Metabolism Open 13 (2022) 100147

Contents lists available at ScienceDirect

Metabolism Open

journal homepage: www.sciencedirect.com/journal/metabolism-open

An immediate post op and follow up assessment of circulating adipo-cytokines after bariatric surgery in morbid obesity

Astha Sachan [a], Archna Singh [a], Sakshi Shukla [a], Sandeep Aggarwal [b], Ishfaq Mir [a], Rakhee Yadav [a,*]

[a] Department of Biochemistry, 3rd Floor, Main Teaching Block, All India Institute of Medical Sciences, New Delhi, 110029, India
[b] Department of Surgical Disciplines, 1st Floor, CMET, All India Institute of Medical Sciences, New Delhi, 110029, India

ARTICLE INFO

Keywords:
Obesity
Bariatric surgery
Insulin resistance
Adipo-cytokines
Inflammation

ABSTRACT

Background: Bariatric surgery has emerged as a promising treatment for improving adipose tissue dysfunction in obesity, but the mechanisms for such amelioration are still not known. This study comprehensively explores a panel of adipo-cytokines in individuals with obesity undergoing bariatric surgery, in conjunction with markers of insulin resistance, at three time points i.e., pre-op, immediate post-op and 6 months post-surgery.

Methods: It is a case-control prospective study among obese individuals undergoing bariatric surgery (BMI ≥35 kg/m2, n=30) and non-obese subjects (BMI <25 kg/m2, n=30), comparing the levels of serum adiponectin, resistin, C-Reactive Protein (CRP), Interleukin (IL)-6 and 8, Monocyte chemoattractant protein (MCP)-1 and Tumor necrosis factor (TNF)-α between them. The same were followed at immediate and 6-month post-op periods in the former group. The serum markers were correlated with the markers of Insulin resistance like HOMA-IR, HOMA-β and QUICKI.

Results: A significant increase in adiponectin was seen after weight loss in obese group (17.54 ± 1.31 μg/mL at baseline vs 68.76 ± 1.84 μg/mL at 6- month post-surgery). CRP being an acute phase protein showed significant higher levels at immediate post-op period but declined even below its baseline at 6 months after surgery (33.34 ± 16.85 μg/mL at baseline vs 59.85 ± 23.12 μg/mL at immediate post-op vs 9.66 ± 1.84 μg/mL at 6 months post-operatively). Few inconsistencies were observed in the trajectories of IL-6 and TNF-α, while other pro-inflammatory markers indicated resolution after surgery.

Conclusion: Bariatric surgery alleviated the systemic inflammation, correlating with improved insulin resistance in individuals with obesity.

1. Introduction

The global burden of obesity has dramatically increased in the past decades in all age groups. If this trend continues, then it is speculated that more than 18% of men and 21% of women will be obese by 2025 [1]. Notably, its prevalence in the developing world is rising at an unprecedented rate, as compared to the Western world [2]. Besides, obesity brings with it a plethora of co-morbidities like type 2 diabetes, cardiovascular complications and a predilection for certain cancers. In fact, to prevent the premature deaths from such non-communicable diseases, World Health Organisation (WHO) has set the goals for the governments across the globe to prevent further rises in the obesity by 2025 [3].

While dietary changes, lifestyle modifications and pharmaceutical interventions have been known to exhibit significant weight loss and other health benefits, bariatric surgery has recently emerged as the most effective treatment for obesity and its associated comorbidities [4,5]. The mechanisms by which insulin resistance and inflammation are resolved after bariatric surgery, is largely unknown [6]. The inflammatory hypothesis, which is one of the probable theories put forward in this regard, states that most of the health hazards associated with obesity are caused by the presence of a low-grade systemic inflammation, which is created by the obesity associated adipose tissue dysfunction [7].

Thus, it becomes fundamentally acceptable to presume that bariatric surgery leads to a considerable reduction in the adipose tissue mass which further results in resolution of inflammation. Upon reviewing the

* Corresponding author. Room no. 3040, 3rd floor Main Teaching Block, AIIMS, Ansari Nagar, New Delhi, 29, India.
E-mail addresses: astha.7.sachan@gmail.com (A. Sachan), arch_singh@ymail.com (A. Singh), sakshishukla802@gmail.com (S. Shukla), sandeep_aiims@yahoo.co.in (S. Aggarwal), ishfaqashraf42@gmail.com (I. Mir), rakheeyadav@aiims.edu (R. Yadav).

https://doi.org/10.1016/j.metop.2021.100147
Received 11 September 2021; Received in revised form 31 October 2021; Accepted 31 October 2021
Available online 1 November 2021

scientific literature, it is evident that there is an altered expression of inflammatory cytokines and adipokines, such as increased production of pro-inflammatory and decreased production of anti-inflammatory adipo-cytokines in obesity [6,8,9]. In our cross-sectional study also, we found that the levels of CRP, IL-8, MCP-1 and TNF-α were higher in individuals with obesity as compared to non-obese [10]. Pro-inflammatory adipokines like IL-6 and 8, TNF-α and CRP have been found to be involved in increasing vascular reactivity, thrombosis, angiogenesis, insulin resistance, sympathetic nervous activity and inflammation, while anti-inflammatory adipokine like adiponectin were have been found to be protective against obesity and related complications [11].

At the same time, there are inconsistencies as well, which were seen in the levels of the various adipo-cytokines in obesity across studies [12]. Nevertheless, this brings out the fact that the interplay of inflammatory cytokines with the metabolic functions in obesity is more complex than expected. A cross talk amongst various cytokines adds another layer of intricacy to the overall outcome of the balance of pro- and anti-inflammatory responses in obesity. This is also evident from the significant heterogeneous results obtained from a considerable quantum of population-based follow up studies which point towards the conflicting results in the levels of various adipo-cytokines after bariatric surgery [6,13,14]. Thus, scientific community across the globe strongly believes that the association of metabolic functions of adipose tissues with inflammatory system is dynamic, complex and need a comprehensive evaluation in obesity.

Although many studies have explored the changes in the inflammatory status with the weight loss after bariatric surgery, none have taken into account the immediate post-operative period in evaluating the trajectory of their changes. Considering some of the inflammatory markers to be acute phase proteins, their status at the immediate post-op period needs to be compared with pre-op and follow-up levels.

With this background, we assessed the inflammatory adipo-cytokines namely IL-6, IL-8, CRP, TNF-α, MCP-1, adiponectin and resistin, in serum of the morbidly obese individuals undergoing bariatric surgery at baseline and compared with non-obese individuals. The same parameters were evaluated in obese group at immediate post op and at 6-month follow up periods. The panel of adipocytokines was assessed on the Luminex multiplex immunoassay in combination with drop array technology, owing to its advantages over conventional ELISA. We aimed to evaluate how the changes in their levels correlated with the type of surgeries and insulin response thereby, bringing out the important insights towards our understanding of a complex interplay of the so called "meta-inflammation" [8] in obesity.

2. Material and methods

2.1. Study design and participants

This was a prospective case-control study where the participants were recruited from the Department of Surgical Disciplines (Laparoscopic and Bariatric Surgery), AIIMS, New Delhi in a period from Jan 2018–Dec 2019. All participants belonged to the age group 18–60 years. The case group (n=30) comprised of individuals who were obese (BMI ≥35 kg/m2) undergoing bariatric surgery. They underwent restrictive bariatric surgery using either gastrectomy, or gastric bypass. The control group (n=30) included age matched individuals (18–60 years' age) with BMI within normal range (<25 kg/m2), undergoing laparoscopic cholecystectomy, hernia repair etc. similar to our previous cross-sectional study [10]. Subjects were excluded if they had concomitant known acute or chronic disorder of the immune system, like auto-immune disorders. Individuals were also excluded if they had any contraindications for general anaesthesia, psychological instability or those who did not give a consent for participation. Written informed consent was obtained from all participants of both groups, and the study was approved by the AIIMS Ethical Committee.

2.2. Anthropometric measurements and physical examination

All study participants underwent comprehensive medical evaluation including history and physical examination. The age, sex, height (cm), weight (Kg), BMI (Kg/m2) and waist circumference (cm) were recorded. Anthropometric measurements were again taken in the individuals with obesity who underwent the bariatric surgical procedure, at the time of follow up, which was at six months post-operatively.

2.3. Sample collection

Fasting venous blood samples were obtained in all the study participants pre-operatively. For the measurement of plasma glucose and Insulin, grey-capped vacutainer containing fluoride-oxalate as additive was used and for the measurement of serum cytokines, red capped plain vacutainer without additive was used (Evacuated blood collection Tubes; BD). Plasma/serum was separated after centrifuging at 3000 rpm for 15 min. Plasma was used for measuring glucose and Insulin, while serum was transferred and stored in sterile Eppendorf tubes at −80 °C for the subsequent cytokine analysis [10]. Sample collection and processing was done in a similar manner when procuring venous blood in obese group of individuals undergoing bariatric surgery, at two more time points: at time of discharge from hospital, and at 6-month follow up visit.

2.4. Analytical methods

Plasma Glucose was estimated using Randox GOD-PAP glucose estimation kit (Randox laboratories, Crumlin, UK) according to manufacturer's protocol [15]. Insulin was estimated using chemiluminescence based immunoassay in a Liaison autoanalyzer (Diasorin, Saluggia, Italy). Serum HbA1c was determined using HPLC [15]. We used multiple well-established markers of Insulin resistance like HOMA-IR, HOMA-β while QUICKI as an index for insulin sensitivity. These were used to find a correlation of insulin sensitivity with our study parameters (levels of serum adipo-cytokines) in obese group at different time points. All these are calculated markers and utilise the values of fasting plasma glucose and insulin for their calculation. So, HOMA-IR was calculated by using formula: HOMA-IR=insulin (μU/ml) × [glucose (mmol/L)/22.5] [16]. A value of ≤2.5 of HOMA-IR indicated high insulin sensitivity, whereas a value >2.5 indicated insulin resistance [15]. HOMA-β was calculated as 20 × insulin (μU/ml)/[glucose (mmol/L)-3.5] [17] and QUICKI was calculated as 1/log (I0) + log (G0)], where I0 is fasting insulin (microunits per millilitre) and G0 is fasting glucose (milligrams per decilitre). A value of <0.34 suggests insulin resistance and <0.30 means diabetes mellitus [18]. A quantitative analysis of the circulating adipo-cytokines at baseline in both the study groups and at immediate post-op (at the time discharge) and at 6th month follow up period in only group comprising individuals with obesity, was done. A panel was made for the measurement of serum Adipo-cytokines: Adiponectin, Resistin along with inflammatory cytokines like IL-6, IL-8, CRP, MCP-1, and TNF-α. These were assayed using R&D Systems Luminex Multiplex Immunoassay (Minneapolis, MN, USA) as per the manufacturer's protocol in combination with Drop array technology and were read in a BioPlex 200 reader (BIORAD Hercules, CA, USA). This system has an edge over the individual cytokine assay using conventional ELISA. Its major strength lies in the fact that it minimises reaction to reaction variations as might be seen while using separate ELISA for each cytokine and can be done in serum samples as low as 25 μL [10,19,20].

2.5. Study outcome

The primary aim of our study was to evaluate the changes in the circulating adipo-cytokines from baseline to immediate post op and at 6-month follow up period, which would help in ascertaining their effect on

weight loss after bariatric surgery. The secondary goal was to compare these changes on the basis of type of surgery and insulin response and to find a correlation with the insulin resistance at each time point.

2.6. Statistical methods

GraphPad Prism (Version 8.0.1) software was used for all the statistical analysis in our study. Shapiro-Wilk/Kolmogorov-Smirnov test was applied for testing the normality distribution in the data. Continuous data was reported as mean ± standard error of mean (SEM). Differences between the two groups were compared using the student's *t*-test for normally distributed parametric variables whereas non-parametric variables were analysed using the Mann–Whitney *U* test or Wilcoxon signed rank. The correlation between variables was calculated using Pearson's method for parametric variables and the Spearman Rho correlation test for non-parametric contrasts. A p value of <0.05 was considered to be statistically significant.

3. Results

3.1. Clinical characteristics of the study participants

A total of 60 subjects were enrolled in this study, 30 in each group. Group-I was called Case group, and had individuals with obesity, while Group-II was named the Control group, and consisted of individuals with normal BMI. The Group-I individuals underwent bariatric surgery procedures and were assessed again at immediate post-op (at the time of discharge) and 6-month follow up period. All 30 participants were assessed at pre-op and post-op period but 12 out of these 30 did not complete the 6-month follow-up assessment due to non-compliance. The Group-I had 80% women and 20% men while the Group-II comprised of 64% women and 36% women. The average age of participants in these groups, was 38.33 ± 1.99 and 39.8 ± 2.72 years respectively. The average BMI of Group-I was 46.11 ± 1.18 kg/m^2 as opposed to 24.1 ± 0.76 kg/m^2 in the Group-II (Table 1).

3.2. Baseline biochemical characteristics of the study groups

Biochemical parameters like fasting plasma glucose and insulin levels were significantly higher in Case group as compared to the Control group, along with a significant difference in the markers of insulin resistance like HOMA-IR, HOMA-β and QUICKI in both groups (Table 1). This clearly indicates that insulin resistance is highly prevalent in obese state. Among adipocytokines, we found higher levels of pro-inflammatory cytokines like CRP, IL-8, MCP-1, Resistin and TNF-α in Group-I as compared to Group-II with a significant difference only in MCP-1 (Table 1). On the other hand, IL-6 showed significantly lower values in individuals with obesity which was opposite to our expectations. Adiponectin was found to be significantly lower in the obese group when compared with non-obese counterparts, which was in line with our expectations, since adiponectin is known to be one of the most potent insulin sensitising adipokines.

Table 1
Anthropometric Characteristics, Biochemical Parameters and Serum Adipocytokines in obese and non-obese group.

	OBESE (n=30)	NON-OBESE (n=30)
Age	38.33 ± 1.99	39.8 ± 2.72
Anthropometric Characteristics:		
Body Weight (kg)	120.2 ± 4.90	61.1 ± 2.2***
BMI (Kg/m^2)	46.11 ± 1.18	24.1 ± 0.76***
Clinical Parameters:		
Fasting Serum Glucose (mg/dL)	100.3 ± 3.53	89.38 ± 3.01*
Fasting Serum Insulin (μU/L)	20.28 ± 2.45	10.75 ± 1.78***
HOMA-IR	4.727 ± 0.60	2.098 ± 0.25***
HOMA-B	272.3 ± 49.78	119.2 ± 20.23**
QUICKI	0.31 ± 0.00	0.36 ± 0.01***
Serum Inflammatory Cytokines:		
CRP (μg/mL)	33.34 ± 16.86	6.69 ± 1.35***
TNF-α (pg/mL)	26.7 ± 4.71	23.15 ± 3.83
IL8 (pg/mL)	130.3 ± 50.66	37.47 ± 6.8
MCP1 (ng/mL)	0.98 ± 0.07	0.73 ± 0.05**
IL6 (pg/mL)	23.39 ± 4.03	39.97 ± 34.9***
Serum Adipocytokines:		
Adiponectin (μg/mL)	17.54 ± 1.32	45.94 ± 7.7***
Resistin (ng/mL)	44.10 ± 5.67	36.12 ± 6.11

All data have been represented as Mean ± SEM.
p value obtained by using student *t*-test (unpaired) for parametric and Mann-Whitney-U test for non-parametric variables between two groups (p value <0.05*, <0.01**, <0.001***).

3.3. Changes in clinical and analytical parameters in patients with obesity when followed in post op-period

Group-I individuals who underwent bariatric surgery, were evaluated again, at stipulated time points in our study. In immediate post-operative period, the anthropometric and glycemic assessment did not differ from the baseline measurements. At the time of follow-up at 6th month, the average %TWL (total weight loss); calculated as {(weight lost/initial weight) x100}, was 30.05%, bringing down the mean BMI from 46.11 ± 1.18 kg/m^2 to 33.82 ± 0.89 kg/m^2 (Table 2). Additionally, there was a significant improvement in all the glucose homeostasis parameters like fasting plasma glucose, insulin, HbA1c along with calculated insulin resistance markers (Table 2).

We measured the panel of adipocytokines thrice in Group-I i.e., at pre-op, post-op (at the time of discharge) and at 6-month follow-up visit. Out of 30, only 18 participants were compliant to visit hospital for the 6th month follow-up. Assessment of serum cytokines postoperatively (at the time of discharge) revealed a significant reduction in serum TNF-α (p value<0.01) with a reduction in the levels of IL-8. On the other hand, CRP and IL-6 were found to be increased at this time. CRP is an acute phase protein and its levels at immediate post-op period were expected

Table 2
Follow up changes in Anthropometric Characteristics, Biochemical Parameters and Serum Adipo-cytokines in individuals with obesity undergoing bariatric surgery (n=30).

	BASELINE (n=30)	POST-OP (n=30)	FOLLOW UP (n=18)
Anthropometric Characteristics:			
Body Weight (Kg)	120.2 ± 4.98	–	88.10 ± 4***
BMI (Kg/m^2)	46.11 ± 1.18	–	33.82 ± 0.89***
Glucose Homeostasis:			
Fasting Serum Glucose (mg/dL)	104.3 ± 3.44	–	87.71 ± 2.273***
Fasting Serum Insulin (μU/L)	20.28 ± 2.45	–	7.89 ± 1.01***
HbA1c	6.18 ± 0.1	–	5.48 ± 0.12***
HOMA-IR	4.72 ± 0.6	–	1.80 ± 0.24***
HOMA-B	272.3 ± 49.78	–	130.9 ± 23.36**
QUICKI	0.31 ± 0.00	–	0.36 ± 0.09***
Serum Inflammatory Cytokines:			
CRP (μg/mL)	33.34 ± 16.85	59.85 ± 23.12	9.66 ± 1.84***
TNF-α (pg/mL)	26.7 ± 4.71	20.82 ± 3.58**	33.83 ± 4.98
IL8 (pg/mL)	130.3 ± 50.66	85.10 ± 26.33	93.49 ± 23.58
MCP1 (ng/mL)	0.98 ± 0.07	0.94 ± 0.08	0.88 ± 0.12
IL6 (pg/mL)	23.39 ± 4.03	34.21 ± 12	50.86 ± 5.24**
Serum Adipocytokines:			
Adiponectin (μg/mL)	17.54 ± 1.31	17.90 ± 1.31	68.76 ± 12.85***
Resistin (ng/mL)	44.10 ± 5.67	42.75 ± 5.8	29.4 ± 4.45

All data have been represented as Mean ± SEM.
p value obtained by using student *t*-test (paired) for parametric and Wilcoxon signed rank test for non-parametric variables at each time point when compared with baseline (p value <0.05*, <0.01**, <0.001***).

to be higher. At the follow-up visit, the results of bariatric surgery in terms of weight loss, were expected to be maximum. At this point CRP levels decreased significantly and adiponectin improved significantly ($p<0.001$) when compared with their baseline levels respectively, as was hypothesized. Out of the other pro-inflammatory markers; MCP-1, resistin and IL-8 decreased at this time in comparison to the baseline and contrarily IL-6 (p value<0.001) and TNF-α increased.

For further analysis, individuals from Group-I (n=30) were divided into insulin-sensitive (IS) and insulin-resistant (IR) subgroups, taking a HOMA-IR cut-off as 2.5, which is an established level for this purpose [15]. Those individuals with HOMA-IR levels <2.5 were grouped under IS and those with ≥2.5 were grouped under IR (Table 4). Thus, 22 (5 males & 17 females) individuals in Group-I had obesity with IR and 8 individuals (1 male & 7 females) out of 30, were sensitive for insulin function. IR subset demonstrated significant recovery in terms of anthropometric and clinical parameters at 6-month follow-up, while the IS sub-group did not exhibit such stark changes (Table 4). Adiponectin, IL-6 and TNF-α showed significant differences from baseline in group with IR and obesity indicating that bariatric surgery plays more beneficial role in alleviating the insulin resistance and associated inflammation in such individuals.

We also intended to bring out any differences in our study parameters due to the types of bariatric surgeries performed. For this, we divided Group-I individuals into two broad subgroups: First subgroup comprised of 18 individuals (4 males & 14 females) who underwent gastrectomy procedures and second subgroup comprised of 12 individuals (2 males and 10 females) who underwent gastric bypass surgical procedures (Table 3). Individuals of both sub-groups exhibited significant weight reduction at 6 month follow up visit (%TWL being 32.78% and 26.83% respectively) and showed an improved glycaemic profile with significant differences in fasting insulin, HOMA-IR and QUICKI in both these sub-groups when compared with their baseline levels. Adiponectin increased significantly and the levels of pro-inflammatory cytokines decreased at each time points in both these subgroups when compared with their baseline levels. Significant differences were seen at 6-month follow-up visit in the levels of MCP-1, resistin and IL-6 in those who underwent gastric bypass surgeries (Table 3) but unexpectedly levels IL-6 and TNF-α (p value <0.01) were raised in gastrectomy sub-group.

Overall, a significant increase in the adiponectin level was seen consistently after weight loss at follow-up in individuals with obesity (i.e., Group-I) and in each of the sub-groups. Secondly, CRP being an acute phase protein was found to be much higher at immediate post-op period but decreased even below its baseline levels at 6 months after weight loss. This trajectory was seen invariably for obese group and each of the sub-groups. Lastly, some inconsistencies were noted in the levels of the IL-6 and TNF- α whereas all other pro-inflammatory markers indicated resolution after surgery (Tables 2–4).

3.4. Correlation analysis of metabolic profile with serum adipo-cytokines

Since the variables in our study displayed a non-normal distribution in different groups, Spearman's correlation coefficient was used to find out the correlation between the adipo-cytokines and clinical characteristics at different time points. CRP correlated significantly with the fasting insulin ($r=0.510$; $p<0.01$) and HOMA-IR ($r=0.556$; $p<0.001$) in the Group-I at baseline. Additionally, IL-8 correlated significantly with weight ($r=0.307$; $p<0.05$) and BMI correlated with MCP-1 ($r= 0.420$; $p<0.01$) at this time of evaluation. After weight loss at the time of follow up, a significant negative correlation was observed between some of the inflammatory markers with baseline clinical characteristics like resistin ($r=-0.618$; $p<0.01$) and IL-6 ($r=-0.449$; $p<0.05$) with pre-op weight respectively and MCP-1 ($r=-0.491$; $p<0.05$) and TNF-α (-0.432; $p<0.05$) with HOMA- β likewise respectively. Consequently, it can be stated that the subjects with obesity, with higher BMI, weight and those with insulin resistance pre-operatively show much lower values of the pro-inflammatory cytokines at follow-up.

4. Discussion

The present study aims to evaluate the changes in the circulating adipo-cytokines before and after bariatric surgery induced weight loss, in obese individuals. We intended to follow such subjects till the 6th month of follow up visit when an effective weight loss is expected, particularly including an assessment at the immediate post op period. Besides, our study also seeks to assess the improvement in metabolic and anthropometric parameters together with the indices of insulin resistance at such time points and their correlations with the inflammatory markers.

While comparing the baseline parameters, the Case group i.e.,

Table 3
Follow up changes in Anthropometric Characteristics, Biochemical Parameters and Serum Adipo-cytokines in individuals with obesity (n=30) undergoing different bariatric procedures.

	GASTRECTOMY			GASTRIC BYPASS		
	BASELINE (n=18)	POST-OP (n=18)	FOLLOW UP (n=9)	BASELINE (n=12)	POST-OP (n=12)	FOLLOW UP (n=9)
Anthropometric Characteristics:						
Body Weight (Kg)	118.2 ± 6.23	–	85.73 ± 5.71***	123.1 ± 8.21	–	90.69 ± 5.77**
BMI (Kg/m^2)	45.99 ± 1.45	–	33.21 ± 1.18***	46.29 ± 2.05	–	34.48 ± 1.38***
Glucose Homeostasis:						
Fasting Serum Glucose (mg/dL)	99.26 ± 5.10	–	92.98 ± 5.48	101.8 ± 4.56	–	92.33 ± 4.28
Fasting Serum Insulin (μU/L)	16.47 ± 3.25	–	5.99 ± 0.78**	25.98 ± 3.18	–	10.37 ± 1.85***
HbA1c	5.75 ± 0.13	–	5.58 ± 0.21	6.13 ± 0.24	–	5.57 ± 0.23
HOMA-IR	3.61 ± 0.72	–	1.36 ± 0.2**	6.45 ± 0.81	–	2.33 ± 0.42***
HOMA-B	264.2 ± 75.93	–	101.8 ± 15.58	284.7 ± 52.43	–	165.8 ± 46.88
QUICKI	0.33 ± 0.01	–	0.37 ± 0.01**	0.30 ± 0.007	–	0.34 ± 0.01**
Serum Inflammatory Cytokines:						
CRP (μg/mL)	29.66 ± 24.03	50.02 ± 31.89	10.48 ± 5.47	37.21 ± 26.56	61.58 ± 31.77	6.77 ± 2.40***
TNF-α (pg/mL)	15.62 ± 2.5	12.78 ± 1.87	37.80 ± 8.77**	43.32 ± 9.48	32.88 ± 7.35	29.87 ± 4.97
IL8 (pg/mL)	74.41 ± 33.74	68.04 ± 31.06	67.86 ± 19.64	214.2 ± 114.9	110.7 ± 47.24	119.1 ± 42.58
MCP1 (ng/mL)	0.94 ± 0.08	0.82 ± 61.5	0.80 ± 0.06	1.04 ± 0.11	1.01 ± 0.15	0.70 ± 0.08*
IL6 (pg/mL)	17.82 ± 4.06	43.41 ± 19.75	93.98 ± 80.64	31.73 ± 7.64	20.40 ± 4.03	7.73 ± 2.13**
Serum Adipocytokines:						
Adiponectin (μg/mL)	17.44 ± 1.92	19.01 ± 2.01	42.59 ± 15.64**	17.68 ± 1.68	16.25 ± 1.24	94.93 ± 16.94***
Resistin (ng/mL)	32.27 ± 2.88	37.11 ± 3.24	34.8 ± 5.55	61.85 ± 12.05	51.227 ± 13.83	23.99 ± 6.78*

All data have been represented as Mean ± SEM.
p value obtained by using student *t*-test (paired) for parametric and Wilcoxon signed rank test for non-parametric variables at each time point when compared with baseline (p value <0.05*, <0.01**, <0.001***).

Table 4
Follow up changes in Anthropometric Characteristics, Biochemical Parameters and Serum Adipo-cytokines in individuals with obesity undergoing bariatric surgery (n=30) divided into IS and IR based on the HOMA-IR cut off.

	INSULIN RESISTANT OBESE (IR, n=22)			INSULIN SENSITIVE OBESE (IS, n=8)		
	HOMA IR ≥ 2.5			HOMA IR < 2.5		
	BASELINE (n=22)	POST-OP (n=22)	FOLLOW UP (n=14)	BASELINE (n=8)	POST-OP (n=8)	FOLLOW UP (n=4)
Anthropometric Characteristics:						
Body Weight (Kg)	121.1 ± 6.50	–	90.69 ± 5.21***	117.7 ± 5.02	–	82.17 ± 5.44***
BMI (Kg/m^2)	45.73 ± 1.45	–	34.08 ± 0.98***	47.16 ± 1.99	–	33.21 ± 2.01***
Glucose Homeostasis:						
Fasting Serum Glucose (mg/dL)	96.93 ± 2.94	–	92.94 ± 3.45	112.5 ± 11.94	–	91.96 ± 9.06
Fasting Serum Insulin (μU/L)	## 24.22 ± 2.77	–	9.31 ± 1.28***	## 9.43 ± 2.66	–	4.67 ± 0.66
HbA1c	6.03 ± 0.14	–	5.56 ± 0.17**	5.57 ± 0.25	–	5.63 ± 0.34
HOMA-IR	### 5.7 ± 0.61	–	2.15 ± 0.31***	### 1.18 ± 0.21	–	1.06 ± 0.17
HOMA-B	## 324.4 ± 58.09	–	150.8 ± 31.90**	## 81.08 ± 34.11	–	88.28 ± 21.74
QUICKI	### 0.30 ± 0.01	–	0.35 ± 0.01***	### 0.36 ± 0.02	–	0.39 ± 0.01
Serum Inflammatory Cytokines:						
CRP (μg/ml)	42.90 ± 22.76	55.05 ± 25.07	# 9.46 ± 3.06	33.88 ± 29.26	65.85 ± 54.5	# 12.17 ± 6.14
TNF-α (pg/ml)	30.47 ± 6.03	22.17 ± 4.61	35.53 ± 6.16	16.31 ± 4.88	17.12 ± 4.57	27.92 ± 6.52
IL8 (pg/ml)	131.4 ± 64.87	70.97 ± 27.04	91.13 ± 30.17	127.5 ± 71.79	124.0 ± 66.64	101.7 ± 18.35
MCP1 (ng/ml)	1.02 ± 0.08	0.99 ± 0.09	0.90 ± 0.15	0.87 ± 0.12	0.81 ± 0.11	0.82 ± 0.15
IL6 (pg/ml)	26.60 ± 5.14	40.92 ± 16.19	16.36 ± 6.64*	14.54 ± 4.35	15.74 ± 3.12	5.94 ± 1.94
Serum Adipocytokines:						
Adiponectin (μg/ml)	17.29 ± 1.50	17.09 ± 1.32	70.32 ± 14.25***	18.21 ± 2.88	20.13 ± 3.39	63.33 ± 33.49
Resistin (ng/ml)	44.75 ± 6.92	39.94 ± 6.78	27.14 ± 4.79*	42.30 ± 10.22	50.51 ± 11.96	37.3 ± 11.39

Data is represented as Mean ± SEM. It was analysed using the paired Student's *t*-test for parametric variables and Wilcoxon Sign rank test for non-parametric variables.
* Comparison of post-op & follow up data with baseline within same sub-group i.e., IR or IS; *p <0.05, **p <0.01, ***p <0.001.
# Intergroup comparison of data at respective time points, e.g., follow up CRP of IR with that of IS. #p <0.05, ##p <0.01, ###p <0.001.

Group-I exhibited a diabetogenic profile. The fasting plasma glucose and serum insulin were both found to be significantly higher in Group-I, as compared to Group-II. This led us to evaluate the measures of insulin resistance using HOMA-IR, which is a calculated index as well as HOMA-β, which predicts pancreatic beta-cell function. Both HOMA-IR and HOMA-β were significantly high in Group-I. Combined with raised serum insulin, these findings point towards a prevailing insulin resistance in a non-diabetic obese state because all our participants were clinically non-diabetics. This was consolidated by another calculated index, QUICKI, which is one of the most accurate and useful indices for determining insulin sensitivity [21]. A value of <0.34 suggests insulin resistance and those <0.30 indicates diabetes mellitus. It was found that Group-I had a significantly lower mean QUICKI, lying in the insulin resistant range. In next 6 months, following bariatric surgery and significant weight loss; subjects showed a significant improvement in all the markers and indices of insulin resistance.

Adiponectin plays a vital role in glucose metabolism and its regulation [22,23]. Thus, overexpression of adiponectin has a protective role against insulin resistance. Many previous studies have shown that its expression decreases in obesity and increases with weight loss [23]. In our study also, the most consistent and significant finding at each time-point and in each subgroup was that adiponectin was low in obese state, and raised with weight loss after bariatric surgery. Antagonistic in function to adiponectin is another adipokine named resistin, which is secreted by visceral adipocytes. It increases hepatic gluconeogenesis and insulin resistance [24]. It has also been shown to exert pro-inflammatory effects through enhancing the nuclear factor kappa (NF-KB), light chain enhancer in activated B-cell pathway and increasing the production of IL-6 and TNF-α [25]. In our study we found that circulating resistin was higher in participants with obesity as compared to those without, and its levels decreased with weight loss post-operatively. Resistin is a pro inflammatory adipokine, which is thought to be an important player in progression of obesity and subsequent pathogenesis of Type 2 DM in obese individuals. When studied separately, IR subgroup displayed a significant reduction in its levels with surgically induced weight loss. The evidence of the changes in the levels of resistin in response to bariatric surgery has been inconclusive in literature where some studies reported its significant reductions [26–28], while others reported contradictory findings [29,30].

It is well documented that the chances of contracting the obesity associated co-morbidities increases manifold in presence of an exacerbated inflammatory state. This is due to the fact that adipose tissue secretes a number of pro-inflammatory cytokines; most important being IL-6 and TNF-α which promote its dysfunction and insulin resistance [31]. These may be secreted directly from adipocytes or the immune cells that take up residence in the adipose tissue [32].

IL-6 is a pleiotropic cytokine that has complex effects on obesity-associated metabolic dysfunctions [33] and circulating concentration of IL-6 increases with adipose tissue mass and it has been suggested that about 30% of total circulating IL-6 originates from adipose tissue [34]. Albeit, this is in contradiction with our study, where we found IL-6 to be comparatively lower in individuals with obesity, prior to surgery and to be escalated gradually to significantly higher levels at the time of follow up. Recently, the pro-inflammatory role of IL-6 in insulin resistance associated with human obesity has been challenged, as it has been reported to exert some insulin-sensitising effects in both healthy human individuals and those with type 2 diabetes [35]. It is also known from previous studies that anti-inflammatory effects of IL-6 are brought by its inhibitory action on TNF-α, which is one of the most commonly implicated cytokines in metabolic dysfunctions of obesity and is secreted by macrophages present in adipose tissue's stromal vascular fraction [36]. Furthermore, the reductions in TNF-α are considered to be a protective measure from insulin resistance induced by high-fat diet [37]. Again contrarily, we found that subjects with obesity had higher TNF-α as compared to healthy controls before surgery. But after surgery, as levels of IL-6 started to increase in immediate post-operative period, TNF-α decreased significantly. As time elapsed after surgery and at 6th month follow up assessment, TNF-α portrayed a rising trend with a concomitant increase in IL-6. So, by convention it becomes inevitable to state that the interplay of cytokines is much more complex than was expected earlier. This is also reinforced by findings of the Kartz et al. [38] and Lindegaard et al. [39] who had reported similar discordant results about circulating IL-6 in their studies, and also in a meta-analysis which reported several inconclusive results on exploring the impact of bariatric surgeries on serum IL-6, TNF-α and CRP [6]. Another meta-analysis found while exploring the literature, advocated a decrease in IL-6 levels accompanying the weight loss after bariatric surgery [40].

The most often measured inflammatory marker which has been

consistently correlated with weight, BMI, waist-hip ratios and insulin resistance is CRP, so much so that it has become the marker of "meta-inflammation" [6,41]. While comparing our study subjects, we found Group-I had higher circulating CRP than Group-II. The serum levels spiked in immediate post-op period which we speculate could be attributed to the acute phase reactant characteristic of CRP and might be consequent to the invasive procedure which the individuals underwent. At the time of follow up at 6th month, the serum levels were notably lower than baseline. As CRP also correlated positively to the serum insulin and HOMA-IR prior to the surgery, it could predictably be commented that CRP is associated with the development of insulin resistance. Furthermore, IL-6 was postulated to be a driver of CRP synthesis and its secretion from liver [41]. Our data disputed such a relation between the two, since CRP levels dropped with increasing weight loss, while IL-6 continued to rise.

In our study, MCP-1 and IL-8 were also analysed as part of the study panel in all study groups. IL-8 has previously been shown to increase in obesity, thereby, contributing to the ongoing low-grade systemic inflammation in obesity [42]. It plays a role in local immune activation. It acts by promoting recruitment of various immune cells like neutrophils and macrophages to the stromal vascular fraction, subsequently worsening the inflammation. Previous studies have observed a significant difference between the individuals with BMI ranging from normal to overweight, in terms of serum level of IL-8 [19,43]. In our study also, we found notably higher IL-8 levels in subjects who were obese as compared to control group. Assessment for prospective changes in its levels revealed a declining trend after surgery at various time points, though the differences were not significant. IL-8 also correlated to weight preoperatively, hence pointing towards the role of obese adipose tissue in promoting its own dysfunctions. One of the most common immune cells infiltrating adipose tissues are macrophages and their recruitment to adipose tissue is facilitated by MCP-1 secreted by adipose tissue itself [41,44]. In our study MCP-1 was found to be significantly higher in obese state, decreased with weight loss and this drop was consistent in all the groups and sub-groups especially significantly in those who underwent gastric bypass surgeries.

When the subgroup with gastrectomy procedure was compared with the subgroup of individuals with gastric bypass surgeries, the improvement in weight and BMI was equally significant. But closely following the serum markers, it was noted that gastric bypass surgery had a better outcome in terms of resolution of insulin resistance over the course of study. This was reflected in the glycaemic parameters namely serum insulin, HOMA-IR and QUICKI; as well as serum estimation and comparison of the adipo-cytokines, CRP, IL-8, MCP-1, Resistin and IL-6 (Table 3).

5. Conclusion

Overall, our study has put forward that consequent to bariatric surgery, there is alleviation of the pro-inflammatory milieu in the body with concurrently significant improvement in insulin resistance. So far, prospective studies assessing the changes in various inflammatory adipokines and cytokines following bariatric surgery have measured them at different time points like 3, 6, 12 and 24 months post-operatively [45–47]. Those catering to the assessment at less than 3 months post-operatively are very few and to the best of our knowledge none has taken into account the assessment at immediate post op period. Another distinction that this study holds is the comparison of outcome of bariatric procedures according to the type of surgery, where gastric bypass was found to be more beneficial as compared to gastrectomy.

Considering the complexity of the metabolic and inflammatory functions of adipose tissue along with the dynamicity and associated co-morbidities in obesity, our study will add more evidence to the trajectories of the changes in these parameters right from the beginning of the bariatric surgery. This will further enhance our knowledge whether improvement in cytokine profile eventually translate into a significant clinical benefit with regard to obesity related morbidity and mortality. Our study did not take into consideration the dietary and physical activity patterns of the participants in the time following bariatric surgery. The variations in the glycaemic profiles and insulin sensitivity, especially in individuals who had diabetes or insulin resistance at baseline, could also be affected by concomitant anti-diabetic treatments. This is another confounding factor that may be looked into. Future prospective studies with a bigger sample size, stringent follow up and a range of biomarkers which could best predict the outcomes of bariatric surgery in terms of resolution of co-morbidities would bring out more objectivity to the spectrum of benefits of such weight loss surgeries.

Author contributions

RY, SA, and ArS conceptualised and designed the study; AsS, SS, and IM acquired the data; RY and AsS performed analysis and interpretation of data; AsS analysed data statistically; RY and AsS drafted the manuscript; RY, SA, and ArS critically revised the manuscript; RY obtained funding, provided administrative, technical, or material support, and supervised the study.

CRediT authorship contribution statement

Astha Sachan: acquisition, Formal analysis, drafting of the manuscript; statistical analysis. **Archna Singh:** Study concept and design, critical revision of the manuscript. **Sakshi Shukla:** acquisition of data. **Sandeep Aggarwal:** Study concept and design; critical revision of the manuscript. **Ishfaq Mir:** acquisition of data. **Rakhee Yadav:** Study concept and design, Formal analysis, drafting of the manuscript, critical revision of the manuscript; obtained funding, administrative, technical, or material support, Supervision.

Declaration of competing interest

Authors declare no conflict of interest.

Acknowledgements

This work was funded by IMRG (Project code: A-536), by AIIMS. We thank SA for providing, and Mr. Mudassir for collecting patient samples from Dept of Surgical Disciplines. We also thank Dr Sivasankar Baalasubramanian (Executive Director, Indoor Biotechnologies India Pvt. Ltd., Bangalore) for analysing the samples for the planned array of cytokines.

References

[1] Di Cesare M, Bentham J, Stevens GA, et al. Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19.2 million participants. Lancet 2016;387:1377–96.
[2] Hruby A, Hu FB. The epidemiology of obesity: a big picture. Pharmacoeconomics 2015;33:673–89.
[3] World Health Organisation. About 9 voluntary global targets. https://www.who.int/nmh/ncd-tools/definition-targets/en/.
[4] Colquitt JL, Pickett K, Loveman E, Frampton GK. Surgery for weight loss in adults. Cochrane Database Syst Rev 2014;8. Art. No.: CD003641.
[5] Poirier P, Cornier MA, Mazzone T, et al. Bariatric surgery and cardiovascular risk factors. Circulation 2011;123:1683–701.
[6] Askarpour M, Khani D, Sheikhi A, Ghaedi E, Alizadeh S. Effect of bariatric surgery on serum inflammatory factors of obese patients: a systematic review and Meta-Analysis. Obes Surg 2019;29:2631–47.
[7] Cornejo-Pareja I, Clemente-Postigo M, Tinahones FJ. Metabolic and endocrine consequences of bariatric surgery. Front Endocrinol 2019;10:626.
[8] Hotamisligil GS. Inflammation and metabolic disorders. Nature 2006;444(7121):860–7. https://doi.org/10.1038/nature05485.
[9] Sams VG, Blackledge C, Wijayatunga N, et al. Effect of bariatric surgery on systemic and adipose tissue inflammation. Surg Endosc 2016;30:3499–504.
[10] Sachan A, Singh A, Shukla S, Aggarwal S, Mir I, Yadav R. Serum adipocytokines levels and their association with insulin sensitivity in morbidly obese individuals undergoing bariatric surgery. J Obes Metab Syndr 2020;29(4):303–12.
[11] Ohashi K, Shibata R, Murohara T, Ouchi N. Role of anti-inflammatory adipokines in obesity-related diseases. Trends Endocrinol Metabol 2014;25(7):348–55.

[12] Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol 2011;11(2):85–97.
[13] Rakotoarivelo V, Lacraz G, Mayhue M, et al. Inflammatory cytokine profiles in visceral and subcutaneous adipose tissues of obese patients undergoing bariatric surgery reveal lack of correlation with obesity or diabetes. EBioMedicine 2018;30:237–47.
[14] Zhang C, Zhang J, Liu Z, Zhou Z. More than an anti-diabetic bariatric surgery, metabolic surgery alleviates systemic and local inflammation in obesity. Obes Surg 2018;28:3658–68.
[15] Vincent V, Thakkar H, Aggarwal S, Mridha AR, Ramakrishnan L, Singh A. ATP-binding cassette transporter A1(ABCA1) expression in adipose tissue and its modulation with insulin resistance in obesity. Diabetes, Metab Syndrome Obes Targets Ther 2019;12:275–84.
[16] Singh Y, Garg MK, Tandon N, Marwaha RK. A study of insulin resistance by HOMA-IR and its cut-off value to identify metabolic syndrome in urban Indian adolescents. J Clin Res Pediatr Endocrinol 2013;5(4):245–51.
[17] Sung KC, Reaven GM, Kim SH. Utility of homeostasis model assessment of beta-cell function in predicting diabetes in 12,924 healthy Koreans. Diabetes Care 2010;33(1):200–2.
[18] Katz A, Nambi SS, Mather K, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. JCEM 2000;85(7):2402–10.
[19] Harakeh S, Kalamegam G, Pushparaj PN, et al. Chemokines and their association with body mass index among healthy Saudis. Saudi J Biol Sci 2020;27:6–11.
[20] Dalmas E, Rouault C, Abdennour M, et al. Variations in circulating inflammatory factors are related to changes in calorie and carbohydrate intakes early in the course of surgery-induced weight reduction. Am J Clin Nutr 2011;94:450–8.
[21] Chen H, Sullivan G, Quon MJ. Assessing the predictive accuracy of QUICKI as a surrogate index for insulin sensitivity using a calibration model. Diabetes 2005;54(7):1914–25.
[22] Trujillo ME, Scherer PE. Adiponectin–journey from an adipocyte secretory protein to biomarker of the metabolic syndrome. J Intern Med 2005;257(2):167–75.
[23] El Husseny MW, Mamdouh M, Shaban S, et al. Adipokines: potential therapeutic targets for vascular dysfunction in type II diabetes mellitus and obesity. J Diabet Res 2017;2017:8095926.
[24] Steppan CM, Lazar MA. Resistin and obesity-associated insulin resistance. Trends Endocrinol Metabol 2002;13(1):18–23.
[25] Singla P, Bardoloi A, Parkash AA. Metabolic effects of obesity: a review. World J Diabetes 2010;1(3):76–88.
[26] Salman MA, Salman AA, Nafea MA, et al. Study of changes of obesity-related inflammatory cytokines after laparoscopic sleeve gastrectomy. ANZ J Surg 2019;89(10):1265–9.
[27] Edwards C, Hindle AK, Fu S, Brody F. Downregulation of leptin and resistin expression in blood following bariatric surgery. Surg Endosc 2011;25(6):1962–8.
[28] Marantos G, Daskalakis M, Karkavitsas N, Matalliotakis I, Papadakis JA, Melissas J. Changes in metabolic profile and adipoinsular axis in morbidly obese premenopausal females treated with restrictive bariatric surgery. World J Surg 2011;35(9):2022–30.
[29] Nieva-Vazquez A, Pérez-Fuentes R, Torres-Rasgado E, López-López JG, Romero JR. Serum resistin levels are associated with adiposity and insulin sensitivity in obese Hispanic subjects. Metab Syndr Relat Disord 2014;12(2):143–8.
[30] Wolfe BE, Jimerson DC, Orlova C, Mantzoros CS. Effect of dieting on plasma leptin, soluble leptin receptor, adiponectin and resistin levels in healthy volunteers. Clin Endocrinol 2004;61(3):332–8.
[31] Mathieu P, Poirier P, Pibarot P, Lemieux I, Després JP. Visceral obesity: the link among inflammation, hypertension, and cardiovascular disease. Hypertension 2009;53(4):577–84.
[32] Phillips CL, Grayson BE. The immune remodel: weight loss-mediated inflammatory changes to obesity. Exp Biol Med 2020;245(2):109–21.
[33] Kraakman MJ, Allen TL, Whitham M, et al. Targeting gp130 to prevent inflammation and promote insulin action. Diabetes Obes Metabol 2013;15(Suppl 3):170–5.
[34] Mohamed-Ali V, Goodrick S, Rawesh A, et al. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. J Clin Endocrinol Metab 1997;82(12):4196–200.
[35] Pedersen BK, Febbraio MA. Point: interleukin-6 does have a beneficial role in insulin sensitivity and glucose homeostasis. J Appl Physiol 2007;102(2):814–6.
[36] Shoelson SE, Ferrero L, Naaz A. Obesity, inflammation, and insulin resistance. Gastroenterology 2007;132:2169–80.
[37] Johar D, Ahmed SM, El Hayek S, et al. Diabetes-induced proteome changes throughout development. Endocr Metab Immune Disord - Drug Targets 2019;19(6):732–43.
[38] Kratz M, Hagman DK, Kuzma JN, et al. Improvements in glycemic control after gastric bypass occur despite persistent adipose tissue inflammation. Obesity 2016;24:1438–45.
[39] Lindegaard KK, Jorgensen NB, Just R, Heegaard PM, Madsbad S. Effects of Roux-en-Y gastric bypass on fasting and postprandial inflammation-related parameters in obese subjects with normal glucose tolerance and in obese subjects with type 2 diabetes. Diabetol Metab Syndrome 2015;7:12.
[40] Askarpour M, Khani D, Sheikhi A, Ghaedi E, Alizadeh S. Effect of bariatric surgery on serum inflammatory factors of obese patients: a systematic review and meta-analysis. Obes Surg 2019 Aug;29(8):2631–47.
[41] Heinrich PC, Castell JV, Andus T. Interleukin-6 and the acute phase response. Biochem J 1 February 1990;265(3):621–36.
[42] Després JP, Lemieux I. Abdominal obesity and metabolic syndrome. Nature 2006;444(7121):881–7.
[43] Kim CS, Park HS, Kawada T, et al. Circulating levels of MCP-1 and IL-8 are elevated in human obese subjects and associated with obesity-related parameters. Int J Obes 2006;30:1347–55.
[44] Cao H. Adipocytokines in obesity and metabolic disease. J Endocrinol 2014;220:T47–59.
[45] Min T, Prior SL, Dunseath G, Churm R, Barry JD, Stephens JW. Temporal effects of bariatric surgery on adipokines, inflammation and oxidative stress in subjects with impaired glucose homeostasis at 4 Years of follow-up. Obes Surg 2020;30(5):1712–8.
[46] Farias G, Netto BDM, Boritza K, Bettini SC, Vilela RM, Dâmaso AR. Impact of weight loss on inflammation state and endothelial markers among individuals with extreme obesity after gastric bypass surgery: a 2-year follow-up study. Obes Surg 2020;30(5):1881–90.
[47] Goktas Z, Moustaid-Moussa N, Shen CL, Boylan M, Mo H, Wang S. Effects of bariatric surgery on adipokine-induced inflammation and insulin resistance. Front Endocrinol 2013;4:69.