ESTIMATION LEVELS OF SOME HORMONES IN MYELODYSPLASTIC SYNDROMES PATIENTS

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SUMMARY

Objective: Some hormones play an influence on the course of malignancy. Adiponectin has been related mostly to the risk of “myeloid” cell-line hematological malignancies, whereas leptin has been shown to enhance progenitor cell proliferation and drive leukemic cell development in vitro. Resistin promote inflammation so we investigated in this study about the role of adiponectin, leptin and Resistin levels in the etiopathogenesis of Myelodysplastic syndromes (MDS), and relationship with demographic characteristic of patients.

METHODS: This study performed on 50 patients suffering from myelodysplastic syndromes (MDS) including (29 males, 21 females) and 25 healthy individuals as control group (15 males, 10 females). Age of patients ranged from (20-83 yrs.). During the period from June 2019 to the December 2020 at the laboratory of DNA in the department of Biology, Faculty of Science, Babylon university / Iraq. Levels of Adiponectin, Leptin, and Resistin were estimated by ELISA assay technique according to manufacturer's description.

RESULTS: Results of demographic study showed that the males percentage value of myelodysplastic syndrome was more than females percentage value (58% and 42% respectively). Also, the age of the samples in this study were arranged from 20-85 years old older men and women more affected than control group. The results of body mass index showed a significant decrease in patients with myelodysplastic syndrome when compared with control group. Levels of hormones the results showed significant differences in some hormones level between the myelodysplastic syndrome patients and control group, Adiponectin and Resistin were a significantly increased in patients of myelodysplastic syndrome compared with control group. While there was no significant differences in leptin hormone between patient and control groups.

CONCLUSION: Estimation levels of some hormones such as Adiponectin, Leptin, and Resistin accompanied with demographic characteristic and body mass index may use as useful marker for detecting Myelodysplastic syndromes.

Keywords: Myelodysplastic syndromes (MDS), Adiponectin, Leptin, and Resistin

I. INTRODUCTION

Myelodysplastic syndromes (MDS) are clonal stem cell malignancies identified by cytopenias, insufficient hematopoiesis, dysplasia in one or more myeloid cell lineages, and an increased risk of developing acute myeloid leukemia (AML). It is divided into subtypes based on the percentage of blasts in the bone marrow and peripheral blood, the type, degree, and number of dysplastic cell lineages, the presence or absence of ring sideroblasts (RS), and the presence of specific chromosomal abnormalities. The average age at MDS diagnosis is seventy one years. [1].

Obesity has been linked to a higher rate of myelodysplastic syndromes. (MDS). Obesity is defined as having a body mass index (BMI) of 30 or above, and it is a complex and multifaceted illness with hereditary and environmental predispositions, resulting in a wide range of health consequences that impact approximately 1-3 of the world's people. [3]. Obesity has been related to a variety of illnesses, especially carcinoma [4]. Among fact, obesity has been linked to an increased risk of MDS in the elderly [5].

Adiponectin, an adipocyte hormone, controls metabolic processes and enhances insulin sensitivity [6]. Adiponectin is a 244-amino-acid polypeptide belonging to the complement 1q family of proteins [7]. Adiponectin is a cytokine...
generated by adipocytes that activates particular receptors in a variety of organs via autocrine, paracrine, and endocrine signaling. Adiponectin is involved in the control of cell survival, cell proliferation, and apoptosis [8,9]. The pathophysiological role of adiponectin inside the human body is important, such as in metabolic activities, by acting on the glucose and lipid metabolism in peripheral tissues like skeletal muscles, also in the organ and liver. The development of cardiovascular problems of obesity in adulthood is related with low Adiponectin levels because adiponectin possesses anti-inflammatory, anti-tergogenic and insulin-sensitizing actions [9]. Adiponectin has been linked mainly to the risk of hematological malignancies of the “myeloid” cell line such as childhood acute myeloblastic leukemia, myelodysplastic syndromes (MDS) [10, 11], and myeloproliferative disorders including chronic myelogenous leukemia (CML) [12].

Leptin (LEP), which includes 167 amino acids, was identified in 1994 and contains 16 kDa proteins, which are produced and released by white adipose tissue and act in the brain to regulate energy homostasis. This hormone has been identified to control intakes of food and is produced by adipocytes [13]. Leptin, for example, has a number of vital activities and specialized responsibilities in managing body mass, in addition to bone remodeling, and has a part in regulating body weight and food intake [14]. Leptin exerts there effects through its specific receptor that localized to the cell membrane and present in a variety of hematopoietic cells, such as hematopoietic progenitor cells, erythropoietic, myeloid and lymphoblastic cell lines. Leptin participate in the regulation of normal hematopoiesis, leptin and its receptor have been implicated in hematopoietic malignancies pathogenesis and progression. So it is important to estimation the expression of leptin and its receptor in malignant blood diseases and the effect of leptin/leptin receptor signaling on blast cells of these disorders [15]. This leptin circulation is positively correlated to leptin mRNA and adipose tissue protein levels. This hormone also affects the function of neuroendocrine and energy consumption and has subsequently offered important insight into obesity [16].

Resistin is hormone-based protein, 12 kDa and rich in human macrophages, rich in cysteine polypeptides. It is the founding member of the hormone family of resitine-like molécules (RELM), with 108 amino acid peptides; it circulates as dimeric protein in human blood consisting of two polypeptides of 92 amino acids, [17].

Resistin hormones inhibit insulin's capacity to promote cellular glucose uptake and have a role in obesity, insulin resistance, diabetes [18]. Some researchers feel that resistin is more important in inflammatory processes in humans than it is in insulin resistance, because serum resistin levels correlate better with subclinical inflammation than with insulin resistance. Furthermore, investigations have shown that human resistin alone can increase inflammation [19], whereas other research have shown that human resistin can inhibit inflammation in response to a lethal endotoxin challenge [20].

These inconsistent findings indicate a contextual and disease-specific proinflammatory or antiinflammatory role of resistin. Resistin has a pathogenic function in the promotion of insulin resistance, atherosclerosis, and hypertension in general. Resistin levels have a positive relationship with central/visceral obesity (but not BMI) in humans, and they play a role in proinflammatory processes [21, 22].

II. MATERIALS AND METHODS

Study design: This case-control study was carried out during the period from June 2019 to the December 2020 at the laboratory of DNA in the department of Biology, Faculty of Science, Babylon university / Iraq. Samples of myelodysplastic syndrome were who attended to the Blood Disease Center/ Medical City/ National Center for Research and Treatment of Hematology/ Al-Mustansiriyah University/ Baghdad province, Merjan Teaching Hospital in Babylon province, AL-Hussein Center for Oncology Treatment and Blood Disease / Al-Hussein Medical City/ Karbala province, and AL-Furat AL-Awset for Tumors and Blood Disease, Najaf province, Iraq. In additional, present study was in agreement with ethics was obtained from all Participating patients.

Sample Collection: The samples were females and males with age range (20-83) all patients and control were from the same ethnic group (Arabic). The present study include (75) blood samples which grouped as following: control group (25) samples (15 males, 10 females), patients group including (50) blood samples (29 males, 21 females). Myelodysplastic syndrome was diagnosed by physicians according to hematological and molecular parameters.

Methods

Body mass index (BMI)
BMI was calculated as weight in kilograms divided by height in meters squared. BMI 3 categories—18.5 ≤ 25, 25 ≤ 30, and ≥30—which are consistent with the definitions of normal weight, overweight, and obesity proposed by the World Health Organization (23).

**Estimation Hormones level**

Two ml of blood was collected from each patient put in gel tube and allow the sample to clot for a few minutes at room temperature, followed by serum separation from the clot by centrifugation for 15 minutes at 1000 g. Then the serum was divided into several eppendorf tubes, labeled and stored at -40 °C. Levels of Adiponectin, Leptin, and Resistin was estimated by ELISA assay technique. Reagent preparation and procedure prepared according to manufacturer's description, Adiponectin (Elabscience company, USA) Leptin, and Resistin (Demeditics Diagnostics GmbH, Germany).

**Statistical analysis:** Statistical Package for Social Sciences version 23 (SSPS 23) computer software together with Microsoft Excel 2010 were depended for statistical analysis. The results were expressed as numbers, range and mean ± SD (standard deviation). Results which had a probability value (P value) less than 0.05 were considered statically significant.

**III. RESULTS**

**Subjects Demographic Characteristics**

The results of the present study showed that the males percentage value of myelodysplastic syndrome was more than females percentage value (58% and 42% respectively). Also, the age of the samples in this study were arranged from 20-85 years old men and women (Table1) (Fig.1). Patients and control groups were distributed according to the age category (<=40, 41-61, 51-61+), and gender (Fig.2, 3 respectively).

Table (1): Population characters of myelodysplastic syndrome patients and control groups.

<table>
<thead>
<tr>
<th>Population</th>
<th>Sample size</th>
<th>Age ± SD</th>
<th>Gender</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>50</td>
<td>57.55 ± 20.35</td>
<td>male</td>
<td>29</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>65.3 ± 14.7</td>
<td>female</td>
<td>21</td>
<td>42</td>
</tr>
<tr>
<td>Control</td>
<td>25</td>
<td>57.44 ± 5.85</td>
<td>male</td>
<td>16</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>55.56 ± 2.79</td>
<td>female</td>
<td>9</td>
<td>36</td>
</tr>
</tbody>
</table>

SD= Standard Deviation; No.= Number; %= percent

Figure (1): Percentage rate between male and female of myelodysplastic syndrome patients and control groups.
Figure (2): Age mean ± SE comparison between patients of myelodysplastic syndrome and control groups.

Figure (3): Percentage of age categories for myelodysplastic syndrome patient and control groups.

Figure (4): Percentage of gender for myelodysplastic syndrome patient and control groups.
Body Mass Index In Myelodysplastic Syndrome Patients

The results of body mass index showed a significant decrease \((p<0.05)\) in patients with myelodysplastic syndrome \(20.1960\pm2.38943\) when compared with control group \(24.4440\pm1.65254\) (Figure 5).

![BMI comparison](image)

**Figure 5:** BMI mean ± SE comparison between patients and control groups

### Hormone parameters

The results of the present study showed significant differences in some hormones level between the myelodysplastic syndrome patients and control group as shown in table (3). Adiponectin and Resistin were a significantly increased \((p<0.05)\), in patients of myelodysplastic syndrome compared with control group. While there was no significant differences in leptin hormone between patient and control groups.

Table (2): Mean ± SD of hormones between control and MDS patient groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hormones (ng/ml)</th>
<th>Control group (No.25) Mean ±SD</th>
<th>MDS group (No.50) Mean ±SD</th>
<th>Mean Differences</th>
<th>SE</th>
<th>T-test value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adiponectin</td>
<td>7.1300±1.29408</td>
<td>31.3014±9.21683</td>
<td>24.1714</td>
<td>P 1.30346 C 0.25882</td>
<td>18.1889 8</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td></td>
<td>Leptin</td>
<td>4.6724±1.73042</td>
<td>3.9872±2.27170</td>
<td>-0.6852</td>
<td>P 0.32127 C 0.34608</td>
<td>-1.45103</td>
<td>0.152</td>
</tr>
<tr>
<td></td>
<td>Resistin</td>
<td>138.1240±40.18569</td>
<td>409.5752±91.03437</td>
<td>226.4512</td>
<td>P 12.87420 C 8.03714</td>
<td>14.9207</td>
<td>&lt;0.000</td>
</tr>
</tbody>
</table>

SD= Standard Deviation; SE= Standard Error ; * = P value is significant at \(p \leq 0.05\)

### IV. DISCUSSION

**Subjects Demographic Characteristics**

Myelodysplastic syndrome is typically considered as a neoplasm because of frequent genetic aberrations, limited patient survival, and progression to acute myeloid leukemia (AML) [24]. Moreover, MDS is a collection of disorders with a wide range of outcomes. Some patients have a near-normal life expectancy without severe clinical symptoms and signs, while others die of complications associated with bone marrow failure, including infection, hemorrhage, and iron overload [25].
Aging is one of the most major risk factors for myelodysplastic syndrome development. Exonic mutations accumulate in hematopoietic stem cells as a result of mistakes in DNA replication and spontaneous mutations from normal metabolic byproducts (e.g., transformation of cytosine to thymidine via oxidative deamination from free radicals) [26]. Around 10% of people over the age of 70 have clonal mutations in genes linked to myeloid neoplasia, such as DNMT3A, TET2, and SF3B, and these people have a 0.5 percent to 1% probability of accumulating more mutations each year, leading to MDS or another hematological neoplasm [27]. The prevalence of MDS increases with age, with the majority of cases identified after the age of 60 [28]. MDS is more commonly diagnosed in males excepted in 5q- syndrome, which is more common in females [29].

The current study came in agreement with study conducted by Wang et al. (2019)[30], who demonstrated that the mean age of MDS patients at diagnosis was 76 years (range of interquartiles, 66-83 yrs). At the time of diagnosis, female patients were not statistically different from male patients (median 77 years vs. 75 years, respectively). The number of male and female patients gradually increased as they grew older. When compared to patients in the ≥60s, 60s-69s, and 70s-79s age groups, patients aged 80 had the highest prevalence of MDS. MDS cases in the general population grew substantially as people became older. Men, on the other hand, were found to have more yearly instances of MDS than women. The study also found that survival varied as a function of gender across the MDS histological subtypes

Moreno Berggren et al.[31], discovered that gender has a substantial influence on the incidence rate of MDS patients, as well as difference in survival across genders within subgroups by age at diagnosis, race, marital status at diagnosis, and MDS subtypes. Male patients showed considerably lower lifespan compared to females, which Asian and European MDS cohorts confirmed. Male patients with MDS have greater death rates, which might be explained by the presence of more comorbidities at the time of diagnosis [32]. Other possible causes for male MDS patients’ lower survival include the connection with molecular abnormalities such as quicker methylene aging and shorter telomeres[33], both of which are frequently associated with shorter survival [34]. Females showed higher levels of cytidine deaminase (CDA) than male MDS patients. Decitabine (DAC) and azacitidine (AZA), two 5-methylated cytidine analogs, may be rapidly inactivated by CDA [35].

Body Mass Index In Myelodysplastic Syndrome Patients

The current measure for measuring anthropometric height/weight features in adults is the body mass index (BMI). It's commonly utilized as a risk factor for the onset or occurrence of a variety of health problems. Because of its widespread acceptability in identifying distinct categories of body mass as a health problem, the BMI has been beneficial in population-based investigations. However, it is becoming more common [36].

Obesity, defined as an abnormal or excessive buildup of body fat, has been linked to an elevated risk of cancer development in the bone marrow and other organs. Obesity may have an inductive or selective impact on a malignant clone due to its metabolic, endocrine, and inflammatory effects [37]. While the influence of excess body fat on patient survival at the time of cancer diagnosis appears to be well-defined, the impact of excess body fat on patient survival at the time of cancer diagnosis appears to be less well-defined [38] show that obesity increases survival in a mouse model of myelodysplastic syndrome (MDS) in the absence of therapy. They provide biological explanations for this survival advantage. A connection has been discovered between the fatty acid binding protein FABP4 and a cellular pathway that leads to DNA damage caused by saturated fatty acids that accumulate in the blood of obese persons [39].

The use of Ob/Ob mice in which the Lep gene is disrupted, as recognized by Kraakman et al.,(2018) [38], is a drawback of their work. Leptin levels are higher in overweight people, and this pro-inflammatory adipokine has been demonstrated to impact tumor cell activity and its microenvironment. A proliferative and anti-apoptotic effect of leptin has also been depicted on AML blast cells [40]. As a result, the lack of leptin in the tested model may modify the natural history of the illness in an overweight environment, necessitating confirmation in a different model of obesity where leptin secretion is maintained. The discovery that the lack of leptin causes increased survival in MDS mice might lead to the creation of leptin antagonists, including leptin analogs [41]. Poynter et al.(2016) [42] discovered strong correlation with overweight and MDS in female.

Obesity and hematologic malignancy are linked biologically, although the mechanism has yet to be discovered. Changes in the metabolism of endogenous hormones such sex steroids, insulin, insulinlike growth factors, leptin, adiponectin, and fetuin-A have been postulated [43]. Insulin-like growth factor-1 (IGF-1) appears to be particularly
relevant as it is known to increase in response to obesity-related insulin tolerance[44]. In both myeloid and lymphoid leukemia cell lines, it has mitogenic activity. Leptin levels, which are also higher in obese people, have been found to impact hematopoietic cellular proliferation as well as myeloid leukemia cell lines [42]. Multiple obesity-related metabolic changes, potentially in the context of changes in the bone marrow microenvironment, are most likely to blame for the link [45]. Adipocytes have been found to adversely affect hematopoiesis and their quantity in the bone marrow rises with age [46].

Overweight and obesity in MDS have been documented in two prior studies and one case control study [11, 5,47]. The risk estimations in the million women research were based on a 10 kg/m2 rise in BMI, so they are not directly comparable to ours; nonetheless, the findings do suggest a substantial increase in MDS risk in obese women [47].

Hormone Parameters

Adipocytes secrete active biological molecules, mainly including leptin, resistin and adiponectin [48]. Adipose tissue is no longer thought of as a passive energy storage organ, but rather as an active endocrine organ that secretes hormones that regulate physiological and pathological processes like appetite, insulin sensitivity and resistance, endocrine function, inflammation, hematopoiesis, immunity, and angiogenesis [49]. Adiponectin, leptin, and resistin are examples of proinflammatory and anti-inflammatory adipokines, as well as adipokines and cytokines such as TNF- and IL-6 [50].

Dalamaga et al., (2007) [10] discovered that elevated serum adiponectin levels were associated with a reduced risk of MDS before and after adjusting for age, gender, BMI, and serum leptin levels. These results confirm a previously hypothesis that adiponectin is an adipocyte-secreting hormone that causes apoptosis and suppresses myeloid cell growth [51], in addition, they agreed with a similar research that looked at adiponectin levels in individuals with myeloblastic leukemia. [52].

Adiponectin, which is expressed specifically in human white adipose tissue adipocytes and is inversely related to adiposity, may suppress proliferation of myelomonocytic progenitors by inducing apoptosis, which is mediated primarily by downregulation of Bcl-2 expression and activation of caspase group apoptotic enzymes. Exogenous adiponectin treatment inhibits the development of myelomonocytic leukemia cells. Furthermore, adiponectin inhibits macrophage precursor development and suppresses phagocytosis caused by targeted reduction of TNF-α transcription [ 51]. As a result, the TNF-overproduction in the bone marrow may be caused by decreased adiponectin levels seen in MDS. Recent research has found that progenitor cells in the bone marrow of MDS patients overexpress IL-6 and TNF. Increased TNF-production appears to lead to excessive intramedullary progenitor cell death and increased bone marrow angiogenesis, both of which are thought to be important cofactors in MDS and progression to AML (Flores- [53,54]. Early MDS pathogenesis is characterized by intrinsic apoptosis, which explains why peripheral cytopenia occurs despite hypercellular bone marrow [55]. Furthermore, adiponectin's anti-inflammatory properties include the suppression of IL-6 synthesis, which is mediated in part via nuclear factor-κB inhibition[50,56].

Leptin, a 16-kDa protein expressed by the obese gene, is mostly generated by white adipocytes and has a positive relationship with body fat accumulation [49]. In accordance with the findings of Dalamaga et al.,(2007) [10] the current investigation discovered that mean leptin levels were virtually comparable in MDS patients and controls. Another research looked at a significantly smaller number of patients (60 patients and controls with MDS and AML) [40]. Only individuals with leptin levels in the normal range were shown to have a reduced risk in the third quartile. Importantly, after controlling for age, gender, and BMI, decreased leptin concentrations were seen in low-risk MDS patients with a normal or excellent prognostic karyotype. According to recent research, leptin increases leukemic cell growth in vitro. The leptin receptor (OB-R, mostly the short isoform), which has homologies to cytokine and hematopoietic growth factor receptors, is expressed in the majority of AML, as well as secondary AML and MDS, in normal CD34+ progenitor cell [10]. Therefore, leptin generated by hematopoietic stem and/or progenitor cells, and its OB-R receptors might operate as a growth factor/receptor-linking system in paracrine. Leptin also uprules the phagocytic function and production of cytokine, for example, in monocytes and macrophages TNF (early), IL-6(late) and IL-12 [49].

In vivo leptin has proven to be a permissive factor in human regulation of the endocrine and immunological function in particular. All such activity in humans is thus confined largely to leptin-sensitive people (i.e. leptin less than 10–15 ng/ml), whereas in leptin-sustaining, normal neuroendocrine and immunological function are not affected by
leptin-sustained subjects. Leptin in human beings further, the biological activities of leptin are resistant with hyperleptinemic obese people and bone marrow cells of subjects with extremely high leptin circulation owing to leptin resistance might react to circulation of leptin in a way similar to that of leptin deficiency [57,58].

Compared with control group, the result of resistin was considerably higher in patients with MDS. Resistin was first found as an insulin resistance hormone, however further research of mouse and human have shown contradicting findings suggesting the physiological role of resistin might primarily be linked to inflammation [59].

The current study contradicts the findings of Dalamaga et al., (2008) [11], who found that MDS patients have decreased levels of resistin, most likely as a compensatory reaction to the elevation of other inflammatory markers associated with myelodysplasia. Resistin, an adipokine of resistin-like molecules (RELM), was first shown to produce insulin resistance or liver sensitivity to insulin without altering the sensitivity of the peripheral insulin [60]. Human data, on the other hand, is debatable. Resistin is in humans, unlike mice, shown at lower adipocyte levels, but at higher blood-monocytes circulating [61]. Furthermore, increased serum resistin levels in obese or insulin-resistant patients have not been detected in human investigations (Lee et al.,2003) [59]. Resistin is mostly recognized as an inflammatory factor linked to TNF-a and IL-6, and it has been shown to upregulate a number of adhesion molecules and cytokines [60].

A decrease in blood leptin level in acute leukemia patients was observed by Fantuzzi and Faggioni, (2000) [62]. They indicate that the reason is very complex and associated with serious sickness, changed energy balance, and consequences of disease. Although elevated blood leptin levels and OB-R expression were discovered in a wide variety of cancers with associated obesity, reports have also been made that the lymphoid and myeloid malignancies had decreased serum leptin levels.

Aref et al. (2013) [63] found that serum leptin levels in AML were substantially lower than normal controls (P=0.00). The decrease in leptin concentrations in AML patients has been described by Wallace et al.(1998) [64], while normal fat tissue has been used to guard from the body the hunger for weight-gaining and weight loss avoidance. This might be because of the failure to regulate feedback pathways in hematic malignancy patients. According to Tabe et al. (2004) [65], leptin has been found to increase AML cell proliferation while simultaneously having an anti-apoptotic impact. It elevates the number of progenitor cells and spontaneous AML blast growth, as well as the release of IL-1beta, IL-6, TNF-alpha, and granulocyte-macrophage colony stimulating factor by AML blast. Pamuk et al.[66] was discovered a greater amount of resistin in lymphoma patients, suggesting that resistin may be linked to disease etiology, immunological change, and the inflammatory response during disease progression. Resistin levels were also higher in multiple myeloma patients who took corticosteroids more intensely and often than others, indicating a steroid function. The amount of resistin is affected by steroid use [67].

V. CONCLUSION

Estimation levels of some hormones such as Adiponectin, Leptin, and Resistin accompanied with demographic characteristic and body mass index may use as useful marker for detecting Myelodysplastic syndromes.

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