

Immunological & metabolic responses to a therapeutic course of *Basti* in obesity

Urmila Thatte, Shubhada Chiplunkar*, Supriya Bhalerao**, Aditi Kulkarni†, Raman Ghungralkar†, Falguni Panchal**, Shamal Vetale*, Pradeep Teli*, Dipti Kumbhar** & Renuka Munshi**

*Department of Clinical Pharmacology, Seth GS Medical College & KEM Hospital, *Chiplunkar Laboratory, Advanced Centre for Treatment, Research & Education in Cancer (ACTREC), Tata Memorial Centre, **Department of Clinical Pharmacology, TNMC & BYL Nair Charitable Hospital & †Department of Kayachikitsa, MA Podar Ayurvedic Hospital, Mumbai, India*

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Background & objectives: *Basti* (medicated enema) is a popular Ayurvedic intervention recommended for obesity. However, there are no data to show whether any physiological or biochemical changes occur following this treatment. This study was conducted to identify the immunological and metabolic changes in obese individuals after a therapeutic course of *Basti*.

Methods: Thirty two obese individuals (18 and 60 yr) with a body mass index (BMI) ≥ 30 kg/m² who received a therapeutic course of 16 enemas (*Basti*) followed by a specific diet and lifestyle regimen for a period of 32 days as their treatment for obesity, were enrolled in the study. Clinical examination, measurement of immune and metabolic markers were done before (S1), immediately after (S2) and 90 days after the completion of therapy (S3).

Results: A significant reduction ($P < 0.001$) in weight, BMI, upper arm and abdominal circumference was seen at S3, along with a decrease in serum interferon (IFN)- γ ($P < 0.02$), interleukin (IL)-6 ($P < 0.02$) and ferritin ($P < 0.05$) and increase in IgM levels ($P < 0.02$). Peripheral blood lymphocytes (PBLs) stimulated with anti-CD3 monoclonal antibodies showed significant increase in reactive oxygen species (ROS) generation and calcium flux after *Basti*. All organ function tests revealed no changes.

Interpretation & conclusions: Our study documents that a therapeutic course of *Basti* modulates immune responses by regulating pro-inflammatory cytokines, immunoglobulins and functional properties of T-cells. These changes are associated with a reduction in the body weight which is maintained even after three months of treatment. The study also documents the safety of *Basti* procedure.

Key words *Basti* - immunological markers - metabolic markers - obesity - *Panchakarma*

Obesity is defined by the World Health Organization (WHO)¹ as a body mass index (BMI) equal to or more than 30 kg/m². It is associated with an elevated risk of cardiovascular disease^{2,3} and type 2 diabetes due to co-existing metabolic abnormalities including impaired fasting glucose, hyperinsulinaemia and dyslipidaemia (characterized by low level of HDL and high plasma triglycerides)⁴. Increasing adiposity is also associated with low micronutrient levels due to inadequate nutrient intake and/or alterations in nutrient metabolism⁵. One of the hallmarks of obesity is an increase in circulating markers of oxidative stress and low grade inflammation characterized by increased levels of circulating C-reactive protein (CRP) and/or other inflammatory cytokines such as tumour necrosis factor alpha (TNF- α) and interleukin-6 (IL-6)^{6,7}. Adipocytokines such as TNF- α or IL-6 are also reported to be elevated with increasing adiposity^{8,9} and are closely correlated with CRP, a known inflammatory marker¹⁰.

Apart from the definite benefits from change in lifestyle, diet and regular exercise, various pharmacological interventions have been investigated with limited success. For example, orlistat which is approved for use in obesity¹¹ leads to a modest weight loss of 2.9 kg after 1 to 4 yr and its use is associated with many gastrointestinal side effects¹². For the extremely obese patient with established complications, surgery may be the most appropriate intervention and may be lifesaving¹³. However, due to its cost and the risk of complications, the search is still on for other effective and safe treatments. Alternative therapies are also used widely without the necessary evidence of benefit or safety.

Ayurveda, the Indian traditional medicine system has described *Basti* (medicated enema) as an intervention for many disorders especially lifestyle disorders including obesity¹⁴. Ayurvedic texts describe the indications and contraindications for *Basti* therapy¹⁵. However, no study has yet attempted to document its safety and whether this intervention has any effect in obese individuals. In absence of data to show whether any physiological or biochemical changes occur following this treatment, we conducted this study to assess the immunological and metabolic variables in obese individuals after a therapeutic course of *Basti*.

Material & Methods

Thirty two consecutive patients attending the Internal Medicine Out Patient Department (OPD) of MA Podar Ayurvedic Hospital, Mumbai, India,

for treatment of obesity who were prescribed *Basti* treatment as per Ayurvedic criteria¹⁶, were invited to participate in this study. Patients in the age groups of 18 to 60 yr with a BMI \geq 30 kg/m² and waist-hip ratio \geq 1.0 in men and \geq 0.9 in women were invited to participate in the study after obtaining their written informed consent. Acutely ill patients with severe cardiac, respiratory, hepatic and renal dysfunction and pregnant or lactating women were excluded from the study. The study was carried out during the period from August 2007 to December 2010.

As this was a hypothesis generating proof of concept study where we wished to study whether any changes occurred in metabolic and immune parameters in patients who were receiving *Basti* as therapy for obesity, a sample size of at least completed 30 patients was considered adequate for analysis.

Following written informed consent to participate, a detailed clinical history was taken from each individual and relevant physical examination including recording of weight and measurement of anthropometric data like waist-hip ratio, upper arm circumference and abdominal circumference was carried out. Blood sample (45 ml) was collected aseptically from the ante-cubital vein and processed for measurement of various metabolic and immunological parameters. The study protocol was approved by the Institutional Ethics Committee of MA Podar Ayurvedic Hospital, Mumbai.

Samples were coded at the clinical site and dispatched to the laboratory. After collection of blood sample at baseline (S1), the prescribed therapeutic course of 16 *Bastis* in a regular pattern as described in *Charak Samhita* (known as *kala basti*¹⁵) was initiated.

On day 1, *Anuvasan Basti* (oil enema) with sesame oil of 240 ml was administered through the anal canal with the help of syringe and a rubber catheter. The next day *Asthapana Basti* (enema with decoction) consisting of *til tail* 120 ml, *Triphala kwatha* (decoction of *Terminalia bellerica*, *Terminalia chebula* & *Phyllanthus emblica*)-700 ml, cow's urine -150 ml, honey 10 g and Black salt 10 g was administered. This course of alternate *Anuvasana* and *Asthapana Basti* was administered six times and then *Anuvasan Basti* four times on consecutive days. During the course, each participant was observed for symptoms of proper, inadequate or over effects of *Anuvasana Basti* and *Asthapana Basti* daily.

After completion of this course of 16 days, the participants were advised a diet of food items that

are easy to digest like *khichadi*, *moong dal*, *roti* and requested to avoid fried and carbohydrate rich foods, cold drinks, *etc.* during the course of *Basti*. They were also asked to alter their lifestyle regimen (*e.g.* to avoid sitting and standing for the prolonged period, excessive/loud speaking, strenuous travelling/ walking for long distance, day time sleep, suppression of natural urges, sex, overexposure to breeze, sunlight, smoke, dust, use of pillow of unsuitable thickness, *etc.*) for a period of 32 days as per the recommendations of Ayurveda¹⁵. At the end of this period the course of *Basti* was considered complete. Clinical examination and blood investigations were repeated (S2) at this point as in the baseline visit.

The final blood sample (S3) was collected 90 days after S2 along with clinical examination to assess whether the effect of *Basti* was maintained. During this period, no dietary or lifestyle modifications or medications for weight control were recommended. Thus, the participant was under observation for a total of 138 days after being recruited in the study. The day Sample 1 (S1) was collected was designated as day 0, S2 was collected on day 48 (16 *Basti* days and 32 days of lifestyle and diet restrictions) and S3 on day 138. Anthropometric parameters such as weight, BMI, waist-hip ratio, upper arm and abdomen circumference were measured at the time of S1, S2 and S3 collections.

Isolation of peripheral blood lymphocytes (PBLs) was done from the heparinized peripheral venous blood by Ficoll-Hypaque (Sigma-Aldrich, USA) centrifugation method¹⁷.

Immunophenotyping of peripheral blood lymphocytes (PBLs): PBLs were incubated with phycoerythrin (PE) labelled mouse anti-human monoclonal antibodies (MAb) targeted against CD3, CD4, CD8, CD14, CD19, CD56, CD80, CD86, CD209, CD11c, $\alpha\beta$ -T cell receptor (TCR) and $\gamma\delta$ -TCR (BD Pharmingen, USA) for surface staining as per manufacturer's instructions. Activation markers were assessed by using dual colour flow cytometry. PBLs were stained with fluorochrome conjugated monoclonal antibodies against activation markers such as fluorescein 5-isothiocyanate (FITC) conjugated CD25, CD69, CD71 and PE-conjugated HLA-DR (BD Pharmingen, USA) and further incubated with FITC or PE conjugated mouse anti-human monoclonal antibody targeted against CD3. Appropriate isotype controls were used in all experiments. PBLs were acquired on FACSCalibur flow cytometer

(Becton Dickinson, USA). The data were analyzed with CellQuest software (Becton Dickinson, USA).

Estimation of intracellular calcium release: PBLs (1×10^6 /ml 0.01M PBS) were loaded with 5 μ M Fluo-3-AM for 30 min at 37°C. One μ g of anti-CD3 MAb was added as stimulant and fluorescence intensity was measured immediately on the FACSCalibur flow cytometer for the determination intracellular calcium release at different time points as previously described¹⁸.

Measurement of intracellular reactive oxygen species in lymphocytes: Reactive oxygen species (ROS) level in PBL was measured using 2', 7' dichlorofluorescein diacetate (DCFH-DA) dye (Sigma, USA). PBLs were loaded with 4 μ M dye and incubated for 30 min at 37°C. Anti-CD3 MAb (1 μ g) was added as stimulant and increase in mean fluorescence intensity was measured from 0 to 45 min, as described earlier¹⁹. Analysis was performed using Cell Quest software and results were expressed as mean fluorescence intensity (MFI).

Lymphocyte turnover by Annexin FITC staining: PBLs were incubated with PE labelled mouse anti-human monoclonal antibodies targeted against CD3 and CD19 (BD Pharmingen, USA) for surface staining. Lymphocyte turnover (apoptosis) was further monitored by Annexin V FITC (BD Pharmingen, USA) staining method as per manufacturer's guidelines and the data analyzed using the Cell Quest software.

Lymphocyte-proliferation assay: Proliferative responses in PBLs were analyzed using tritiated thymidine incorporation assay, according to standard protocol²⁰. Anti-CD3 MAb (1 μ g) was used as a stimulant. Tritiated thymidine incorporation was measured in a liquid beta scintillation counter (Model 1900, Packard, USA) as counts per minute (cpm). Stimulation index (S.I.) was calculated as the ratio of mean cpm of cultures stimulated with anti-CD3 MAb and unstimulated cultures.

Cytokine and immunoglobulin ELISA: PBLs (1.5×10^5 /200 μ l cRPMI/well) were stimulated with 1 μ g anti-CD3 MAb for 48 h at 37°C. Cell-free supernatants were collected and analyzed for secreted cytokines interferon gamma (IFN- γ), interleukin (IL)-10, IL-4, IL-8, TNF- α , IL-6 and IL-1 α using Opt-EIA sandwich ELISA kit (BD, Biosciences, USA) as per the manufacturer's instructions. Serum samples were also analyzed for the above mentioned cytokines using the same kits.

Immunoglobulins, IgG and IgM levels in serum samples from obese individuals were measured using the IgG and IgM detection kit (Bethyl Laboratories Inc., Montgomery, TX, USA) as per the manufacturer's recommendations.

Cytotoxicity assay: The cytotoxicity of lymphocytes was monitored by ^{51}Cr -release assay. PBLs (effectors) were co-cultured with ^{51}Cr -labelled K562 targets (5000 cells per well) in effector to target E:T cell ratios 40:1, for 4 h at 37°C, according to standard protocol²¹. The percentage of cytotoxic activity was calculated using the following formula: % specific lysis = (sample cpm - spontaneous cpm) / (maximal cpm - spontaneous cpm) × 100.

Complete blood count (CBC) was done on haematology analyzer SYSMEX KX-21 (Sysmex Corporation, Kobe, Japan) and erythrocyte sedimentation rate (ESR) was done by the Westergren method²².

Fasting blood sugar, liver function tests [serum bilirubin, serum alanine transaminase (ALT), aspartate transaminase (AST) alkaline phosphatase (ALP), γ -glutamyl transferase GGT], renal function tests (blood urea nitrogen, serum creatinine, protein, albumin and uric acid), minerals (serum calcium, phosphorus, iron and copper), lipid profile (serum cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol) were analyzed using standard biochemical kits (AMP Diagnostics, Austria) on a fully automated biochemical analyzer (Biochemical Systems International SRL, Italy). Fasting insulin, vitamin B12 and endocrine markers (T3, T4, TSH, cortisol) were measured using standard radioimmunoassay kits (Shinjin Medics Inc., South Korea). Homeostatic model assessment-insulin resistance (HOMA-IR) index was calculated using the formula [Sugar (mmol/l) × Insulin]/22.5. Glycosylated haemoglobin and high sensitive (*hs*)-CRP were measured using Nycocard Reader (Axis Shield, Norway). Vitamin A levels were measured using standard HPLC kit (Recipe Chemicals, + Instruments, GmbH Germany). Ferritin levels were measured using standard ELISA kits (Calabazas, CA, USA).

For assurance of quality, both internal and external quality control (obtained from Christian Medical College, Vellore, India and BIO RAD, USA) samples were processed. For maintaining the integrity of metabolic data all the kits procured for analyzing the parameters were kept constant throughout the study.

Statistical analysis

Immunologic variables - The results were analyzed using SPSS software (Version 15.0, SPSS Inc., USA) or GraphPad Prism software (version 5.01), (GraphPad Software, Inc., USA). Overall significance was assessed by using one-way ANOVA or Friedman test; Wilcoxon signed rank test was applied for multiple comparisons at $P < 0.02$ (after applying the Bonferroni correction). Analysis of serial measurements (*viz.*, intracellular Ca^{2+} flux and ROS) was done using MedCalc software (version 11.4.4), (MedCalc Software bypa, Belgium). The Spearman's rank correlation between various parameters was analyzed at $P < 0.05$.

Clinical and metabolic variables - All results were analyzed using GraphPad InStat software (version 3.5) and GraphPad Prism software (version 5.01). The normally distributed data were expressed as mean \pm SD (95% confidence interval) and analysed using paired 't' test. The data not normally distributed were expressed as median (IQR) and statistical analysis was done by Wilcoxon signed rank test.

Results

Of the 74 participants who were prescribed *Basti* treatment during the study period, 48 were included. Of these, 15 participants did not come for S2 and S3 collections and were excluded from the analysis. One of the participants withdrew due to adverse events.

Of the 32 patients who completed the study, 25 were women. The mean age (\pm SD, range) of the participants was 42.5 (\pm 8.44, 22.00 - 58.00) yr. A significant ($P < 0.001$) decrease occurred in weight, BMI, upper arm and abdominal circumference immediately after the course of *Basti* (at S2) and this was maintained at S3. The waist hip ratio remained unaltered throughout the course (Table I).

As shown in Fig. 1, a marked decrease in levels of IFN- γ (3.771 ± 4.63 to 1.54 ± 2.23 pg/ml; $P < 0.02$) and IL-6 (111.2 ± 152.05 to 52.85 ± 111.24 pg/ml; $P < 0.02$) was observed at S2 as compared to S1. IL-6 levels remained significantly low in serum even at S3 compared to S1 (27.79 ± 79.29 pg/ml; $P < 0.002$). A reduction in IL-1 β levels was observed at S3 and a gradual decrease in IL-8 levels was noted, although not significant. No significant changes in levels of TNF- α , IL-4 and IL-10 were noted.

During the course of *Basti* therapy (S1, S2, S3), no significant alterations were observed in the percentages of T cell subsets (CD3, CD4, CD8, $\alpha\beta$ -TCR and $\gamma\delta$ -

Table I. Effect of a course of *Basti* on anthropometric data

Parameter	Sampling days		
	S1 (day 0)	S2 (day 48)	S3 (day 138)
Weight (kg)	83.5 (77.63 - 91.5)	81.50* (72.25 - 90.5)	80.50* (72 - 90.5)
BMI (kg/m ²)	34.44 ± 3.25 (33.27 - 35.61)	33.24 ± 3.38** (32.02 - 34.46)	33.22 ± 3.63** (31.91 - 34.53)
Waist-hip ratio	1.00 ± 0.07 (0.98 - 1.02)	1.00 ± 0.08 (0.98 - 1.03)	1.01 ± 0.08 (0.98 - 1.04)
Upper arm circumference (cm)	34.0 (32.13 - 36)	32.25* (30.63 - 34)	32.75* (31 - 34.38)
Abdominal circumference (cm)	109.12 ± 9.81 (105.59 - 112.66)	103.84 ± 10.35** (100.11 - 107.58)	102.51 ± 11.24** (98.45 - 106.56)

Result expressed as mean ± SD (n=32) (95 % confidence interval) for normally distributed data and Median (IQR) for data not normally distributed. **P*<0.001 as compared to S1 using Wilcoxon signed rank test; ***P*<0.001 as compared to S1 using paired t test

TCR), natural killer (NK, CD56), B cells (CD 19), macrophages (CD14), dendritic cells (CD209), and regulatory T cells (CD4 CD25). The activation status of T lymphocytes as measured by the expression of early (CD3 CD69) and late (CD3 CD25) activation markers was also not altered (Table II).

A marked increase in production of IFN- γ (*P*<0.02) was observed in culture supernatants of

PBLs stimulated with anti-CD3 MAb at S2, which further increased (*P*<0.002) at S3 as compared to that noted at S1. In contrast, a gradual reduction in IL-8 production was noted in PBL-culture supernatants upon anti-CD3 MAb stimulation (*P*<0.02) (Fig. 2a). There was a positive correlation between IL-6 and IL-8 levels at S1 (*r* = 0.87), S2 (*r* = 0.57) and S3 (*r* = 0.87). No significant changes in levels of cytokines IL-6, IL-1 β ,

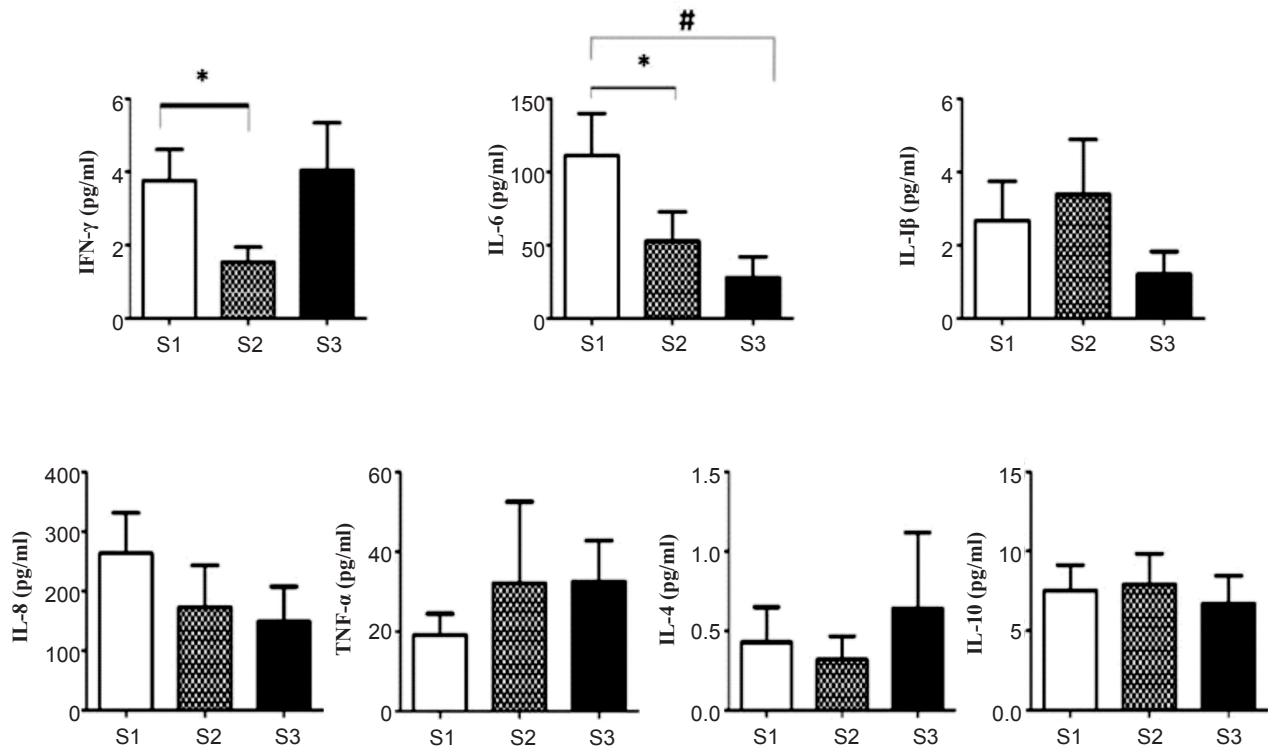


Fig. 1. Effect of various cytokines in serum of obese individuals. Values represented in pg/ml ± SD (n=32). **P*<0.02, #*P*<0.002.

Table II. Expression of immunophenotypic markers and activation status of lymphocytes

Marker	Sampling days		
	S1 (day 0)	S2 (day 48)	S3 (day 138)
CD3 ⁺	74.19 ± 8.35	71.70 ± 7.99	71.69 ± 8.73
CD4 ⁺	47.23 ± 6.67	49.03 ± 6.46	48.49 ± 7.06
CD8 ⁺	28.94 ± 6.79	26.22 ± 7.78	27.01 ± 9.25
CD56 ⁺	18.39 ± 12.93	15.51 ± 6.27	16.35 ± 7.04
CD19 ⁺	8.84 ± 4.47	8.03 ± 4.16	9.52 ± 10.04
CD14 ⁺	3.89 ± 6.89	2.85 ± 3.12	4.69 ± 5.39
CD80 ⁺	2.83 ± 1.52	2.84 ± 1.81	2.86 ± 1.99
CD86 ⁺	5.02 ± 3.06	4.77 ± 4.47	4.88 ± 3.02
CD209 ⁺	0.58 ± 1.46	0.41 ± 0.58	0.31 ± 0.67
CD11c ⁺	11.65 ± 6.01	10.31 ± 5.48	11.16 ± 7.54
αβTCR ⁺	54.75 ± 17.08	55.68 ± 17.71	53.93 ± 16.70
γδ TCR ⁺	18.27 ± 12.97	17.27 ± 11.83	16.71 ± 9.73
CD3 ⁺ CD25 ⁺	3.99 ± 3.54	4.31 ± 3.96	3.62 ± 2.99
CD3 ⁺ CD69 ⁺	3.33 ± 6.59	1.33 ± 0.92	2.26 ± 2.47
CD3 ⁺ CD71 ⁺	1.46 ± 1.95	1.99 ± 3.02	0.95 ± 0.54
CD3 ⁺ HLA-DR ⁺	5.82 ± 5.72	6.06 ± 5.90	4.36 ± 4.48

Results expressed as mean % positive cells ± SD (n=32)
TCR, T cell receptor

TNF- α , IL-4 and IL-10 were observed in PBL-culture supernatants before and after stimulation with anti-CD3 MAb.

A significant increase in generation of ROS was observed over a time-period of 45 min at S2 compared to that at S1. When ROS-generation was measured at S3, levels returned to values observed at S1. Also, a rapid increase in calcium flux was noted at 20 seconds after stimulation with anti-CD3 MAb in PBLs at S2, while peak in calcium release was noted at 30 seconds after addition of stimulus (anti-CD3 MAb) at S3. No significant modulations in NK-cytotoxicity were observed in obese individuals undergoing *Basti* therapy (*i.e.* at S2 and S3). Following *Basti* therapy, PBLs exhibited higher proliferative response to TCR-agonist at S2 compared to S1 but the difference was not significant (Fig. 2b). There was a gradual increase in turnover of T-cells with slight reduction in B-cells at S2 (Fig. 2c).

As seen in Fig. 3, the levels of IgM were markedly elevated from 1.06 ± 0.68 mg/ml at S1 to 3.76 ± 4.08 mg/ml at S2 ($P < 0.02$) with a significant reduction at

S3 (1.67 ± 1.10 mg/ml; $P < 0.02$) as compared with S1. A marginal decrease in levels of IgG was at S2 with significant drop ($P < 0.02$) at S3. A positive correlation between IgG and IL-6 levels was observed at S2 ($r = 0.39$) and S3 (0.66).

No significant change was observed on the metabolic parameters like fasting blood sugar, HbA_{1c}, HOMA-IR, liver function, renal function, serum phosphorus, iron, sodium, potassium, copper, vitamin B₁₂, T3, T4, thyroid stimulating hormone (TSH) and any of the variables of lipid metabolism after the course of *Basti*.

A decrease was observed in serum insulin at S2 compared to S1 and this reduction was maintained at S3. A significant increase was observed in serum calcium at S2 as compared to S1. However, this effect was not sustained over time. There was a significant decrease in serum ferritin at S2 and S3. A significant decrease was observed in serum cortisol levels at S2 and S3 as compared to S1 (Table III).

Discussion

This was a systematic study carried out to assess certain immunological or metabolic changes in obese individuals after a therapeutic course of *Basti*. A significant decrease was observed in weight, BMI, upper arm and abdominal circumference in obese individuals and this decrease was maintained over a period of three months. The mean weight loss of 3.09 kg at the end of *Basti* treatment (day 48 from baseline) was comparable to the various pharmacological interventions like sibutramine, orlistat, phentermine, diethylpropion or bupropion and fluoxetine in obesity²³. Further, no side effects were observed during our study over a period of three months indicating the safety of the procedure.

The *Basti* treatment lasted 16 days followed by a 32 period of diet and lifestyle change which was common to all patients. It appeared that the whole package of *Basti* and diet in the prescribed package led to a sustained weight loss associated with changes in upper arm and abdominal circumference.

According to Ayurveda, the ingredients of *Basti* reach up to the small intestine and get absorbed through the gut wall, are distributed in the body and thus exert systemic effects²⁴. Our study confirmed that the therapeutic procedure brought about a weight loss that was sustainable. However, one cannot comment on its mechanism of action at this point of time. Perhaps as an indicator toward the mechanism of action, was the

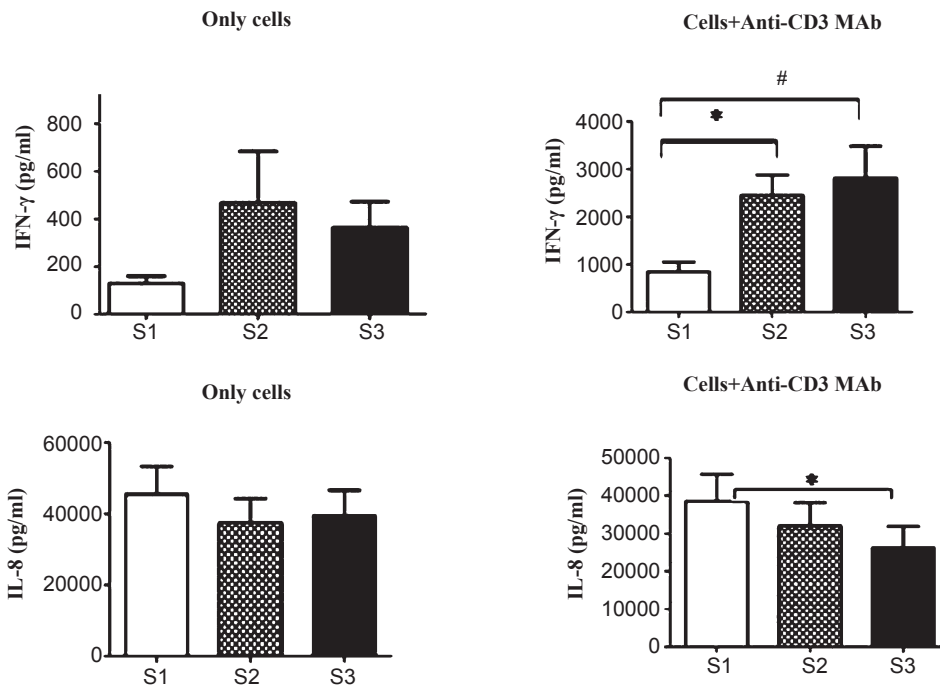


Fig. 2. Functional responses of lymphocytes from obese individuals. **2(a).** Measurement of cytokine production in cell-free culture supernatants of unstimulated peripheral blood lymphocytes (PBLs) and after stimulation with anti-CD3 MAb (1 μ g) [IFN- γ and IL-8]. Figures represent mean values in pg/ml \pm SD (n=32) (* P <0.02, # P <0.002).

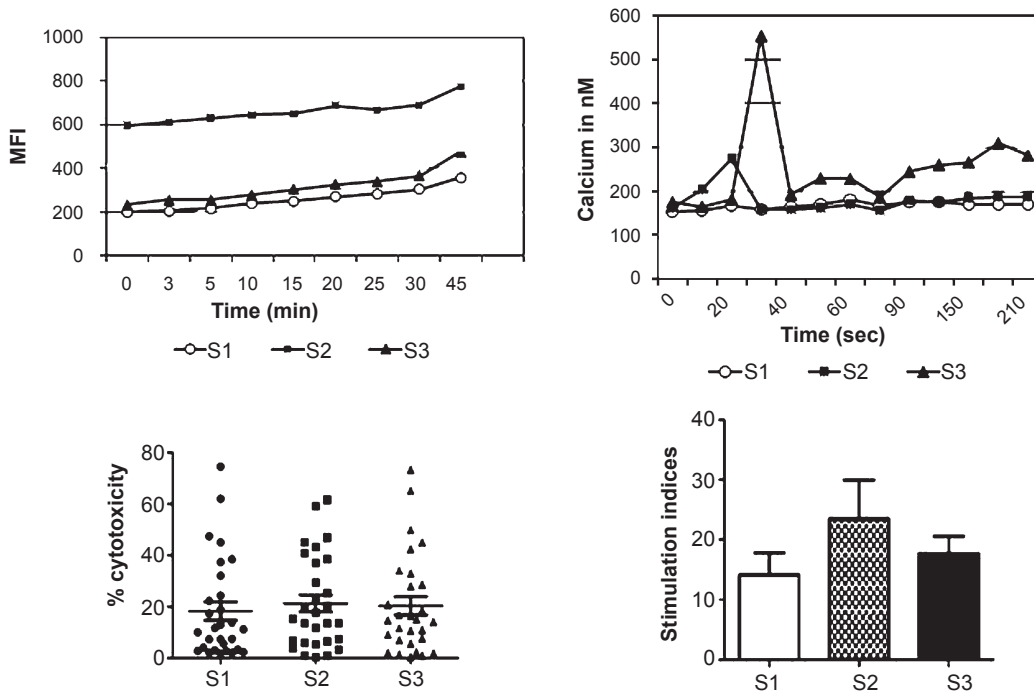


Fig. 2(b). Generation of ROS, intracellular Ca²⁺ release, NK-cytotoxicity and proliferative response of PBLs upon stimulation with anti-CD3 MAb at different time points. MFI, mean fluorescence intensity.

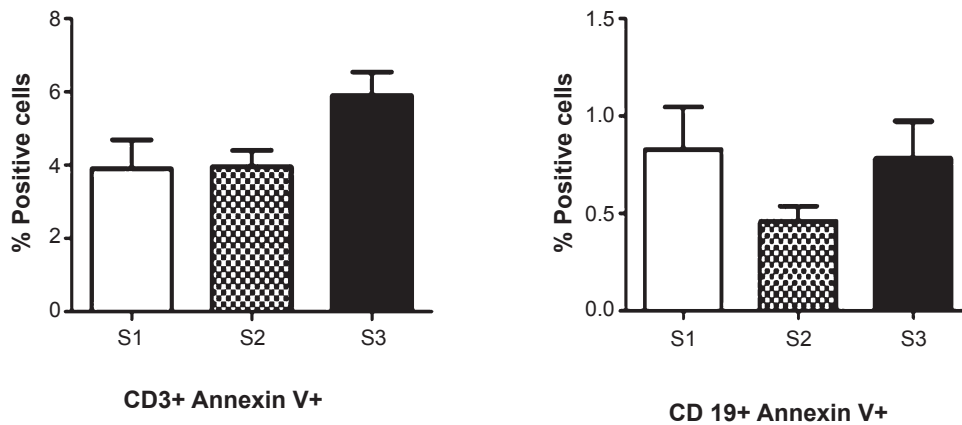


Fig. 2c. Measurement of apoptosis of PBLs using Annexin V FITC staining. Figure represents mean of per cent positive cells \pm SD (n=32).

observation that the elevated serum levels of IL-6 and IFN- γ of these obese patients reduced significantly after *Basti*. The low grade chronic inflammation in obesity is characterized by accumulation of macrophages and other activated immune cells (T cells, B cells, eosinophils, neutrophils, mast cells and natural killer cells) in the adipose tissue. M1 macrophages are characterized by enhanced pro-inflammatory cytokine production (TNF- α , IL-6 and IL-1). On the other hand, M2 macrophages are characterized by the production of anti-inflammatory cytokines such as IL10. Adipokines/cytokines released from activated immune cells contribute to insulin resistance that is associated with obesity²⁵. In obese individuals, increased production of adipokines influences multiple functions such as appetite and energy balance, immunity, insulin sensitivity, angiogenesis, blood pressure and lipid metabolism including production of high amount of TNF- α and IL-6²⁶. Enhanced activity of TNF and IL-6

are involved in the development of obesity-related insulin resistance²⁷. Macrophages and dendritic cells infiltrate into expanding adipose tissue²⁸, where they produce IFN- γ . *Basti* therapy downregulated levels of IL-6 and IFN- γ at S2. The effect was sustained in case of IL-6 even 90 days after the therapy. A gradual reduction in IL-8 level was also observed following *Basti* therapy. IL-8 has been shown to be produced and released from human adipose tissue and/or adipocytes suggesting IL-8 involvement in obesity²⁹. Our data showed that serum insulin fell significantly as did HOMA-IR. Thus, the results demonstrate that *Basti* treatment ameliorates Th1 type inflammatory cytokine response in obese individuals that may attenuate adipose tissue inflammation and insulin resistance without disrupting other immune functions.

It was interesting to note that functional properties of T-cells such as proliferative response, intracellular calcium flux and generation of ROS were modulated in

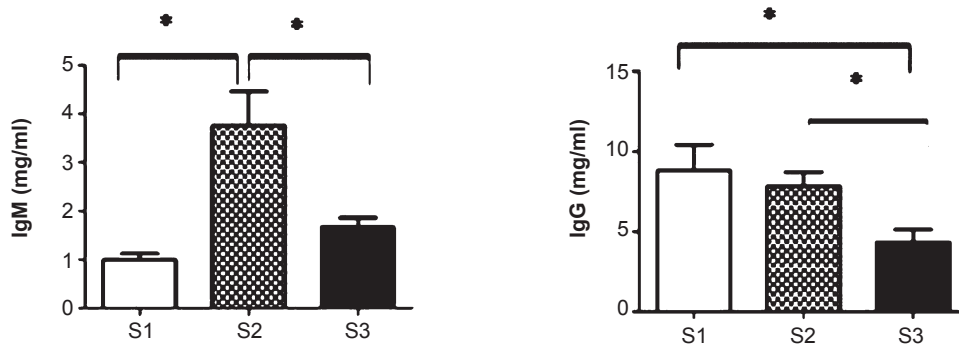


Fig. 3. Effect of IgM and IgG immunoglobulin levels in serum of obese individuals. Figures represent mean values of IgM and IgG in mg/ml \pm SD (n=32) (* P <0.02).

Table III. Effect of a course of *Basti* on metabolic parameters

Parameter	Sampling days		
	S1 (day 0)	S2 (day 48)	S3 (day 138)
Serum insulin (pmol/l)	119.80 (14.22 - 18.57)	104.24 (13.42 - 18.60)	109.38 (14.85 - 19.05)
Serum calcium (mmol/l)	2.17 ± 0.21 (2.09 - 2.25)	2.26 ± 0.18@ (2.19 - 2.32)	2.22 ± 0.18 (2.16 - 2.29)
Serum ferritin (pmol/l)	80.89 (18-77)	64.04** (16.25 - 44)	67.41** (15.75 - 43.75)
Serum cortisol (nmol/l)	389.02 (11.24 - 17.52)	319.49* (9.06 - 14.89)	293.28** (7.12 - 13.45)

Result expressed as mean ± SD (n=32) (95% confidence Interval) for normally distributed data and Median (IQR) for not normally distributed data. *P<0.05, **P<0.001 as compared to S1 using Wilcoxon rank test; @P<0.05 as compared to S1 using paired t test

obese individuals after undergoing *Basti* therapy. Fat accumulation is known to increase systemic oxidative stress independent of hyperglycaemia and may relate to the dysregulated production of adipocytokines³⁰. Moreover, regulation of intracellular Ca²⁺ ([Ca²⁺]_i) plays a key role in obesity, insulin resistance and hypertension. Our results demonstrated increased generation of ROS and higher levels of intracellular calcium in PBLs of obese individuals at S2, which returned to baseline at S3.

A significant increase in serum calcium was seen after the course of *Basti*. Though serum calcium decreased after 90 days, it was still higher than baseline level. There have been several isolated reports of an inverse relationship between dietary and serum calcium and indices of obesity³¹. Our results confirm these findings.

No clinically significant changes were observed in haematological parameters, as well as liver and renal function tests over the three month study period in any of the patients indicating safety of the intervention.

Serum ferritin reduced significantly after the *Basti* treatment and this decrease was maintained up to 90 days. Serum ferritin is generally considered as reflective of iron storage, but it is also increased in response to inflammation and is considered as a measure of chronic inflammation³². There is increasing evidence indicating that higher ferritin levels are observed in obesity (which is associated with increase in multiple markers of low grade inflammation)³³ which further contribute to insulin resistance. The reduction seen in ferritin levels, therefore, indicates the beneficial effects of *Basti* in obesity.

Serum cortisol levels decreased after the 48 days treatment period, which further decreased after 90 days. The primary reason for endocrine malfunction in human obesity is hypersensitivity and/or hyper-responsiveness of the hypothalamo-pituitary-adrenal (HPA) axis. The decrease in cortisol levels observed in our study suggests normalizing of the HPA axis. The *Basti* treatment, however, did not demonstrate significant change in levels of any other hormone.

In conclusion, our findings showed that in obese individuals, the Ayurvedic *Basti* (enema) treatment modulated immune responses by regulating pro-inflammatory cytokines, immunoglobulins and functional properties of T-cells. These changes were associated with a reduction in the body weight which was maintained even after three months. Additionally, the study documented the safety of *Basti* procedure adequately.

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Reprint requests: Dr Urmila Thatte, Department of Clinical Pharmacology, 1st Floor, M.S. Building, Seth GS Medical College & KEM Hospital, Parel, Mumbai 400 012, Maharashtra, India
e-mail: urmilathatte@gmail.com