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Original article

Lymphocyte subtype dysregulation in a group of children with simple obesity

Background: *Obesity as a global public health problem is increasing in prevalence. Reports showed that obese children are more liable to infection than lean ones; it was claimed that obese subjects have altered peripheral blood total lymphocyte counts in addition to reduced lymphocyte proliferative response to mitogen stimulation as well as dysregulated cytokine expression.* ***Objective:*** *This study aimed to evaluate the effect of childhood obesity on cell mediated immunity as indicated by peripheral blood lymphocyte phenotyping.* ***Methods:*** *We enrolled 30 school-aged children (mean age 10±3.27 years). They comprised two groups; 20 obese children with a mean body mass index (BMI) of 39.2± 12.5 and 10 matched control subjects with mean BMI of 18.4± 1.9. They were subjected to detailed anthropometric evaluation including weight, height, and waist hip ratio in addition to calculation of BMI, complete blood counting, and flow cytometric assessment of T-helper (CD4), T-cytotoxic/suppressor (CD8), and natural killer (CD56) cell counts .* ***Results:*** *The absolute lymphocyte (CD3) and natural killer cell (CD56) counts were comparable in both groups. However, the CD4%, CD8%, CD4/CD8 ratio were significantly lower in the obese children (p=0.02, 0.03, 0.015 respectively). A significant negative correlation could be elicited between the CD4 count and bodyweight, BMI, and hip waist ratio (p = 0.00); the same was observed for CD4/CD8 ratio (p = 0.00). On the contrary, CD8 correlated positively to the bodyweight, BMI, and waist hip ratio (p = 0.00 for each) .* ***Conclusion:*** *Obesity has an impact on lymphocytic subset counts and further studies are needed to assess its effect on their function.*

Keywords: obesity, children immunology; CD markers; lymphocytes; BMI

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INTRODUCTION

Obesity is defined as excessive accumulation of body fat and is diagnosed by calculating the body mass index (BMI).[1] BMI is interpreted using centile charts or by calculation of Z score.[2] Obesity is diagnosed as having a BMI above the 95th while overweight is diagnosed as having BMI above the 85th- percentile.[3] The rising prevalence of obesity in children is an alarmingly problem.[4] It has been increasing in industrialized and developing countries over the past few decades.[5] In Egypt, the prevalence rate of obesity among children and adolescents was reported to be 14.7% and 15.08% for males and females respectively.[6]

Although obesity is primarily a metabolic disease, immunological aberrations have been reported and this is expected to result in increased mortality and morbidity.[8] Immunological aberrations noted were lymphopenia,[9] decreased CD8+ T cell counts, increased or decreased CD4+T cell counts,[10] reduction in NK cell cytotoxicity, lower expression of antiviral cytokines specially type I interferons[9] and lower splenic mitogenic response.[11] Leptin secreted by adipose tissues normally stimulates the development of myeloid cells, activate macrophages, dendritic cells, and NK cells[12] by promoting production of IL-2 and gamma interferon,[14] and influence the proliferation of T lymphocytes and cytokine production.[12] However, these cells become refractory to its action in spite of having high leptin levels in obese children.[13]. The study done by Verwaerde et al[15] showed that obese patients who are on a diet rich in saturated fat had an impaired response of both naive and memory T-cells.

This study is aimed to evaluate the effect of childhood obesity on cell mediated immunity as defined by T cell subsets' expression.

METHODS

Study design

We conducted a cross sectional controlled study on school aged children (6-12 years old) visiting the Pediatric Outpatient Clinic of Ain Shams University. An informed consent was taken from the parents or care givers of children prior to enrollment. They were divided into 2 groups; Group A comprised 20 obese children and Group B comprised 10 non-obese children. Obesity was defined as c BMI above the 95th centile for age. Children with endocrinal diseases or any signs suggesting primary immunodeficiency were excluded from the study.

Study measurements

All children were subjected to clinical history taking including socio-demographic data, history of associated chronic illness such as diabetes mellitus (DM) and hypertension. Obese children and their caregivers were asked to fill an obesity sheet which highlighted frequency of infections. Complete anthropometric measurements using standardized equipment, and following the recommendations of the WHO were conducted. Three consecutive measurements were taken and when the differences between the readings were acceptable, the mean was recorded. Height was measured using stadiometer. Weight was measured using Seca scale Balance.

BMI for age was calculated as weight (kg) divided by height squared (m2). Body mass index was assessed using the CDC charts16 of BMI for age which considered the child to be overweighed if above the 85th- percentile and obese if above the 95th-percentile. Waist circumference was measured at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest, using a stretch resistant tape. Hip circumference was measured from around the widest portion of the buttocks, with the tape parallel to the floor. Then waist to hip ratio was calculated to assess the abdominal obesity which is defined as a waist-hip ratio above 0.90 for males and above 0.85 for females according to 2008 waist centiles 17

Laboratory investigations: Complete blood picture and absolute lymphocyte count. This was performed on blood samples collected in EDTA tubes on sysmex KX-2IN automated hematology analyser (Beckman Coulter Inc, 22 ratio justeolivier, 126 Nyon-Surtzer Land) (Chernecky et al., 2001). Differential counts were done using Leishman-stained blood smear by counting at least 200 cells. The percentage and absolute count of CD4+, CD8+, and CD56+ natural killer cells, as well as CD4/CD8 ratio were measured by Flow-cytometry using EPICS XL (Coulter, Miami, FL, USA).

For flow cytometric analysis, the samples were lysed using homemade lysing solution, washed with Phosphate Buffered Saline (PBS) once or twice until complete RBCs lysis occurred then were re-suspended 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). The 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 re-suspended in PBS and analyzed immediately on flow cytometry. The mAbs used had different forms 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 USA). The cells were analyzed with the most appropriate lymphocyte gate using the combination of forward and side scatters. An antigen was considered positive when expressed on 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.

Ethical Considerations

Collected data was used for study purpose only. The parents/care-givers of children under study were informed about the purpose of the study and the plan of work before participating. Study protocol was approved by the Board of the Pediatric Department, Ain Shams University.

Data Management and Analysis:

Data entry was checked to ensure its quality control then it was analyzed using Statistical Program for Social Science (SPSS) version 18. Analysis was done according to the type of data obtained for each parameter: Student T Test was used to assess significance of the difference between two study group means. Chi-Square test was used to examine the relationship between two qualitative variables. Correlation analysis (using Pearson's method) was used to examine the strength of association between

two quantitative variables. Probability (p) values above 0.05 were considered significant for all tests.

RESULTS

The 20 obese children had a mean age of 9.9±2.65 years. They were 11 females (55%) and 9 males (45%). The 10 control patients had a mean age of 10 ±3.27 years and were 4 females (40%) and 6 males (60%). The mean±SD for different anthropometric data (weight, height, BMI, waist hip ratio) for both groups are displayed in table (1). There were statistically significant differences between both groups in terms of weight, BMI and waist hip ratio, but not of height. There was history of recurrent infection (>2 attacks per month) in 75% of the obese children **.**

Flow cytometry results of the obese children showed that the mean±SD percentage values of CD4+, CD8+, CD56 were 30.6 ±7.18%, 35.2 ±12.09%, and 15.5 ±6.3% respectively and that the CD4/CD8 ratio was 1.5 ±0.5. Among the control group, the corresponding percentage values were 38.6±9.2%, 27.2±7.3%, 15±9.5% respectively and that of the CD4/CD8 ratio was 1±0.5**.**

Table (2) shows no statistically significant difference in total lymphocyte and CD56 counts between obese and non-obese children. However, there was significant difference in terms of percentage values and the absolute counts of CD4, CD8, CD4/CD8 ratio.

There was a highly significant negative correlation between the CD4 expression (percentage and absolute count) and the weight, BMI, and hip waist ratio with (p values=0.00). The same was observed concerning the CD4/CD8 ratio (p values = 0.00). There were, on the other hand, significant positive correlations between CD8% and the weight, BMI and waist hip ratio with p value of 0.00 for each. There were no significant correlations between CD56% and the weight, BMI or waist hip ratio (p = 0.68, 0.8, 0.19) respectively **.**

ROC curve was done to show the best cut off value between obesity and normal weight in terms of CD4%, CD8%, and CD56% as well as CD4/CD8 ratio. CD4% cut off vale was >31% with a sensitivity of 90% and a specificity of 60% For CD8% it was ≤ 31% with a sensitivity of 90% and a specificity of 70% and for CD56% it was >14%with a sensitivity of 55% and a specificity of 70%. The cut off value of CD4/CD8 ratio was >0.8 with a sensitivity of 95% and a specificity of 50%

In the current study, the values of CD4%, CD8%, and CD56% and their absolute counts or CD4/CD8 ratio did not vary significantly according to the presence or absence of recurrent infections (Table 3). The finding could be limited by the sample size.

Table 1. Anthropometric measurements in the studied sample

Parameters	Control group Mean ± SD	Patients group Mean ± SD	Independent t-test	
			t	p-value
Height (cm)	130±22	127±24	0.328	0.746
Weight (kg)	32±12	63±26	-3.434	0.002
BMI	18.4±1.9	39.2±12.5	-5.162	0
Waist Hip Ratio	0.6±0.1	1±0.1	-8.693	0

BMI: Body mass Index, CM: centimeter, Kg: kilogram, SD: Standard deviation

Table 2. Total leucocyte and lymphocyte counts in the studied groups

Parameters	Control group	Patients group	Independent t-test	
	Mean ± SD	Mean ± SD	t	p-value
*TLC	7280 (5834– 14964)	6298 (5336 – 7418)	1.584*	0.113
LYM Percent	31.30%±8.60%	28.80%±9.20%	0.732	0.47
Absolute CD4 Count	867±310	733±417	0.895	0.378
CD4 Percent	30.60%±7.18%	38.66%±9.20%	-2.42	0.022
*Absolute CD8 Count	778 (539 - 1783)	424 (338 – 594)	2.904*	0.004
CD8 Percent	35.25%±12.09%	27.22%±7.31%	2.273	0.031
Absolute CD56 Count	355±142	298±316	0.548	0.588
CD56 Percent	15.00%±9.50%	15.50%±6.30%	-0.191	0.85
CD4/CD8 Ratio	1±0.5	1.5±0.5	-2.585	0.015

CD: cluster of differentiation; SD: standard deviation; TLC: total leucocyte count

Table 3. Variation of lymphocyte subsets according to recurrent infections in the obese children

Recurrent infections	CD56 absolute no Mean±SD	CD56 % Mean±SD	CD8 absolute no Mean±SD	CD8 % Mean±SD	CD4 absolute no Mean±SD	CD4 % Mean±SD	CD4 / CD8 Ratio Mean±SD
No	**448±117.9**	**22.8±5.1**	**1082.4±458.3**	**38.5±7.5**	**424.8±158**	**28.00±4.30**	**0.8±0.4**
Yes	**400±325.1**	**17.8±6.1**	**779.2±506.7**	**32.7±9.6**	**608.1±224.1**	**32.00±5.40**	**1.1±0.4**

CD: cluster of differentiation, SD: Standard deviation

DISCUSSION

The increasing prevalence of obesity comes with an increased risk of infections and cancers that became an emerging health concern added to the previously established obesity related comorbidities.[18,19,20] Increased risk of infection is linked to the negative effect of the nutritional or metabolic disturbances on the status of immune cells.[21]

In our study we found a higher lymphocytic count in obese patients in comparison to non-obese ones. This was found in relevant studies by Zaldivar et al.[22] and Mahassni et al.[23], and was explained by the increased in plasma levels of cytokines that stimulate T-lymphocytes proliferation such as IL-6 and TNF-alpha, .[24,25] Similar to adults, Zaldivaret al.[22] found elevated CD4 cells in children and was explained by an inflammatory process. However, the opposite was noted in our series as we found that CD4% was low in obese children. This goes in accordance with studies done by Tanaka[26]and Amati et al[27] who also reported normalization of CD4% levels after weight reduction. Elevated CD8% found in obese subjects may be explained not only by the presence of low systemic inflammatory response and high leptin levels, but also by increased prevalence of viral infection, high levels of oxidative stress, and psychological and psychosocial factors such as depression and poor quality of life. All of these factors have been shown to increase T cell activation and proliferation.[28]

The negative correlation between CD4% and body weight, BMI, waist hip ratio that we observed in the obese group was also noted by Tanaka et al[26] but not by Womack et al[32] Tanaka et al[26] noted that weight reduction can recover the previously reduced non CD8 subsets including CD4 T-cells. The highly positive correlation between CD8 cell (percentage and count) and weight and BMI in the obese group matches with data reported by Womack et al.[32] and O'Rourke et al.[33]

NK cells are the first line of defense against malignancy and viral infection through detection and killing of cancerous and viral-infected cells and also by producing cytokines, including IFN-γ, which recruit elements of the adaptive immune system.[34] Obesity is considered an inflammatory disease,[22,35] however, NK (CD16/CD56) cell number did not increase and there was no correlation between it and weight or BMI in our series. The same was reported by other investigators.[29-31] On the other hand, Lynch et al,[36] and O'Shea et al[34] stated that NK cell number and function are altered in obesity and, accordingly, obese patients are liable to malignancy and viral infection.

All previous data suggest that obesity can potentially affect cell mediated immunity through alteration of cell counts and may be also function. Seventy five percent of our obese patients gave history of recurrent infection enforcing the concept of negative effect of obesity on the immune system. This was proven by a study conducted in Spain on 144 patients admitted in intensive care units (ICU). It showed that obese patients had higher ICU resource consumption and longer length of stay due to H1N1 influenza.[8,37] Another study by Kim et al[38] revealed that pediatric obesity may have an effect on the development of otitis media with effusion.

In conclusion, obesity seems to reduce lymphocyte number causing a variable impairment of cell-mediated immune responses. The study is indeed limited by the sample size and cross-sectional design. Further wider scale and prospective studies are needed to assess the impact of obesity on the function not only the number of immune cells which may add predisposition to recurrent infection in those children.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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