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Association between Immune Competence and Metabolic Parameters in Obesity

[1]Hala Abd El-Hafez and [2]Sherin M. Abd El-Aziz
[1]Deparment of Internal Medicine,
[2]Department of Clinical Pathology, Faculty of Medicine, Mansoura University, Egypt

Corresponding Author: Hala Abd El-Hafez, Deparment of Internal Medicine, Faculty of Medicine, Mansoura University, Egypt

ABSTRACT

The increased risk of morbidity and mortality in obesity as a result of infections is an emerging health concern which suggests that obesity may affect immune competence. Thus aim of this study was to assess circulating immune cell parameters including T lymphocyte subsets and Natural killer (NK) cells in obese subjects and their relations to metabolic factors. Thirty obese and 20 lean subjects were included in the study. Recorded data involved BMI, Waist Circumference (WC), blood pressure, HOMA-IR, White Blood Cell (WBC) count, Absolute Lymphocyte Count (ALC),CD4 and CD8 T cell count and frequency and natural killer cell frequency (CD56%). CD4, CD8 and CD56 expressions were measured by flow cytometry. Compared with control, obese group had significantly higher CD4%, CD4 count, WBC count and ALC, and significantly lower CD8%, CD8 count and CD56%. In obese subjects, both CD4% and CD4 count had significant positive correlation with BMI ($p = 0.005$, $p<0.001$, respectively) and WC ($p = 0.014$, $p = 0.01$, respectively). CD4 count had significant positive correlation with systolic blood pressure ($p = 0.015$), HOMA-IR ($p = 0.021$) and triglycerides ($P=0.02$). CD8 count in obese subjects had significant negative correlation with BMI ($p = 0.03$), WC ($p = 0.018$), HOMA-IR ($p = 0.035$) and triglycerides ($p = 0.025$), while CD56% had significant negative correlation with BMI ($p = 0.025$), HOMA-IR ($p = 0.04$) and triglycerides ($p = 0.029$). Obesity is associated with alterations in circulating immune cells in the form of increased WBC, total lymphocyte and CD4 levels with decreased CD8 and CD56 levels, such alterations are related to obesity measures and metabolic factors, so immune alterations may identify obese at risk of infection and metabolic disease who should be targeted for weight loss strategies. Further studies are needed to determine the role of weight loss in the improvement of immune function in obese individuals.

Key words: Obesity, T lymphocyte, natural killer, metabolic factors, flow cytometry

INTRODUCTION

The immune response involves innate and adaptive immunity. T lymphocytes play crucial roles in adaptive immune response and they can be categorized as helper and cytotoxic T lymphocytes. Helper T lymphocytes (CD4) express CD4 protein on their surface, they regulate immune response by producing cytokines that activate macrophages, natural killer cells and B lymphocyte. Cytotoxic T lymphocytes (CD8) express CD8 glycoprotein on their surface and they mediate cytotoxic targeting and killing of virally infected and tumor cells and are also implicated in transplant rejection (Janeway *et al*., 2005). Natural Killer (NK) cells are identified by surface markers CD56

and CD16, they constitute a special kind of lymphocytes that bridge the adaptive immune system with the innate immune system and play an important role in body defense against infection and malignancies, they exhibit cytolytic activity against a variety of allergenic targets in a non-specific, contact-dependent, nonphagocytic process which does not require prior sensitization to antigen. NK cells share several properties with conventional cytotoxic T cells (CTL), including similar mechanisms for cytolysis (Scott and Trinchieri, 1995).

Obesity is an increasing worldwide health problem which has been linked to many chronic diseases including type 2 diabetes mellitus, cardiovascular disease and cancers which represent most of the obesity related morbidity and mortality (Flegal *et al.,* 2007; Barnett *et al.*, 2002) these complications are related to obesity associated chronic inflammation in obesity which is attributed to alterations in metabolic and secretory activities of adipocytes, together with chronic inflammation within fat itself (Schelbert, 2009; Hotamisligil, 2006) . In addition, obese individuals are more liable to certain infections (Falagas and Kompoti, 2006; Dossett *et al.*, 2009) and it was reported that infections cause significant increase in mortality among obese subjects (Flegal *et al.,* 2007). These data give rise to the hypothesis that obesity may affect immune competence, however the exact mechanisms for this are not fully understood and the previous data are controversial for some immune parameters including immune cell levels in obese (O'Rourke *et al.*, 2005; Tanaka *et al.*, 2001).

Immune cells including T lymphocytes and NK are cells with high metabolism and turnover (Marti *et al.*, 2001; Rudd *et al.*, 2001; Maclver *et al.*, 2008), so their proliferation and subsequently their roles in immune response can be disturbed by nutrient excess in obesity which may affect immunocompetence in obese. This research hypothesized that immune cell counts may be altered in obese in relation to metabolic parameters. Aim of this study was to assess immune cell levels in particular T lymphocyte subsets and NK in obese subjects and their relations to metabolic parameters.

MATERIALS AND METHODS

The present study included 30 obese subjects (BMI = 30) (14 men and 16 women with mean age of 35.2±6.87 years) and 20 non obese (BMI = 20-24.9) healthy control subjects (9 men and 11 women with mean age of 34.73±6.19 years).Obese subjects were selected from attendants of Obesity clinic of Specialized Medical Hospital, Mansoura University. The study was approved by local Research Ethics Committee and each participant gave informed consent before inclusion in the study.

Exclusion criteria included smoking; pregnancy; advanced liver, pulmonary, or renal disease; known malignancy; diabetes mellitus; current medications (antihypertensive, lipid lowering, hormones and anti-inflammatory),acute or chronic inflammatory or infectious diseases, recent trauma, abnormal WBC count, positivity for hepatitis B and C.

Complete clinical examination was performed for all participants. Height was measured to the nearest 0.5 cm and body weight to the nearest 1 kg while wearing light clothing and no shoes. Body Mass Index (BMI) was calculated by dividing weight in kilogram by height in meters squared. Waist Circumference (WC) was taken at the level midway between the lowest rib margin and the iliac crest at the end of a normal expiration. Blood Pressure (BP) was recorded in the sitting position after 5 min rest, the mean of two measurements was used.

Laboratory methods: From all patients and controls, fasting 7 mL of peripheral blood were aspirated and divided into two tubes; 2 mL were collected on EDTA tubes and immediately

transported to the flow cytometry immunophenotyping laboratory, Clinical Pathology Department, Faculty of Medicine, Mansoura University; for surface antigens staining; and the remaining 5 mL were composed on dry tubes for biochemical analysis. Blood glucose and lipid profile were done first then the remaining serum was stored at -70°C till time of assay of serum insulin.

Flow cytometric analysis: For surface antigen staining, the received samples were lysed using home made lysing solution, washed with Phosphate Buffered Saline (PBS) once or twice until complete RBCs lysis and then resuspended in appropriate amount of PBS. The cells were stained with different fluorescently labeled monoclonal antibodies (mAbs) according to manufacturer recommendations (Dakocytomation, Denmark and Beckman Coulter, France). In brief, cell suspension was mixed with the fluorescently labeled monoclonal antibody (mAb) and incubated in the dark at room temperature for 30 min. Washing with PBS containing 2% bovine serum albumin was done twice and the billet was resuspended in PBS and analyzed immediately on flow cytometry. The mAbs which were used were in different form of fluorochromes; namely fluorescein isothiocyanate (FITC), phycoerythrin (PE) and phycoerythrin-cyanine 5 (PeCY5). Different mAbs against the following surface antigens were used: CD4, CD8 and CD56 .The immunophenotyping was performed on EPICS-XL flow cytometry (Coulter, Miami, Fl). The cells were analyzed with the most appropriate lymphocyte gate using the combination of forward and side scatters. An antigen was considered positive when the expression is at least 20% of the gated cells. White blood cell count and lymphocyte were determined on whole blood using a Coulter cell counter. Absolute numbers of CD4+and CD8+cells were calculated by multiplying percentages by the Absolute number of lymphocytes per microliter (μL^{-1}).

Biochemical analysis: Quantitative determination of blood glucose was done by glucose oxidase method (Trinder, 1969) using Spinreact kit, made in SPAIN. Quantitative determination of insulin was done by enzyme amplified sensitivity immunoassay using INS-EASIA kit (Biosource, Europe, Belgium). The assay used monoclonal antibodies directed against distinct epitopes of Insulin (Temple *et al.*, 1992). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated with the formula: HOMA-IR = (fasting insulin $\mu U\ mL^{-1}$) × fasting glucose (mmol/L)/22.5 (Matthews *et al.*, 1985). Serum total cholesterol was done by CHOD-POD using enzymatic colorimetric method (Naito and Kaplan, 1984). Serum triglycerides (TG) were done by GPO-POD using enzymatic colorimetric method (Bucolo and David, 1973) via Spinreact kit made in SPAIN. Serum HDL cholesterol was done by precipitation phosphotungstic acid method (Naito 2003) using Elitech kit, made in FRANCE. Serum LDL cholesterol was calculated as follows: LDL-C=TC-(HDL-C +TG/5) (Friedewald *et al.*, 1972).

Statistical methods: Statistical analysis of data was done by using SPSS program "statistical package for social science". The parametric data were presented in the form of mean and standard deviation (M±SD). T-test was used for comparison of quantitative data of two groups. For comparison between qualitative data, Chi-square test was used. Pearson correlation co-efficiency test was done to study the association between different variables. Significance was considered when p-value equal to or less than 0.05 ($\leq$0.05).

RESULTS

No significant difference as regard age, sex, or diastolic blood pressure between lean and obese subjects. BMI, WC, fasting insulin, HOMA-IR, total cholesterol and triglycerides were significantly

Table 1: Clinical and biochemical characteristics of study groups

Characters	Obese subjects (n = 30)	Lean controls (n = 20)	p-value
Age (years)	35.2±6.87	34.73±6.19	0.8
Male/female	14/16	9/11	0.9
BMI (kg m^{-2})	41.3±5.7	22.6±2.3	<0.001
WC (cm): male	119.5±9.3	90.8±7.8	<0.001
female	107.5±8.1	78.6±7.5	<0.001
SBP (mmHg)	127±15	120±9	0.045
DBP (mmHg)	82±10	77±9	0.075
Fasting glucose (mg dL^{-1})	102.1±22.8	91.8±14.5	0.06
Fasting serum insulin (µU mL^{-1})	11.3±4.8	8.5±3.4	0.02
HOMA-IR	3±1.85	1.9±1.05	0.011
T cholesterol (mg dL^{-1})	187.8±28.2	171.8±21.3	0.03
LDL-C (mg dL^{-1})	114.2±26.1	102.8±18.5	0.08
Triglycerides (mg dL^{1})	130±33.2	112±25.4	0.04
HDL-C (mg dL^{-1})	46.2±5.7	49.3±5.6	0.063

Table 2: Immune parameters in obese and control groups

Parameter	Obese (n = 30)	Lean (n = 20)	p-value
WBC count ×109 L 1	7.41±1.18	6.67±1.21	0.041
Lymphocyte (%)	34.33±5.01	31.45±3.94	0.03
ALC ×109 L 1	2.3503±0.2672	2.0575±0.2969	0.001
CD4 (%)	45.13±4.8475	42.0±4.3286	0.024
CD4 count ×109 L 1	1.0528±0.1545	0.8734±0.08806	<0.001
CD8 (%)	21.7±4.23	25.55±2.41	<0.001
CD8 count ×109 L 1	0.4610±0.08389	0.5224±0.09022	<0.001
CD56 (%)	7.7±1.44	12.3±1.92	<0.001

higher in obese compared to lean subjects (p<0.001, <0.001, 0.02, 0.011, 0.03 and 0.04, respectively) (Table 1).

Compared with lean controls, obese subjects had significantly increased WBC count (7.41±1.18 vs. 6.67±1.21, p = 0.041), lymphocyte% (34.33±5.01 vs. 31.45±3.94, p = 0.03) and Absolute Lymphocyte Count (ALC) (2.3503±0.2672 vs. 2.0575±0.2969 p=0.001) than lean controls. Also higher CD4% (45.13±4.84 vs. 42.0±4.32, p = 0.024) as well as CD4 count (1.0528±0.1545 vs. 0.8734±0.08806, p<0.001) than lean persons. CD8% (21.7±4.23 vs. 25.55±2.41, p<0.001), CD8 count (0.4610±0.0838 vs. 0.5224±0.09022, p<0.001) and CD56% (7.7±1.44 vs. 12.3±1.92, p<0.001) were significantly lower in obese than in lean subjects (Table 2).

In obese subjects, WBC count and ALC showed significant positive correlation with BMI (r = 0.450; p = 0.013 and r = 0.441, p = 0.015, respectively), WC (r = 0.402; p = 0.028 and r = 0.363, p = 0.049, respectively), systolic blood pressure (r = 0.397; p = 0.031 and r = 0.380, p = 0.041, respectively), HOMA-IR (r = 0.423; p = 0.02 and r = 0.403, p = 0.027, respectively) and triglycerides (r = 0.542, p = 0.002 and r = 0.402, p = 0.028, respectively) (Table 3, 4).

CD4% in obese subjects was significantly correlated with BMI (r = 0.497; p = 0.005) and WC (r = 0.445; p = 0.014), also CD4 count was significantly positively correlated with BMI (r = 0.617; p<0.001), WC (r = 0.464; p = 0.01), SBP (r = 0.440; p = 0.015), HOMA-IR (r = 0.420; p = 0.021) and triglycerides (r = 0.422; p = 0.02) (Table 3, 4).

In obese subjects CD8 count showed significant negative relation to BMI (r = -0.399; p = 0.03),WC (r = -0.430; p = 0.018), HOMA-IR (r = -0.390; p = 0.035) and triglycerides (r = -0.410;

Table 3: Correlation of immune cell parameters with anthropometric measures in obese subjects

Parameter	BMI	WC
WBC couut		
r	0.450	0.402*
p-value	0.013	0.028
ALC		
r	0.441	0.363*
p-value	0.015	0.049
CD 4%		
r	0.497**	0.445*
p-value	0.005	0.014
CD 4 count		
r	0.617*	0.464*
p-value	<0.001	0.01
CD 8%		
r	-0.370	-0.348
p-value	0.047	0.059
CD 8 count		
r	-0.399	-0.430*
p-value	0.03	0.018
CD 56%		
r	-0.410*	-0.328
p-value	0.025	0.077

*, **Significant at p≤0.05 and 0.01, respectively

Table 4: Correlation of immune cell parameters with metabolic variables in obese subjects

Parameter	TG	SBP	DBP	HOMA-IR	HDL-C
WBC count					
r	0.397*	0.242	0.423*	0.152	0.542*
p	0.031	0.196	0.02	0.253	0.002
ALC					
r	0.380*	0.056	0.403*	0.177	0.402*
p	0.041	0.767	0.027	0.244	0.028
CD 4%					
r	0.355	0.179	0.278	0.194	0.327
p	0.055	0.345	0.137	0.208	0.077
CD 4 count					
r	0.440*	0.107	0.420*	0.0.269	0.422*
p	0.015	0.573	0.021	-0.17	0.02
CD 8%					
r	-0.21	-0.044	-0.29	0.375	-0.284
p	0.267	0.81	0.12	-0.152	0.13
CD 8 count					
r	-0.134	-0.14	-0.390*	0.423	-0.410*
p	0.39	0.461	0.035	-0.199	0.025
***CD 56%**					
r	-0.139	-0.06	-0.382*	0.291	-0.40*
p	0.463	0.76	0.04	0.0291	0.029

*, **Significant at p≤0.05 and 0.01, respectively

p = 0.025), CD56 had significant negative correlation with BMI (r = -0.410; p = 0.025), HOMA-IR (r = -0.382, p = 0.04) and triglycerides (r = -0.40, p = 0.029) (Table 3 and 4).

DISCUSSION

With the increasing obesity rates, increased risk of infections and cancers is an emerging health concern added to the previously established obesity related comorbidities (Falagas and Kompoti, 2006; Dossett *et al.*, 2009; Calle *et al.*, 2003; Owiredu *et al.*, 2009). Therefore, reduced immune competence in obese is suggested although responsible mechanisms are unknown, however, this may be linked to negative effect of nutritional or metabolic or status on immune cells (Maclver *et al.*, 2008).

In the present study obese subjects had higher WBC counts and lymphocyte% with correlation with BMI. Present results are consistent with previous finding of a relation between (within normal range) both total and differential WBC counts and obesity (Kim *et al.*, 2008), White blood cell and lymphocyte counts were found to increase with increasing BMI and decrease after weight loss (Dixon and O'Brien, 2006), Obesity is associated with increased cytokines which can induce leukocyte differentiation and elevated leukocyte count which produce more cytokines, this increase in WBC count even within the normal range may predispose to increased cardiovascular disease risk in obese (Horne *et al.*, 2005).

T lymphocytes play crucial roles in adaptive immune response and can be categorized according to their functions and phenotypes as helper T lymphocytes (CD4+) and cytotoxic T lymphocytes (CD8+). In the present study ,we found higher frequency and number of CD4 cells in obese subjects compared to lean controls with association between CD4 cell% and count in obese and BMI. In support, others found increase in CD4 cells in morbidly obese women compared to normal weight controls (O'Rourke *et al.*, 2005); Also, being overweight or obese was found to be associated with higher CD4 count (Womack *et al.*, 2007), in contrast another study demonstrated reduced CD4 in obese compared to non obese (Tanaka *et al.*, 2001). The increase of CD4 cells in our obese subjects is most likely can be explained by increased plasma levels of cytokines that stimulate T lymphocyte proliferation ,in particular leptin, can influence the proliferation of immune cells especially Th1 CD4 subsets (Assal *et al.*, 2007; Otero *et al.*, 2006).

In obese subjects, we found significant associations of immune cells including total WBC, lymphocyte% and CD4 counts with HOMA-IR, SBP and triglycerides. Our findings support the link between immune system and metabolic imbalance (Wellen and Hotamisligil, 2005; Nikolajczyk *et al.*, 2011) and in agreement with previous finding of an association between higher total WBC count with insulin resistance in non-diabetic subjects at high metabolic risk (Hanley *et al.*, 2009) as well as the association of features of metabolic syndrome with counts of total WBC, lymphocyte% (Kim *et al.*, 2008) and lymphocyte subpopulations, which suggest the involvement of altered immune state in pathogenesis of atherosclerosis (Tanigawa *et al.*, 2004). Recent data point to the emerging role of T helper cells cytokines in obesity associated inflammation and insulin resistance (Pacifico *et al.*, 2006; Surender *et al.*, 2011). So, It can be suggested that increased levels of CD4 cells in obese may lead to metabolic imbalances by playing a role in obesity associated low grade inflammation, which appears not to be specific for pathogen clearance but instead leads to insuhn resistance and metabolic disease (Hotamishgil, 2006). So alterations in CD4 lymphocyte subset may identify obese individuals at risk of infection as well as cardiovascular disease.

On the other hand, previous data suggest that alteration of metabolic parameters could affect the functional capacity of T cell to respond to infection or to remain as memory cell (Pearce, 2010). Previous data suggest that increased inflammatory signals during primary infection (Smith *et al.*, 2007) together with chronic inflammatory state and altered antigen presentation in

obesity could affect the balance between effector and memory T cells, rendering the obese host susceptible to re-infection (Jameson and Masopust, 2009; Karlsson and Beck, 2010).

In the present study, obese subjects had less CD8 cell frequency, CD8 count and CD56% than lean controls with correlation between CD8 count, CD56 frequency in obese and BMI. This is consistent with previous data showing decreased frequency of CD3+CD8+ cells or cytotoxic T cells (O'Rourke *et al.*, 2005; Lynch *et al.*, 2009) and NK cells (Lynch *et al.*, 2009; O'Shea *et al.*, 2010) in obese. In contrast, one study found that morbid obesity was associated with a higher CD8 count (Womack *et al.*, 2007). Obesity has been associated with decreased thymic output of naive T cells in middle aged obese persons (Yang *et al.*, 2009). Decreased expression of CD8 and CD56 in obese could be induced by altered immune cells development and/or differentiation or lowered apoptotic threshold (O'Rourke *et al.*, 2005; Lynch *et al.*, 2009). Since cytotoxic T and NK cells play crucial roles in body defense against infection and in tumor surveillance, decreased their levels may potentially increase susceptibility to infection and cancer in obese subjects.

Another important finding in the present study is the presence of inverse relations of both CD8 count and CD56 frequency in obese subjects with metabolic factors including HOMA-IR and triglycerides, this goes in line with the previous finding of decreased cytotoxic T cells and NK cells in metabolically unhealthy obese compared to healthy obese (Lynch *et al.*, 2009) and suggests a link between altered levels of both CD8 and CD56 and obesity related metabolic disease, although the exact mechanism is not clear. However, disturbed metabolic state could influence T cell proliferation (MacIver *et al.*, 2008). At the same time, reduction in NK cells may disturb immune response which promote Th-1 cytokines that promote inflammation (Li *et al.*, 2005) that is implicated in obesity related metabolic disease (Hotamisligil, 2006).

In summary, Obesity is associated with alterations in circulating immune cells in the form of increased CD4, lymphocyte and total WBC levels and decreased CD8 and CD56 expression levels, such alterations are related to obesity measures and metabolic factors which suggest that immune alteration may identify obese at risk of infection and metabolic disease who should be targeted for weight loss strategies. Further studies are needed to determine the impact of these immune alterations on long term morbidity and mortality, as well as the role of weight loss in the improvement of immune function in obese individuals.

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