin are lower in obese patients as compared to normal-weight controls. Little is known about the relationship between ATMs and GSH-R in adipose tissue inflammation. In our study, we proposed to investigate the association between CD68 and GHS-R in adipose tissue inflammation by immunohistochemical staining.

Experimental part

Patients

Samples of subcutaneous and visceral adipose tissues were obtained from normal weight females undergoing laparoscopic cholecystectomy (N = 3, age 39±8 years) and from morbidly obese females (N = 5, age 42±7 years) who underwent laparoscopic sleeve gastrectomy (LSG), from 1st of January to 31st of December 2018. All patients gave their consent to participate in the study prior to surgery. The study adhered to the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the University of Medicine and Pharmacy from Iasi, Romania.

Histological examination and morphometry

Tissue samples were fixed in buffered formaldehyde and incorporated into paraffin blocks for the study of histology. Adiposity study was done by using morphometry based on usual histological stains. Morphometry was applied on 5 random high-power fields (HPF, x400) of subcutaneous and visceral adipose tissue. We calculated the mean adipocyte diameter (MAD) of 10 adipocytes of various sizes in a tissue area of 197 229 µm².

Immunohistochemistry of CD68 and GHS-R

Immunohistochemical study was made for assessing the CD68 and GHS-R expression of the macrophages in
adipose tissue samples from obese and normal-weight patients. Monoclonal mouse antihuman CD 68 antibody (Dako, IS613) was used for targeting ATMs. GHSR Polyclonal Antibody (ThermoFisher Scientific, PA5-28752, conc. 1/500) was used. PA5-28752 targets GHS-R1 in IHC (P) and shows reactivity with human samples. The PA5-28752 immunogen is synthetic peptide corresponding to a region within amino acids 1 and 45 of Human GHS-R1.

The two IMH markers were expressed as mean percentage values, reported to adipose tissue area.

Statistical analysis

The statistical analysis was performed using IBM SPSS Statistics 21 Software. Numerical data are reported as means ± SD and compared using Mann–Whitney test. Categorical data are reported as number (%) and compared by the Pearson chi-square test. Statistical significance was set at P < 0.05.

Results and discussions

Histological examination and adipocyte size of subcutaneous and visceral adipose tissue

Histologically, the mean adipocyte diameter (MAD) of subcutaneous and visceral adipose tissue was larger in obese patients (SAT, 88 µm; VAT, 93.3 µm) as compared to normal-weight patients (SAT, 78.9 µm; VAT, 74.3 µm) (Table 1).

Immunohistochemistry of CD 68 and GHS-R

The expression of CD68 was lower in normal-weight patients as compared to obese patients in both SAT and VAT adipose tissue samples (Figs. 1 and 2). Also, the expression of GSH-R was lower in normal-weight patients as compared to obese patients in both SAT and VAT adipose tissue samples (Figs. 1 and 2).

Table 1

| Patients     | Mean Adipocyte Diameter (µm) | CD68 No | CD68 % | GHS-R Mean No | GHS-R % |
|--------------|------------------------------|---------|--------|---------------|--------|
| Normal-weight (N = 3) | SAT 78.9 (31.4) 90-145 | 5.8  | 2.8   | 4.4   | 1.4 |
|               | VAT 74.3 (26.1) 28-121 | 2.2  | 1.1   | 4.8   | 1.1 |
| Obese (N = 5) | SAT 88.0* (21.1) 59-139 | 15.2* | 7.2*  | 14.6* | 7.5* |
|               | VAT 91.3* (15.0) 66-118 | 18.2* | 9.1*  | 18.6* | 8.2* |

SAT* = subcutaneous adipose tissue, VAT, visceral adipose tissue.

* P values <0.05 when compared with normal-weight patients.

Fig. 1. CD68 (minimal immunoreactivity) and GSH-R (minimal immunoreactivity) expression of macrophages in SAT and VAT from normal-weight patients. Section with fatty replacement of exocrine pancreas showing positive staining for GHS-R. Magnification 40x.

Fig. 2. CD68 (minimal immunoreactivity) and GSH-R (minimal immunoreactivity) expression of macrophages in SAT and VAT from obese patients. Section with fatty replacement of exocrine pancreas showing positive staining for GHS-R. Magnification 40x.
Weisberg et al. [12] observed that macrophages originating from adipose tissue represented one of the main forces in development of chronic inflammation of obesity. Adipose tissue also contribute to inflammation, secreting various hormones and cytokines which play crucial roles in obesity-associated metabolic complications [13].

Our study was focused on the relation between CD68 and GSH-R expression of ATM in adipose tissues collected from normal-weight and obese patients. We found increased CD68 immunohistochemical expression in ATs from obese patients. This finding is consistent with other research reports [14] showing increased macrophage infiltration in obese adipose tissue. The increased CD68 expression is associated with large adipose cells.

In a recent study, GHSR knockout mice fed with high-fat diet (HFD) showed improved insulin sensitivity through improved adipose inflammation. Deletion of GHS-R would abolish the effect of acylghrelin and subsequently inhibit improved adipose inflammation. Deletion of GHS-R would abolish the effect of acylghrelin and subsequently inhibit increased macrophage polarization to M1 phenotype, and finally improve insulin sensitivity [15].

There is few information about the GSH-R expression in human tissues [16]. Our current data showed positive GSH-R immunohistochemical expression of ATM in obese patients. Moreover, our study showed an association between macrophage infiltration and increased GSH-R expression in adipose tissue.

Some reports noticed that, GHS-R1a ablation could promote an anti-inflammatory process under both diet-induced and age-associated adipose inflammation [17, 18]. Sun et al. also noticed that GHS-R1a deletion activates anti-inflammatory macrophages, while increased GSH-R1a is associated with inflammatory macrophages [19].

Taken together these results could suggest that ATM switch express GSH-R may have a pro-inflammatory phenotype. The immunohistochemical analysis could be a useful test to assess the macrophage functions and to predict the following development of obesity-associated metabolic complications [20-27].

Conclusions
Our study suggested that increased immunohistochemical expression of GHS-R of ATMs is associated with increased macrophage infiltration in adipose tissue. These immunohistochemical markers could be related to insulin resistance and metabolic complication in obese bariatric patients.

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