Evaluation of inflammatory markers in egyptian obese and nonobese adolescents

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Background
Obesity causes an inflammation state that is related to co-morbidities such as hypertension and insulin resistance. Though, the underlying inflammatory events in obesity are still obscure particularly in pediatric age group.
Objective
To identify the initial stage of inflammation linked to this nutritional disorder.
Materials and methods
A case control observational study involved 45 cases of overweight/obese adolescents and 44 healthy peers of matching age and sex. Serum levels of Interleukin-10, Interleukin-18, and C – reactive protein were estimated. Leukocytes, Neutrophils, Lymphocytes counts were determined, and Neutrophil/Lymphocyte Ratio was calculated.
Results and conclusion
C – reactive protein, Neutrophils, and Neutrophil/Lymphocyte Ratio were significantly higher in overweight/ obese adolescents ($P = 0.000$, $P = 0.015$, and $P = 0.034$, respectively). Interleukin-10 was non-significantly lower whereas IL-18 was non-significantly higher in overweight/ obese adolescents ($P = 0.334$, and $P = 0.427$, respectively). Obesity is accompanied by a low inflammatory state that exists since childhood. Therefore, timely interventions should be adopted to preclude the deleterious consequences in grown up adults.

Keywords:
obesity, inflammation, adolescents, interleukin-10, interleukin-18

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Introduction

Obesity raises the incidence of cardiovascular sequel [1,2] that is triggered by the coexisting inflammatory milieu [3]. Up till now, only few researches have tackled this issue in childhood [4]. The hypertrophy and the hyperplasia of adipocytes, mediated by Macrophage colony-stimulating factor (MCSF) [5,6], result in hypoxia and oxidative stress [7]. Furthermore, the visceral fat is loaded by neutrophils [8–10]. The high Neutrophil to Lymphocyte Ratio (NLR) reflects the degree of inflammation [11]. Additionally, the pro-inflammatory interleukin-1 (IL-1) family is raised [12]. While contradictory levels of the anti-inflammatory IL-10 were found [13,14]. This inflammatory state causes obesity's co-morbidities later on in adulthood [15]. Our study evaluates inflammatiory status in obese adolescents compared to normal peers.

Subjects and methods

The procedures followed were in accordance with the ethical standards of the Helsinki Declaration of 1975, as revised in 2000 (available at https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/). The subjects' parents gave their informed written consent for the participation of their children who gave an assent to be enrolled in the study. The Ethics' committee of the National Research Centre (NRC) approved the study with approval number 16/130.

A case – control study was carried on 89 Egyptian adolescents aged 10–18 years of both genders. The cutoff BMI was calculated according to the World Health Organization (WHO) growth charts. Overweight was BMI ≥ 85th to < 95th percentile. Obesity is defined as a BMI ≥ 95th percentile for age and sex [16]. Consequently, the study participants were divided into two groups. Group A (the cases' group): included 45 overweight/obese adolescents with BMI above or equal to 85th centile. Group B (the control group): Involved 44 healthy, age and gender matched normotensive adolescents who had BMI under the 85th

centile. Inclusion criteria: adolescents of both genders aged 10 to 18 years old. Exclusion criteria: adolescents who have a genetic syndrome or other end ocrine disorder known to cause obesity (e.g. Prader- Willi syndrome, hypothyroidism, Cushing disease). Acute or chronic infections and current medications with a documented effect on the immune system. The overweight/obese adolescents were recruited from the Nutrition- Immunotherapy Clinic at the Medical Research Centre of Excellence (MRCE), and the control group was recruited from the pediatric outpatient clinic in a period of 6 months from January 2019 to June 2019.

The study participants were subjected to history taking with emphasis on age, sex, parents' education, the inquiry about associated morbidities as diabetes mellitus, hypertension, and family history of obesity, diabetes mellitus, and hypertension. Also, physical examination was done including anthropometry. Body weight was measured with light clothes and barefoot on Seca Scale Balance to the nearest 0.1Kg. Height was recorded to the nearest 0.5 cm on a Holtain portable stadiometer. Calculation of BMI was done using the equation [weight (Kg)/ height^2 (m^2)]. Data were plotted on WHO curves via the software AnthroCalc v2.24 Home [16]. Waist circumference was measured at the midpoint between the upper edge of the iliac crest and the lower edge of the rib cage on the mid axillary line using a flexible and an inelastic tape. Blood pressure was measured after a 5- minutes rest in the semi- sitting position with an appropriately sized cuff using Riester sphygmomanometer, Germany. Data were plotted through the software AnthroCale v2.24 Home, and the participants were diagnosed to have hypertension according to the guidelines of the American Academy of Pediatrics [17].

Laboratory investigations comprised blood sampling in the morning after an overnight fasting (at least 8 h) to measure glucose, insulin. Insulin resistance was calculated from the HOMA- IR using the formula: [fasting insulin (μU/ml)×fasting glucose (mg/dl) /405] [18]. The criterion of Insulin Resistance was a HOMA- IRË 4.0 for adolescents [19]. Serum leptin, adiponectin, IL-18, and MCSF were assessed by Enzyme – Linked Immunosorbent Assay (ELISA) Kits (NOVA, No.18, Kayuan Road, Daxing Industry Zone, Beijing, China). IL-10 was measured using ELISA kits (Elabscience, http://www.elabscience.com). CRP was measured using ELISA kits (Immunospec kits).

All statistical calculations were done using computer program SPSS (Statistical Package for the Social Sciences) statistical program version (21.0). Quantitative data were statistically represented in terms mean, standard deviation, and median. Comparisons between different groups in the present study were done using Mann-Whitney Test for comparing non-parametric data, and independent sample T- test for normally distributed data. Correlations between various variables were done using spearman correlation coefficient. Reliability was calculated using Cronbach' alpha and test-retest reliability correlation. A probability value (P value) less than or equal to (0.05) was considered statistically significant.

Results and discussion

The cases' group (group A) included 6 overweight and 39 obese adolescents. The two groups cases and controls were well matched in term of age ($P=0.446$) and had a similar gender distribution ($P=0.267$).Waist circumference, systolic and diastolic blood pressure centiles were significantly higher in overweight/ obese group in comparison to the control group ($P=0.001$, $P=0.005$, and $P=0.019$, respectively). Four participants of cases' group have missed blood pressure readings and thirteen of them were diagnosed to have stage 1 hypertension according to AAP, 2017 criteria [17]. Control group were normotensive. The clinical data of the participants were shown in (Table 1).

Fasting blood glucose levels for all participants were <126 mg/dl. In the cases' group, 38 participants had HOMA-IR values and 8 of them had insulin resistance (21.1%) (HOMA-IR mean±SD=2.72±2.57). Leptin was significantly higher in overweight/obese adolescents with a median value of 250 pg/ml compared to a median of 90 pg/ml in controls.

Table 1 The demographic and clinical data of the participants

	Overweight/ obese (Number = 45)	Control group (Number = 44)	*P* values
Gender (M/F)	13/32	17/27	0.267
Age (years)	13.05±2.61	12.62±2.60	0.446
Waist circumference (cm)	89.17±12.66	60.68±8.18	0.001
Systolic blood pressure centile	52.95±32.60	31.66±22.64	0.005
Diastolic blood pressure centile	65.93±28.52	54.32±21.49	0.019

M/F: male/female.

Within the group of cases, the ratio between those having a Leptin value higher than the median was 16%: 28% in overweight versus obese candidates respectively. Although median Adiponectin was significantly lower in the study group (0.5 ng/ml) compared to (0.825 ng/ml) in the control group ($P=0.001$). The overweight/obese candidates had lower serum level of IL-10 and higher level of MCSF compared to controls but they did not reach statistical significance ($P=0.334$, and $P=0.905$) respectively. Serum level of IL-18 was nonsignificantly higher in overweight/obese compared to controls ($P=0.427$). In addition, the overweight/obese children had significantly higher level of CRP in comparison to controls ($P=0.000$). Participants with high BMI showed non-significantly higher leukocytes count and non-significantly lower platelets count compared to controls ($P=0.625$, $P=0.888$, respectively). Whereas, neutrophils and neutrophils / lymphocytes ratio were significantly higher in obesity compared to controls ($P=0.015$, and $P=0.034$, respectively). Lymphocytes were significantly lower in cases compared to controls ($P=0.015$). The data were shown in (Tables 2 and 3).

CRP was significantly positively correlated with BMI (r =0.443 $P=0.005$), and had non-significant positive correlations with insulin, HOMA-IR and Leptin (r =0.132 $P=0.442$, r=0.131 $P=0.454$ and r =0.125 $P=0.621$).Whereas, CRP had non-significant negative correlation with Adiponectin in overweight/obese participants (r=–0.046 $P=0.829$). As shown in (Table 4).

There were no significant correlations between neutrophil/lymphocyte ratio and either CRP or BMI in cases ($r=0.166$ $P=0.313$, and $r=0.202$ $P=0.188$, respectively). On the other hand, a significantly negative correlation was found between MCSF and IL-10 (R=–0.398 $P=0.008$) among cases. Otherwise there were no significant correlations between MCSF and other indices (as illustrated in Table 5).

IL-10 had significant positive correlations with insulin and HOMA-IR ($r=0.411$ $P=0.005$, and r=0.383 $P=0.018$, respectively) and non-significant positive correlation with Adiponectin ($r=0.159$ $P=0.41$). Whereas it had non-significant negative correlations with both BMI and Leptin in overweight/obese adolescents (r =–0.199 $P=0.195$, and r=–0.181 $P=0.408$, respectively). A highly significant negative correlation was detected between IL-10 and systolic blood pressure. As shown in (Table 6).

The study showed non-significant positive correlations between IL-18 and BMI, insulin, and HOMA-IR in obesity (r=0.001 P=0.995, r=0.168 $P=0.269$, and $r=0.143$ $P=0.392$, respectively).

Obesity represents a state of chronic low-grade inflammation. Up till now; there is no existing battery of markers to outline the inflammatory stages of obesity and/or to define the cut-off values for inflammation [20]. The current study showed that overweight/obese adolescents had significantly elevated serum levels of Leptin and significantly lower serum levels of Adiponectin compared to controls. Leptin and Adiponectin are two significant adipokines that represent a critical relation between metabolism, energy homeostasis, and immunity. Leptin has principally pro-inflammatory effects whereas adiponectin shows immune regulation with mainly anti-inflammatory effects as regards obesity-associated inflammation and related diseases for instance atherosclerosis [21].

Table 2 Comparison between overweight/obese adolescents and controls as regards serum levels of IL-10, IL-18, CRP, and MCSF

Parameters	Overweight/ obese (*n* 45)	Control group (*n* 44)	*P* value
	Mean±S.D		
IL-10 (pg/ml)	1.71±3.46	2.57±4.96	0.334
IL-18 (pg/ml)	13.96±13.27	12.02±7.60	0.427
CRP (ng/ml)	63.12±32.89	16.04±20.50	0.000
MCSF (pg/ml)	30.59±39.85	23.75±26.12	0.905

Table 3 Comparison between overweight/obese adolescents and controls as regards blood indices

Parameters	Overweight/ obese (Number = 45)	Control group (Number = 44)	*P* value
	Mean±S.D		
Platelets (10^9/l)	285.87±62.35	288.33±99.78	0.888
Leukocytes (10^9/l)	7.08±2.04	6.88±1.82	0.625
Neutrophils (%)	50.01±10.64	43.79±13.09	0.015
Lymphocytes (%)	41.16±10.32	47.12±12.31	0.015
Neutrophil/Lymphocyte ratio	1.39±0.80	1.07±0.59	0.034

Table 4 Correlations between CRP and the following parameters using spearman correlation coefficient in overweight/obese group

Parameters	R (Correlation Coefficient)	*P* value	
BMI	0.443[c]	0.005	P[a]
INSULIN	0.132	0.422	P[a]
HOMA-IR	0.131	0.454	P[a]
Leptin	0.125	0.621	P[a]
Adiponectin	–0.046	0.829	N[b]

[a]Positive Correlation, [b]Negative Correlation, [c]Correlation is significant at the 0.01 level.

Table 5 Correlations between MCSF and the following parameters using spearman correlation coefficient in overweight/obese group

Parameters	R (Correlation Coefficient)	*P* value	
IL-10	–0.398[c]	0.008	N[b]
IL-18	0.065	0.678	P[a]
CRP	0.100	0.554	P[a]
Leukocytes	0.084	0.593	P[a]
Neutrophils	0.093	0.552	P[a]
Lymphocytes	–0.122	0.437	N[b]
BMI	–0.093	0.557	N[b]

[a]Positive Correlation. [b]Negative Correlation. [c]Correlation is significant at the 0.01 level.

Table 6 Correlations between IL-10 and the following parameters using spearman correlation coefficient in overweight/obese group

Parameters	R (Correlation Coefficient)	*P* value	
INSULIN	0.411[d]	0.005	P[a]
HOMA-IR	0.383[c]	0.018	P[a]
Adiponectin	0.159	0.410	P[a]
Leptin	–0.181	0.408	N[b]
BMI	–0.199	0.195	N[b]
Systolic BP	–0.412[d]	0.007	N[b]
Diastolic BP	–0.186	0.244	N[b]

[a]Positive Correlation. [b]Negative Correlation. [c]Correlation is significant at the 0.05 level. [d]Correlation is significant at the 0.01 level.

The present study showed non-significantly lower circulating level of IL-10 in the obese group compared to the controls. This was in line with other studies. Change and colleagues, 2013 reported that overweight and obese adolescents had lower serum IL-10 compared with their normal weight counterparts [22]. While other studies showed that obese subjects had significantly elevated serum concentration of IL-10 in comparison to the non-obese group [23]. There are many contradictions in the literature regarding IL-10 in obesity. IL-10 is a cytokine that is secreted by plenty different cells, comprising B cells, T-helper 2 (Th2) cells, and macrophages. It has a significant role in regulation of immune response and limitation of inflammation [24]. IL-1ß is a pro-inflammatory cytokine that is increased in obesity. A decrease in serum IL-10 in adolescents with high body mass index might be contributing to the IL-1β mediated inflammatory milieu in adiposity [25].

The obese candidates showed nonsignificantly higher circulating level of IL-18 in comparison to controls. This was in agreement with other studies. Schipper and colleagues, 2012 reported that plasma level of IL-18 was higher in obese children than in controls [26]. IL-18 is a fundamental immune response regulator and a pleiotrophic pro-inflammatory cytokine that has an essential role early in the inflammatory cascade process [27]. Nonfat cells in human adipose tissue participate for most of the release of IL-18 emphasizing the significance of IL-18 in obese group [28]. Very few studies revealed a relationship between IL-18 and obesity in the youth, and recent reports designate that IL-18 concentration may have a linkage to obesity and modest weight loss has a valuable influence of decreasing IL-18 [29].

Our data showed that serum level of CRP was significantly higher in obesity. This was in line with other studies. Vargas and colleagues, 2016 revealed increased serum CRP in obese individual [30]. Also, Märginean and colleagues, 2019 reported that CRP was significantly higher in overweight/ obese children in contrast to the control group [31]. CRP is an acute phase inflammatory marker largely produced in the liver, and this process is controlled by pro-inflammatory cytokines [32]. Our elevated CRP levels amongst the adolescents with high body mass index established the linkage between inflammation and CRP in obese individuals. Additionally to being a sensitive marker of inflammation, CRP has direct pro-inflammatory effect. It increases reactive oxygen species and increases the release of pro-inflammatory cytokines [33]. Our study showed a significant positive correlation between CRP and BMI within the overweight/obese group. Similarly, Paepegaey and colleagues, 2015 reported that CRP increased significantly with BMI proposing that expanded and inflamed adipose tissue is the chief source of increased CRP in obesity, via producing cytokines, which potentiate hepatic release of CRP [34]. Similarly, Zimmerman and colleagues, 2011showed that for each 10% increment in BMI, the CRP steps up by 19-20% [35]. Our data showed that CRP had non-significant positive correlations with insulin and HOMA-IR among obese adolescents. Similarly, Vargas and colleagues, 2016 reported that insulin level in obesity positively correlated with the CRP [30]. Kitsios and Colleagues, 2013 revealed that serum levels of CRP directly correlated with insulin resistance in overweight/obese children and

adolescents [36]. These correlations advocated that CRP might have an essential role prompting co-morbidities in obesity and proposed a probable additional role of insulin in CRP levels and inflammation [30]. This study reported non-significant negative correlation between CRP and Adiponectin in obesity. This was in line with other studies. Vargas and Colleagues, 2016 showed that Adiponectin was low in adiposity and it negatively correlated with CRP [30]. CRP increases in response to an inflammatory process [37], and Adiponectin is an anti-inflammatory marker [38]. The decreased Adiponectin level denotes the loss of the anti-inflammatory protective influence with subsequent rise of CRP triggering inflammatory cascade in obesity [30].

Our data showed in Table 2 that serum values of MCSF were non-significantly higher among obese. Adipose tissue grows principally by hyperplasia [39]. MCSF is the first recognized factor that actively encourages hyperplasia of human adipose tissue. MCSF is produced by adipocytes, and is up regulated under conditions in which adipose tissue growth is pathologically or physiologically enhanced [6].

Our data in Table 3 showed that leukocytes counts were nonsignificantly higher while platelet counts were non-significantly lower in the high BMI group compared to controls. Neutrophils, and neutrophil / lymphocyte ratio were significantly higher with obesity in comparison to lean ones. Similarly, Märginean and colleagues, 2019 revealed higher leukocyte levels in the overweight / obese group in comparison to the control group. Though, they could not find significant difference between the two groups regarding neutrophils levels and neutrophil / lymphocyte ratio [31]. Aydin and Colleagues, 2015 revealed that neutrophil / lymphocyte ratio in the obese group was significantly higher in comparison to healthy control [40]. Obesity has been related with high counts of white blood cells [41]. Though, the precise mechanism of this relationship is unknown. Leukocytes are linked to obesity- induced chronic inflammation state via induction of the production of cytokines and other products able to induce inflammation [42], and equally involved in development of related comorbidities [30]. Adiposity related inflammation results in a multitude of immune reactions, including contribution of neutrophil in the early phases followed by involvement of macrophage and polarization of mast cell [43]. The total number of circulating neutrophils is increased in obese subjects and the rise in neutrophil count was confirmed to be directly related to the degree of the obesity and they are included in obesity- related inflammation. The high activity of neutrophil and the release of cytokines by neutrophils within the peripheral blood system augment the extent of inflammation and thus influence the surrounding tissue [44]. Additionally, neutrophils infiltrate arteries mainly during early stages of atherosclerosis [45]. Vessel infiltration by neutrophil and vascular inflammation may associate with the BMI and blood pressure. This proposes that neutrophils and chronic inflammation are a probable linkage between chronic hypertension and obesity, as one of its foremost risk factors [46]. So, inflammatory statues could be revealed by neutrophils counts. NLR is a potential inflammation marker linked to low-grade chronic inflammation [47]. NLR was displayed to be directly correlated with the degree of inflammation and might be a marker in the assessment of the severity of inflammation in obesity [40]. As obese individuals exhibit greater levels of Leptin that acts as a survival cytokine for neutrophils, these findings may elucidate the observed higher NLR in obese subjects [48]. We could not find –as shown in Table 4- significant correlations between NLR and either CRP or BMI in our study. Similar to our study, Mărginean and colleagues, 2019 failed to find a correlation between NLR and BMI in their study [31]. Vargas and Colleagues, 2016 could find significant correlation between NLR and CRP in obese subjects [30].

Our data in Table 6 demonstrated significant positive correlations between IL-10 and both insulin and HOMA-IR. Change and Colleagues, 2013 reported similar but non-significant positive correlations [14]. IL-10 has a crucial role in limitation of inflammation. Thus the low IL-10 in obesity favors a pro-inflammatory state that pave the way to glucose intolerance and insulin resistance when grown up. Further research is required in pediatrics' obesity to elucidate the role of IL-10 in the development of type 2 diabetes mellitus and metabolic syndrome in adulthood. Our data showed that IL-10 had non-significant negative correlations with both BMI and Leptin, and non-significant positive correlation with Adiponectin. Similarly, Abdelhamid and Colleagues, 2020 revealed inverse correlation between BMI and IL-10 in obesity [22], Adiponectin prompts the production of the anti-inflammatory mediator IL-10 [49]. IL-10 is produced by Th2 cells. Leptin has a differential effect on T-helper subsets by encouraging production of Th1 cytokine and suppression of Th2 cytokine production [50]. Our data showed non-significant positive correlation between IL-18 and

insulin, BMI, and HOMA-IR. Bruun and Colleagues, 2007 and TSO and Colleagues, 2010 showed significant positive correlations between IL-18 and BMI, Insulin, and HOMA-IR [29,51]. Zirlik and Colleagues, 2007 revealed that circulating levels of IL-18 directly associated with BMI, and insulin resistance [52].

Conclusion

Our study showed that overweight/obese adolescents had significantly higher CRP levels, NLR, and neutrophils count and non-significantly higher IL-18 and leukocytes count. They had non-significantly lower IL-10 levels compared to normal weight adolescents. These findings revealed that the obesity-related inflammatory state has its roots through childhood. The obese adolescents without co-morbidities are already in a state of low-grade inflammation. This favors the need for early interventions in order to preclude the development of adiposity's complications later on in adulthood.

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Conflicts of interest

There are no conflicts of interest.

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