The Egyptian Journal of Hospital Medicine (October 2020) Vol. 81 (7), Page 2310-2317

Study of CD4 and CD8 Subsets in Morbid Obese Population

Fawzia Hassan Abo Ali, Asmaa Saber Mostafa Abdel Rehim,
Hoda Mohamed El Sayed, Shaimaa Hani Fouad, Amira Ramadan Elmahdi*
Department of Internal Medicine, Allergy & Clinical Immunology,
Faculty of Medicine, Ain Shams University, Cairo, Egypt
*Corresponding Author: Amira Ramadan El Mahdi, Internal medicine Department, Faculty of Medicine, Ain Shams University, Abbasiyya, Cairo, Egypt, Mobile: +201121641520, E-mail: dr_ amiraramadan@yahoo.com

ABSTRACT

Background: Immune dysfunction is one of the co-morbidities that became well recognized several years ago in people with obesity. **Objective.** To study the peripheral blood T-cell compartment, the absolute counts and relative frequencies of T-cell subpopulations including CD8+T cells; CD4+Tcells in adults suffering morbid obesity compared to lean subjects. **Patients and methods:** This case-control study included 30 adults with morbid obesity and 20 lean healthy subjects who were age and sex-matched.CD4 and CD8 absolute and relative count together with CD4/CD8 ratio were assessed and compared between the two groups. **Results:** Adults with morbid obesity had higher total leucocyte count (TLC), CD4 percentage, CD4 absolute count, and CD4/CD8 ratio than that of controls. On the other hand, the CD8 percentage was lower in people with morbid obesity. There were positive correlations between CD4% and BMI, waist circumference, cholesterol, triglyceride, LDL, and TLC and neutrophils count. There was a negative correlation between the percentage of CD8 and BMI**.** CD4/CD8 ratio correlated positively with BMI but negatively with systolic blood pressure. **Conclusion:** $CD4^+$ $CD8^+$ T lymphocytes assay could help assess the immune state and recognize obese at risk of immune dysfunction complication as infection and metabolic diseases.

Keywords: CD4, CD8, morbid, obesity, lymphocytes

INTRODUCTION

Obesity is a metabolic disease that has increased in prevalence lately. It is considered a major risk of death in the West [(1)]. Complications of obesity include type 2 diabetes mellitus and cardiovascular disease [(2)].

Immune dysfunction is one of the co-morbidities that appeared several years ago in obese [(3)]. Studies clarified the role of excess adiposity in increased susceptibility of infection and therefore proved a negative impact on the immune system [(4)].

Morbid obesity has been described as a chronic inflammatory disease that contributes to immune dysfunction, as it participates in neutrophil and monocyte dysfunction [(5, 6)]. Although macrophages in the adipose tissue are long known to be the primary cause of chronic inflammation, T cells appeared to accumulate in adipose tissue in an amount that exceeded that of macrophages [(7-10)]. Therefore, studies postulated the importance of T cells in the pathogenesis of adipose tissue inflammation [(11)].

T cells are the main contributors to the adaptive immune system. The main two arms of T cells are the CD4+ T cells, which recognize MHC II-presented antigens, and the cytotoxic CD8+ T cells which are responsible for the MHC I-presented antigens (12,13). Limited number of studies demonstrated the link between obesity and the composition of the peripheral immune system[(14,15)]. Some studies reported an increase in the T-cell compartment proliferation while others demonstrated a decrease in the number[(16-18)].

This work aimed to study the peripheral blood T-cell compartment, the absolute counts and relative frequencies of T-cell subpopulations including CD8+T cells and CD4+Tcells in morbidly obese compared to lean subjects.

MATERIAL AND METHODS

The present study was a case-control study that included 30 apparently healthy morbidly obese adults (BMI ≥40) subjects. Patients were recruited from the outpatient Obesity Clinic, Ain Shams University Hospital, Cairo, Egypt between October 2014 and February 2016. Twenty lean healthy subjects of matched age and sex were also included as controls (BMI 18.5-24.9).

Ethical considerations

The study was approved by the ethical committee of Ain Shams University and written informed consent was obtained from all participants.

Patients who smoked, pregnant females, patients with chronic illness such as advanced liver, pulmonary, renal diseases, or diabetes mellitus, known malignancy, or on current medications (antihypertensive, lipid-lowering, hormones, anti-inflammatory, and immunosuppressive drugs), acute or chronic inflammatory or infectious diseases, recent trauma, and positivity for hepatitis B and C were excluded from the study.

Patient data and Anthropometric Measurement.: For all subjects: age, weight, height, and blood pressure were recorded. Full history taking and clinical examination were done with special emphasis on the history of any chronic disease, current infections, and any current medications. Measurement of mean arterial blood pressure was done using

Received:22 /6 /2020
Accepted:21 / 8/2020

mercury column sphygmomanometer. All subjects were in a sitting comfortable position with a short period of rest preceding the measurement. Body mass index (BMI) was calculated using the Quetelet formula (weight in kilograms divided by the square of height in meters). Height was measured to the nearest 0.5 cm using stadiometer and body weight was measured to the nearest 1 kg while wearing light clothing and no shoes using a scale. 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 using a measuring tape.

Laboratory Investigations:

After 12-14 hours fasting, 7 ml of venous blood was drawn from each patient and control, where 3 ml was placed in an EDTA vacutainer tube (Becton Dickinson, Oxford, UK) for performing complete blood count (CBC) and for $CD4^+$ $CD8^+$ T-lymphocytes estimation by flow cytometry. The rest of the blood was collected into a gel vacutainer tube (Becton Dickinson) to be clotted and centrifuged (1500 g for 15 min) for assay of total cholesterol(TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), Triglycerides (TG), liver functions and kidney functions. After 8 hours fasting, another 2 ml of a venous sample was drawn into a gel vacutainer tube to be clotted and centrifuged for measurement of fasting plasma glucose (FPG). 2 hours after eating, the last venous sample was collected for measurement of 2-hour postprandial blood sugar. The laboratory work was conducted at the Clinical Pathology Department. CBC was done using a Coulter counter (T660) (Beckman. Coulter, California, USA).

Serum blood glucose levels, lipid levels, AST, ALT, creatinine, and BUN were measured on Synchron CX-9 autoanalyzer (Beckman Instruments Inc.; Scientific Instruments Division, Fullerton, CA 92634, 3100, USA).LDL-C was calculated using the Friedewald equation[19].

$CD4^+$and $CD8^+$ T-lymphocytes assay: For the detection of the combined expression of CD4 and CD8 on lymphocytes, two Wassermann plastic test tubes were used; one for the test and the other for the isotypic control. A total of 50µL of EDTA-anticoagulated whole blood were added to each tube, then were lysed using 1 ml lysing solution followed by a wash with phosphate-buffered saline (PBS).

The cell pellet was stained with combinations of the following antibodies (5 µL each): fluorescein isothiocyanate (FITC)-labeled antihuman CD4 (Immunotech SAS, Marseille Cedex, France) and phycoerythrin (PE)-labeled anti-human CD8 (R&D Systems, Minneapolis, MN) in the test tube; and PE isotype control and anti-CD4-FITC in the control tube. Both tubes were then incubated in dark at room temperature for 15 min followed by a single wash with PBS. Data acquisition and analyses were performed on EPICS XL flow cytometer (Coulter Electronics, Florida, USA) using SYSTEM II version 3 software with a standard 3-color filter configuration. Isotype-matched controls were used to set up the gating. Lymphocytes were gated according to their forward and side scatter properties, and $CD4^+$and$CD8^+$ positive T cells were gated out of total lymphocytes (Figure 1).

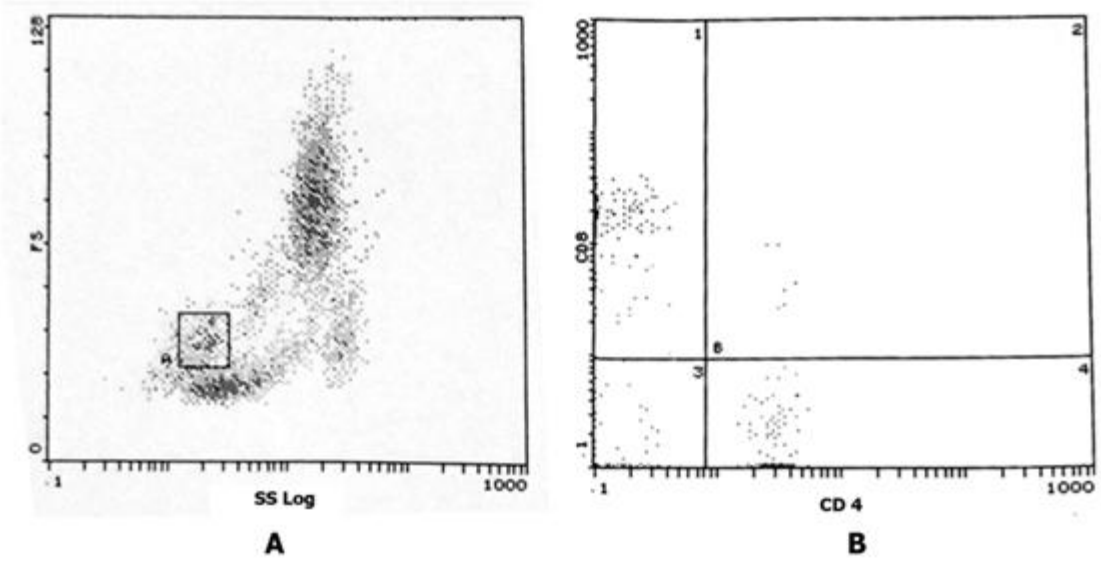

Figure 1: Representative dot plots of flow cytometry and the gating strategy used (A) shows forward and side scatters to gate lymphocytes. (B) Shows that $CD4^+CD8^+$ positive T cells were acquired after gating the lymphocyte population by forward and side scatter properties.

Statistical methods

Analysis of data was done using SPSS (Statistical Package for Social Science) program version 24. To describe the studied sample, quantitative data were

presented as a minimum, maximum, mean, and standard deviation. Qualitative data were presented as count and percentage. The Student t-test was used to compare quantitative data between two groups and the Chi-Square test was used to compare qualitative data. Pearson correlation test was used to compare the correlation between different continuous variables. Linear regression analysis was used to measure the independent effect of different variables on CD4 and CD8 levels. P-value $\leq$ 0.05 was considered statistically significant.

RESULTS

The demographic and clinical characteristics of both groups are shown in Table 1.TLC was statistically significantly increased in cases (p-value<0.033) (table 1) CD4 absolute count, CD4% and CD4/CD8 ratio were statistically significantly increased in cases (p-value<0.001) .On the other hand, CD8% was statistically significantly decreased in morbid obese group (p-value<0.003)(table 2).

CD 4 showed a significant positive correlation with BMI (p-value< 0.001) ,waist circumference (p-value< 0.021) , cholesterol(p-value<0.001), triglyceride (p-value<0.051) and LDL(p-value<0.003),TLC(p-value<0.029) and neutrophils count(p-value<0.036) (table 3). (Fig 2) There was a significant negative correlation between CD8 and BMI (p-value<0.027) (table 4). Fig (3) CD4/CD8 ratio showed a significant positive correlation with BMI (p-value<0.011) and significant negative correlation with systolic blood pressure (p-value<0.041). (table 5). Fig (4).

Table (1): Comparison between cases and controls regarding demographic, clinical data, and laboratory investigations

		Cases (N=30)		Controls (N=20)		t*	P-value
		Mean	SD	Mean	SD		
Age (years)		31.87	3.50	32.25	3.13	0.395	0.694
BMI		43.75	3.46	21.82	1.68	26.282	**<0.001**
Waist circumference (cm)		139.43	10.98	78.98	3.95	27.590	**<0.001**
SBP (mmHg)		115.50	5.62	118.0	4.10	1.815	0.076
DBP (mmHg)		75.00	7.77	78.00	4.10	1.776	0.082
		N	%	N	%	X^{2**}	P-value
Sex	**Male**	3	10.0	1	5.0%	0.408	0.641
	Female	27	90.0	19	95.0		
S. Cholesterol (mg/dl)		198.20	62.64	155.1	25.04	3.381	**0.002**
TG (mg/dl)		109.67	48.28	135.4	8.13	2.859	**0.007**
HDL-C (mg/dl)		61.03	12.60	64.85	13.96	1.005	0.320
LDL-C (mg/dl)		112.73	52.62	83.30	15.45	2.883	**0.007**
TLC (x10^3/ul)		7.56	1.26	6.95	0.67	2.198	**0.033**

Results are expressed in mean ±standard deviation (SD). *Independent samples t-test **Chi-square test (Fisher exact). BMI=Body mass index, SBP= systolic blood pressure, DBP= diastolic blood pressure, TG= serum triglycerides, HDL-C= High-density lipoprotein cholesterol, LDL-C= Low-density lipoprotein cholesterol, TLC= Total leucocytic count.

Table (2): Comparison between cases and controls regarding CD4 count and percentage, CD8 count and percentage, and CD4/CD8 ratio

	Cases (N=30)		Controls (N=20)		t*	P-value
	Mean	SD	Mean	SD		
CD4%	0.63	0.04	0.51	0.02	13.453	**<0.001**
CD4 absolute	1.40	0.34	1.02	0.17	5.159	**<0.001**
CD8%	0.24	0.05	0.28	0.04	3.102	**0.003**
CD8 absolute	0.51	0.16	0.57	0.12	1.313	0.195
CD4/CD8 ratio	2.80	1.02	1.78	0.32	5.108	**<0.001**

*Independent samples t-test

Table (3): Relation between CD4 percentage and demographic, clinical data, and laboratory investigations of cases.

	CD4%	
	r*	P-value
Age	-0.059	0.756
BMI	0.867	**<0.001**
Waist circumference	0.419	**0.021**
SBP	-0.028	0.882
DBP	0.045	0.812
S. Cholesterol	0.579	**0.001**
TG	0.359	**0.051**
HDL-C	-0.094	0.622
LDL-C	0.520	**0.003**
TLC	0.398	**0.029**
Neutrophils	0.385	**0.036**

*Pearson correlation coefficient

Table (4): Relation between CD8percentage and demographic and clinical data of cases.

	CD8%	
	r*	P-value
Age	-0.306	0.100
BMI	-0.404	0.027
Waist circumference	-0.075	0.692
SBP	0.249	0.184
DBP	0.112	0.557

*Pearson correlation coefficient

Table (5): Relation between CD4/CD8 ratio and demographic and clinical data of cases.

	CD4/CD8 ratio	
	r*	**P-value**
Age	0.352	0.056
BMI	0.458	0.011
9-waist circumference	0.087	0.647
SBP	-0.375	0.041
DBP	-0.151	0.427

*Pearson correlation coefficient

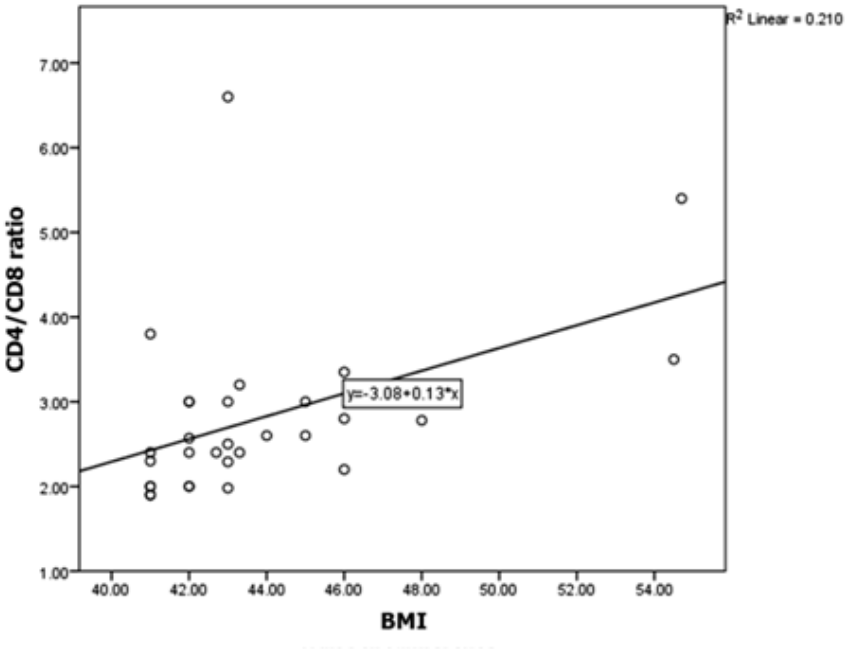

Figure 2: Scatter plot for the correlation between BMI and CD4 percentage among cases

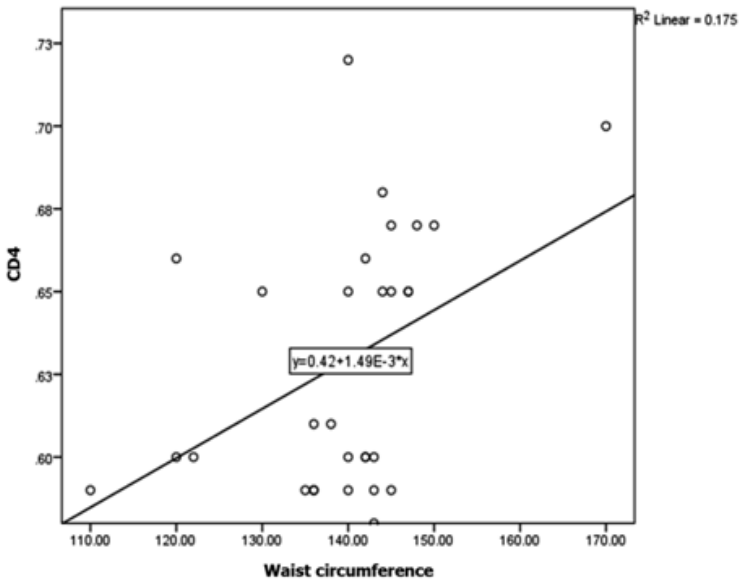

Figure 3: Correlation between waist circumference and CD4 percentage among cases

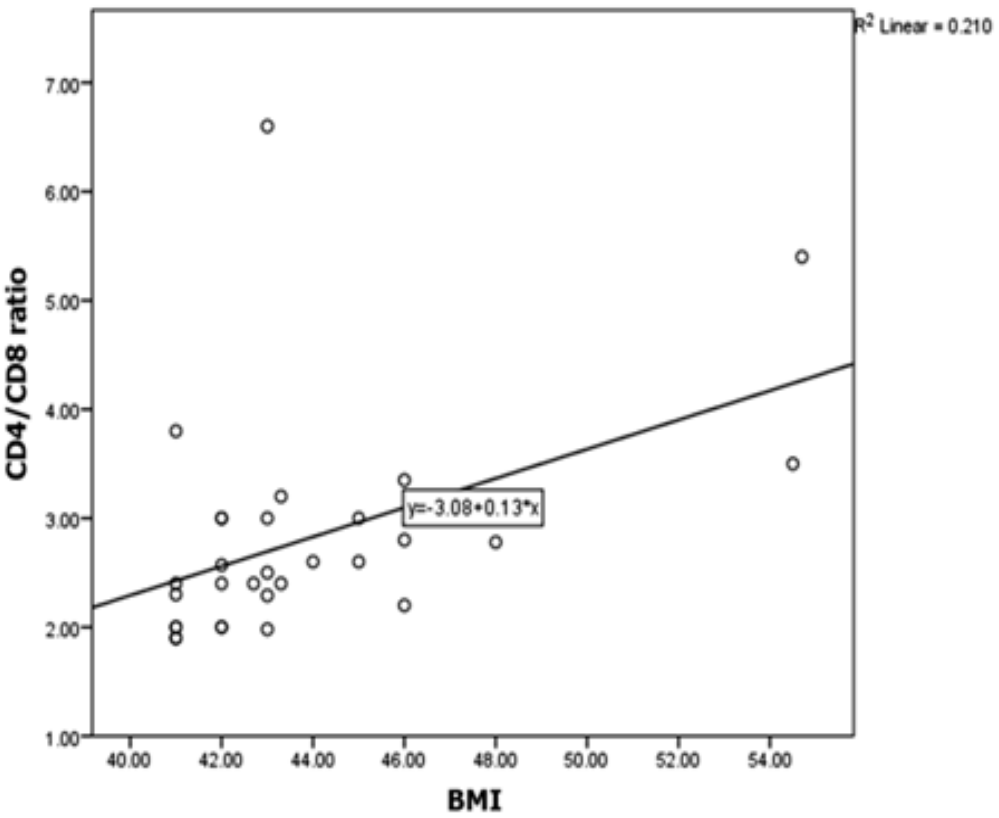

Figure 4: Scatter plot for the correlation between BMI and CD4/CD8 ratio among cases

DISCUSSION

There appears to be a link between obesity and abnormalities in the immune system. When adipose immune interactions occur, in the form of low-grade inflammation, obesity-related comorbidities and long term complications eventually appear [20, 21,22].

T cells seem to be crucial contributors to adipose tissue inflammation [11]. Some studies even supported this fact as they found that insulin sensitivity improved T-cell depletion which in turn led to a decrease in accumulation of adipose tissue macrophage [9,23].

Several researchers studied the composition of the immune system of peripheral blood in obesity. Some studies positively correlated BMI, total white blood cells count, and T cell numbers in peripheral blood [14,24], while other studies showed a decrease in the T cell subpopulations [25]. Furthermore, others claim no difference in T cell subpopulations between normal-weight individuals and obese [26].

In the present study, morbidly obese subjects had higher BMI, waist circumference, total cholesterol, LDL, triglyceride, TLC, CD4 percentage, CD4 absolute count, and CD4/CD8 ratio than that of controls. On the other hand, the CD8 percentage was low in the morbidly obese group. There were positive correlations between CD4% and BMI, waist circumference, cholesterol, triglyceride, LDL, and TLC and neutrophils count. On the other hand, there was a negative correlation between the percentage of CD8 and BMI.

CD4/CD8 ratio correlated positively with BMI but negatively with systolic blood pressure.

As regards total leukocyte count, it was high in the morbidly obese. Similarly, **Kanneganti *et al.*** [27] reported an elevation in total WBC in obese in comparison to control.

Obesity is associated with elevated cytokines and encourage leukocyte differentiation and increase leukocyte count. Indeed, impairment in thymopoiesis, causing malfunctioning of T cell, results in pro-inflammatory responses and metabolic tissue infiltration [28].

In the present study, morbidly obese subjects had higher CD4percentage, CD4 absolute count and CD4/CD8 ratio. On the other hand, CD8 percentage was lower than that of controls.

Similarly**, Agabiti-Rosei *et al.*** [29] found a significant increase in CD4+ effector memory cells in morbidly obese.

Also in a cohort study, **Mauro *et al.*** [30] studied the relationship between obesity and the effector memory phenotype of CD4+ T cells in human and focused on the morbidly obese patients and observed that the percentage of effector memory T

cells was raised in morbid obese as compared to the entire obese population and the lean population.

In a similar study conducted on morbidly obese individuals, **Weerd *et al.* [(1)]** demonstrated that there was a statistically significant increase in the CD4 in peripheral blood and a statistically significant increase in CD4/CD8 ratio. Yet there was no significant change in CD8.

Also, **O'Rourke *et al.* [(31)]** stated that there were alterations in the frequencies of peripheral CD4+ and CD8+ T-cells. There was a statistically significant increase in CD4 and a similar significant decrease in CD8 subsets in obese candidates compared with lean ones. Moreover, there was a statistically significant increase in CD4/CD8 ratio.

Womack *et al.* [(14)], in a large cohort study, found that being overweight or obese was associated with higher CD4, total lymphocyte, and WBC counts. Meanwhile, being morbidly obese was associated with these outcomes, in addition to a higher CD8 count. The associations between weight and immune cell counts became stronger with each successive increase to the next weight category.

Several theories explained the changes in the T cell subsets in obese. One theory postulated that that fat directly affects the CD4 count more than it does on CD8 leading to an increase in CD4. Another theory stated that fat cytokines', specifically leptin, main goal is the activation of CD4 [(14)]. Decreased CD8 levels may be due to change in immune cells development and differentiation. CD8 cytotoxic T cells play important role in prevent infection and cancer, so decreased levels could prone to infection and cancer in obese [(31)].

Our study revealed positive correlations betweenCD4%and BMI, waist circumference, cholesterol, triglyceride, LDL, and TLC and neutrophils count. On the other hand, there was negative correlation between percentage of CD8 and BMI. These findings approve the relationship between immune system and metabolic disturbance.

Studies demonstrated that elevated CD4 cells in obese are associated with increased risk of atherosclerosis, insulin resistance and metabolic syndrome. So it is postulated that high CD4 cells in obese cause metabolic disturbance through its important role in obesity related inflammation. Change in CD4 subset could identify obese at risk of infection, metabolic and cardiovascular diseases [(9-10)].

Interestingly, **Fathy and Morshed [(32)]** found that weight loss patients resulted in a statistically significant decrease in serum leptin levels and CD4 and CD8 T lymphocytes levels after operation.

On the contrary, opposite results were achieved by **Merhi *et al.*[(33)].**They found that no significant changes were found in the CD4+ or CD8+ T cells among all populations after the decrease in BMI three months after surgery.

Travers *et al.* [(34)] demonstrated elevated expression of both CD4 and CD8 in subcutaneous adipose tissue and positively correlated to degree of adiposity.

Duffaut *et al.*'s [(8)] results matched Travers et al.'s that the accumulation of adipose tissue lymphocytes is positively correlated with obesity.

. **Zeyda *et al.*[(35)],** also showed that the CD4 cell numbers in visceral AT of obese individuals were elevated, as compared to lean controls, and were correlated with BMI and markers of systemic or adipose tissue inflammation such as CRP and plasma IL-6.

CONCLUSION

The current study demonstrated that obesity was associated with change in T cells subsets (increased CD4 count and percentage, and decreased CD 8 count and percentage) and their correlations with obesity parameters and metabolic factors identify obese at risk of immune dysfunction, related complications and the importance of weight reduction strategies to overcome these complications.

Large scale study could help study the effect of obesity on T cell subsets at both function and cellular levels and compare T cell function in apparently healthy obese subjects with obese subjects presenting with infections. Studying and comparing tissue T cell number and function with blood T cell number and function, demonstrating the impact of hyperlipidemia on the immune and examining the effect of blood pressure on T cell subsets are recommended. Longitudinal studies are needed to study immune alternations impact on complications and the effect of weight reduction strategies on these changes.

The proper identification of this relationship might open opportunities and new modalities to adjust immunity in obese patients.

Conflict of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper

Author Contributions: All the authors conceived, organized, drafted, reviewed, and approved the manuscript.

REFERENCES

1. **Weerd K, Dik W, Benjamin Schrijver B *et al.* (2012):** Morbidly Obese Human Subjects Have Increased Peripheral Blood CD4+ T Cells With Skewing Toward a Treg- and Th2-Dominated Phenotype. Diabetes, 61:401–408.
2. **Flegal K, Carroll M, Ogden C *et al.* (2010):** Prevalence and trends in obesity among US adults, 1999-2008. JAMA., 303:224–35.

3. **Rocha V, Libby P (2009):** Obesity, inflammation, and atherosclerosis. Nat Rev Cardiol., 6:399–409.
4. **Mancuso P, Lewis C, Sereazani C *et al.* (2010):** Intrapulmonary administration of leukotriene B4 enhances Pulmonary host defense against pneumococcal Pneumonia" Infect Immun., 78:2261-2271.
5. **Cottam D, Schaefer P, Shaftan G *et al.* (2002):** Effect of surgically-induced weight loss on leukocyte indicators of chronic inflammation in morbid obesity. Obes Surg., 12: 335-42.
6. **Cottam D, Schaefer P, Angus L (2002):** The effect of obesity on neutrophil Fc receptors and adhesion molecules (CD16, CD11b, CD62L). Obes Surg., 12: 230-5.
7. **Rausch M, Weisberg S, Vardhana P *et al.* (2008):** Obesity in C57BL/6J mice is characterized by adipose tissue hypoxia and cytotoxic T-cell infiltration. Int J Obes (Lond), 32:451–463
8. **Duffaut C, Zakaroff-Girard A, Bourlier V *et al.* (2009):** Interplay between human adipocytes and T lymphocytes in obesity: CCL20 as an adipochemokine and T lymphocytes as lipogenic modulators. Arterioscler Thromb Vasc Biol., 29:1608–1614
9. **Nishimura S, Manabe I, Nagasaki M *et al.* (2009):** CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. Nat Med., 15:914–920.
10. **Duffaut C, Galitzky J, Lafontan M *et al.* (2009):** Unexpected trafficking of immune cells within the adipose tissue during the onset of obesity. Biochem Biophys Res Commun., 384:482–485.
11. **Sell H, Eckel J (2010):** Adipose tissue inflammation: novel insight into the role of macrophages and lymphocytes. Curr Opin Clin Nutr Metab Care, 13: 366 – 370.
12. **Mazza C, Malissen B (2007):** What guides MHC-restricted TCR recognition? Semin Immunol., 19:225–235.
13. **Raphael I, Nalawade S, Eagar T *et al.* (2014):** T cell subsets and their signature cytokines in autoimmune and inflammatory diseases. Cytokine, 74(1):5-17.
14. **Womack J, Tien P, Feldman J *et al.* (2007):** Obesity and immune cell counts in women. Metabolism, 56:998–1004.
15. **Tanaka S, Isoda F, Ishihara Y *et al.* (2001):** T lymphopenia in relation to body mass index and TNF-alpha in human obesity: adequate weight reduction can be corrective. Clin Endocrinol (Oxf), 54: 347–354.
16. **Janeway C, Travers P, Walport M *et al.* (2005):** Immunobiology" The Immune System in Health and Disease".6th Edn, Garland Science Publishing Inc, New York. Pp. 266.
17. **Karlsson E, Sheridan P, Beck M (2010):** Diet-induced obesity impairs the T cell memory response to influenza virus infection. J Immunol., 184: 3127-3133.
18. **Abd El–Hafez H, Abd El-Aziz S (2012):** Association between Immune Competence and Metabolic Parameters in Obesity. Trends in Medical Research, 7:53-61.
19. **Friedewald W, Levy R, Fredrickson D (1972):** Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without the use of the preparative ultracentrifuge. Clinical Chemistry, 18(6):499-502.
20. **Moulin C, Marguti I, Peron J *et al.* (2009):** Impact of adiposity on immunological parameters. Arq Bras Endocrinol Metabol A., 53:183–189.
21. **Gregor M, Hotamisligil G (2011):** Inflammatory mechanisms in obesity. Annu Rev Immunol., 29:415–445.
22. **Kahn S, Hull R, Utzschneider K (2006):** Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature, 444:840–846.
23. **Winer S, Chan Y, Paltser G *et al.* (2009):** Normalization of obesity-associated insulin resistance through immunotherapy. Nat Med., 15:921–929.
24. **Nieman D, Henson D, Cannarella S *et al.* (1999):** Influence of obesity on immune function. J Am Diet Assoc., 99:294–299.
25. **Kim J, Park H, Kim J *et al.* (2008):** White blood cell count and abdominal fat distribution in female obese adolescents. Metabolism, 57:1375–1379
26. **Fink S, Eckert E, Mitchell J *et al.* (1996):** T-lymphocyte subsets in patients with abnormal body weight: longitudinal studies. In anorexia nervosa and obesity. Int J Eat Disord., 20:295–305.
27. **Kanneganti T, Dixit V (2012):** Immunological complications of obesity. Nat Immunol., 13:707–12.
28. **Yang H, Youm Y, Vandanmagsar B *et al.* (2009):** Obesity accelerates thymic aging. Blood, 114:3803 12.
29. **Agabiti-Rosei C, Trapletti V, Piantoni S *et al.* (2018):** Decreased circulating T regulatory lymphocytes in obese patients undergoing bariatric surgery. PLOS ONE, 14: 1-12.
30. **Mauro C, Smith J, Cucchi D *et al.* (2017):** Obesity-Induced Metabolic Stress Leads to Biased Effector Memory CD4+ T Cell Differentiation via PI3K p110d-Akt-Mediated Signals., Cell Metabolism, 25: 593–609
31. **O'Rourke R, Kay T, Mark H *et al.* (2005):** Alterations in T-cell subset Frequency in Peripheral Blood in obesity". Obesity Surgery, 15: 1463-1468.
32. **Fathy S, Morshed G (2014):** Peripheral blood lymphocyte subsets (CD4+, CD8+ T cells), leptin level and weight loss after laparoscopic greater curvature plication in morbidly obese patients. Arch Med Sci., 5: 886–890.
33. **Merhi Z, Durkin H, Feldman J *et al.* (2009):** Effect of bariatric surgery on peripheral blood lymphocyte subsets in women. Surg Obes Relat Dis., 5:165–171.
34. **Travers R, Motta A, Betts J (2015):** The impact of adiposity on adipose tissue-resident lymphocyte activation in humans. International Journal of Obesity, 39: 762–769.
35. **Zeyda M, Huber J, Prager G *et al.* (2011):** Inflammation correlates with markers of T-cell subsets including regulatory T cells in adipose tissue from obese patients. Obesity (Silver Spring), 19:743–748.