Original Article

Toll like receptors (TLRs) in response to human gut microbiota of Indian obese and lean individuals

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Abstract

Background: The rising incidence of obesity is one of the most serious public health issues in the developed as well as in developing countries like India. Obesity and overweight are most important risk factors for many chronic diseases, including cardiovascular diseases, diabetes and cancer. In this study the body mass index (BMI) cut off was taken as 18.5-22.9 kg/m² for normal, 23.0-24.9 kg/m² for Overweight and >25 kg/m² for obese as per WHO recommendation for Asian Indians, which is different for developed and developing countries. Role of gut microbiota mediated immune response in the development of obesity has been studied but the literature on Indian population are lacking. Therefore, a study was conducted to determine Toll like receptors (TLRs) in response to human gut microbiota of Indian obese and lean individuals using viable colonocytes in a Non invasive technique and Flowcytometry. **Methods:** A total of 20 healthy volunteer (10 obese and 10 lean) were enrolled in the study as per inclusion and exclusion criteria. Viable colonocytes were isolated from fecal samples using a Non invasive technique (SCSR Method). Toll like receptors (TLRs) and immunoglobulin (IgA &IgG) receptor concentration were measured by standard Flowcytometry methods using specific fluorochrome conjugated antibodies. **Results:** Average TLR2 receptor concentration was significantly higher in obese (6.35 %) as compared to lean (2.9 %) ($P = 0.01$). TLR4 receptor concentration was 1.4 % in obese and 1.65 % in lean although the difference was not statistically significant ($P = 0.59$). IgA & IgG receptor concentration was 49.6 % & 11.2 % in the obese and 67.15 % & 8.05 % in the lean respectively but the differences among both the group were not statistically significant. **Conclusion:** The results of the present study will be helpful for physicians and researchers to find some biomarkers which can determine predisposition of the obesity in Indian population and helps to use alternative therapeutics such as probiotics to maintain gut homeostasis and immune modulation to prevent obesity.

Keywords: Colonocytes, flowcytomety, lean, obese, toll-like receptors

Background

Toll-like receptors (TLRs) are the family of pattern-recognition receptors (PRRs) which activate the proinflammatory signaling pathways in response to microbial agents.[1] TLRs seem to be involved in the chronic inflammatory state of obesity. Several studies have demonstrated the role of TLR2 and TLR4 in innate immunity as well as in metabolic function in obesity. Studies on obese human and mice showed that fatty acids induce inflammation occurs due to the TLR4.[2] Toll-like receptor 4 (TLR4) are activated either by bacterial lipopolysaccharide or saturated fatty acids.[3] TLR4-deficient mice are unable to develop diet-induced obesity and the inflammation via nuclear factor κB (NFκB) activation.[4]

Bacterial ingredients are strong TLR ligands. LPS are the ligands of TLR4, which are present in the cell wall of

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gram-negative bacteria.[5,6] Peptidoglycan and lipoteichoic acid are ligands of TLR2 which is present in the cell wall of gram-positive bacteria,[7,8] thus the best resource of TLR ligands are human gut microbiota. TLR2 utilize adaptor protein, the myeloid differentiation factor 88 (MyD88), and TLR4 utilize MyD88 and TRIF [Toll-interleukin 1 receptor (TIR) domain-containing adapter-inducing interferon-β]. It activates the nuclear factor-κB (NF-κB) and generates chemokines and proinflammatory cytokines such as IL-1, IL-6, and TNFα.[9] TLR signaling also leads to production of IgA by intestinal epithelial cells (colonocytes). The present study was aimed to determine receptor concentration of the different PRRs (mainly TLR2 and TLR4) in the colonic epithelial cells of obese and lean Indian individuals that play a key role in the innate immune response against different PAMPs of bacteria.

Materials and Methods

Human volunteer subjects inclusion and exclusion criteria

Healthy human volunteers were enrolled in this study. The study was conducted at All India Institute of Medical Sciences, New Delhi, and was approved by institute Ethics committee. Informed consent was taken by all the volunteers prior to the assessment. Inclusion criteria of obese and lean individual were taken fromWHO guidelines (Asian Indians); for obese individuals, BMI was (≥25 kg/m²) and for lean individuals, BMI was (18.5–22.9 kg/m²). Exclusion criteria were intake of any prebiotic and probiotic in last 6 weeks included history of intake any steroid and antibiotic or gastrointestinal (GI) disorder, for example, inflammatory bowel disease (IBD).

Fecal samples

Fecal samples (from the 20 subjects, i. e., 10 obese and 10 lean) were collected on sterile container and stored at 4°C until processing and all samples were processed within 12 h from the time of collection.

Isolation of live colonocytes

Viable colonocytes were isolated from fecal specimen by somatic cell sampling recovery (SCSR) method (noninvasive Technology, USA). Briefly, 0.5–1.0 gm fecal specimen transferred into SCSR transport medium and passed through a 330 μm nylon mesh, followed by second filter of pore size of 40 μm. The final filtrate was under laid with 10 ml of cushion solution and centrifuged, the light cells moved to interface collected fraction containing cell population as shown in Figure 1.

Staining of colonocytes - surface Antigens and flowcytometery for IgA, IgG, toll-like receptor 2 and 4

The isolated Colonocytes from stool sample (approximately 25,000–50,000 cells) were suspended in 1 ml of PBS and washed with 2 ml of 1% BSA in PBS. The cell suspension was centrifuged at 2,000 rpm for 5 min. at 4°C and supernatant was discarded. The washing was repeated with PBS. The cell pellet was suspended in PBS and 100 μl aliquot was prepared in the running tube. Processing of sample for flowcytometry was performed as per standardized lab protocol. To measure the TLRs, IgA, and IgG receptor concentration on viable colonocytes, TLR2-APC conjugated, TLR4-PE-Cy7 conjugated, IgA-FITC conjugated, and IgG-PE conjugated antibodies were used and at least 5 lakhs events were acquired in BD-FACS Canto, 6 color (BD Biosciences, San Jose, USA). Data analysis was performed by BD-FACS-Diva software. Dot plot representation was shown in Figure 2.

Statistical analysis

Statistical analysis of the data was performed in Stata 12 software. Wilcoxon rank–sum test was performed to assess the difference in expression level of IgA, IgG, TLR-4, and TLR-2 between obese and lean group. $P < 0.05$ was considered as statistically significant.

Results

A total of 20 volunteers (healthy subjects) were enrolled for the study. Of these, 10 volunteers (mean BMI 33.8 ± 3.6) were considered as obese, and remaining 10 volunteers (mean BMI 21.4 ± 1.5) were considered as lean. BMI as well as weight was

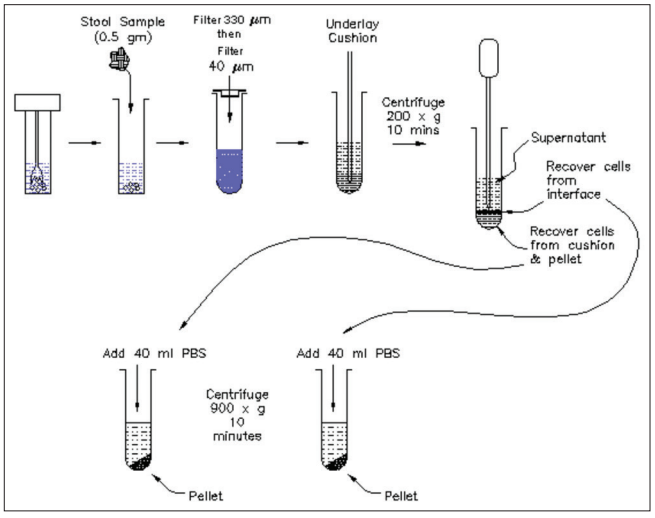
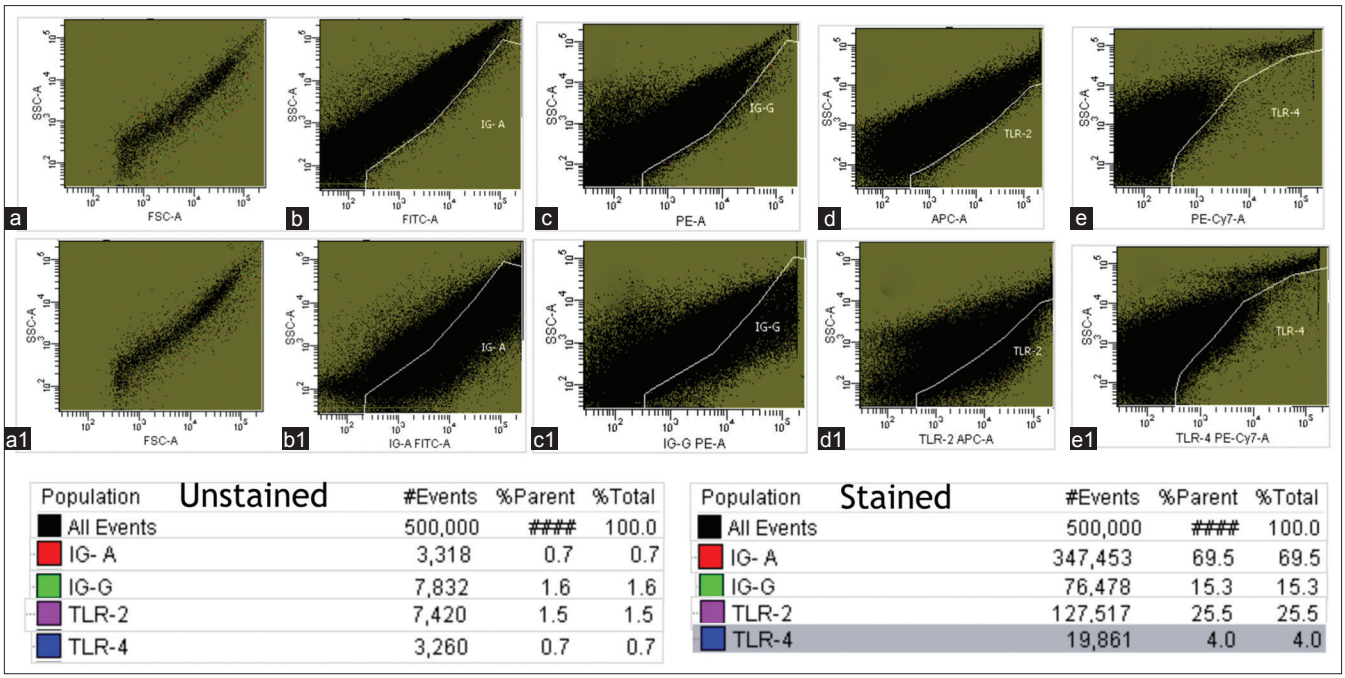


Figure 1: The pictorial presentation of isolation of colonocytes from stool sample

Table 1: Data represent in median (min max) and Wilcoxon rank–sum was applied

Variable	Obese (*n*=10) Mean±SD	Lean (*n*=10) Mean±SD	*P*
BMI	33.8±3.6	21.4±1.5	0.001
Weight	96.2±12.9	60.9±4.9	0.001
Height (cm)	168.6±6.9	168.6±2.9	0.5
IgA	49.6 (32.4–65.2)	67.15 (26.1–90.2)	0.97
IgG	11.2 (2.2–31)	8.05 (3.2–53.8)	0.76
TLR4	1.4 (0.3–21.7)	1.65 (0.7–8.6)	0.59
TLR2	6.35 (2.2–25.5)	2.9 (1.7–9)	0.01



Population	Unstained	#Events	%Parent	%Total
All Events		500,000	####	100.0
IG- A		3,318	0.7	0.7
IG-G		7,832	1.6	1.6
TLR-2		7,420	1.5	1.5
TLR-4		3,260	0.7	0.7

Population	Stained	#Events	%Parent	%Total
All Events		500,000	####	100.0
IG- A		347,453	69.5	69.5
IG-G		76,478	15.3	15.3
TLR-2		127,517	25.5	25.5
TLR-4		19,861	4.0	4.0

Figure 2: Dot plots of flow cytometry demonstrating the expressions of IgA, IgG, TLR2, and TLR4. Relative expressions of markers were analyzed on the basis of their pattern in unstained tube. Figure a and a1): Forward scatter (FSC) and side scatter (SSC) pattern of the cells. Figure b, b1; c, c1; d, d1 and e, e1): Unstained and stained tubes for IgA, IgG, TLR2, and TLR4 expression, respectively

significantly high in obese as compared to lean (P = 0.001). However, there was no any significant difference in height between obese and lean group (P = 0.5) data shown in Table 1.

IgA, IgG, TLR4 and TLR2 receptor concentration

Receptor concentration of IgA was lower 49.6% (32.4–65.2) in the obese than in the lean 67.15% (26.1–90.2) (P = 0.97), receptor concentration of IgG was greater 11.2% (2.2-31) in the obese than in the lean 8.05% (3.2–53.8) (P = 0.76). Receptor concentration of TLR4 was lower 1.4% (0.3–21.7) in the obese than in the lean 1.65% (0.7–8.6) (P = 0.59) but none of these differences were statistically significant and TLR2 receptor concentration in obese was 6.35% (2.2–25.5) significantly greater than the lean 2.9% (1.7–9) (P = 0.01).

Discussion

The studies on mice have shown that high fat diet showed decline in the expression of tight junction proteins such as occluding and zonula occludens-1 (ZO-1) in the small intestine. Therefore, leaky gut absorbs bacterial components and results the stimulation of TLR expressing cells and increase in the plasma LPS levels. Besides this, circulating LPS level also increases in the patients suffering from metabolic syndrome. It increases the gut permeability. A mouse model study showed that tight junction proteins can be maintained by probiotics.[10] This information indicates that normal gut microbiota controls the gut permeability. It prevents the leaks of TLR ligands which can regulate the development of obesity. An alteration to the composition of gut microbiota and increased TLR ligands were observed in obese individuals.[9] TLR ligands are raised in obesity and its signaling is triggered at cell level, resulting in the elevation of proinflammatory cytokines production. Altered gut microbiota play an important role in the development of obesity because it is the main source of TLR ligands.[11]

The goal of this study was to access the host PRR interaction and expression in response to the gut microbial communities. As part of this effort, we tried to examine the receptor concentration of TLR2 and TLR4, which showed that the composition of gut microbiota is different in obese and lean subjects. These differences in the composition of gut microbiota may lead to the rise and fall in the weight and impaired metabolism. Evidence of different animal model shows that obese subjects microbiota have greater capacity to harvest energy compared with microbiota of lean by the activation of different metabolic pathway. In our study, TLR2 receptor concentration showed significant difference, that is, [obese 6.35% vs lean 2.9% (P = 0.01) between obese and lean subjects but TLR4 receptor concentration [obese 1.4% vs lean 1.65% (P = 0.59)] did not show significant difference. The possible explanation of this finding is that TLR2 recognize more PAMPs as compared with TLR4.

IgA feedback is responsible for the human gut colonization by commensal microorganisms. IgG provides important host defense and immunoregulatory functions. A prospective study by Nair *et al.* in 2003[12] showed that colonocyte isolated from human adult stool sample showed IgA and IgG expression. Bamola *et al.* in 2016[13] showed the receptors concentration of immunoglobulins (IgA & IgG) in healthy child and adult among Indian population on viable colonocytes by using flowcytometry. They measured a significant difference between IgA (children 71 ± 0.97 vs adults 66 ± 1.2) and IgG (children

65 ± 1.1 vs adults 62 ± 1.3) receptor concentration. In our study, receptor concentration of IgG was more in obese as compared with lean [obese 11.2% vs lean 8.05% ($P = 0.76$)] and receptor concentration of IgA was less in obese as compare to lean [obese 49.6% vs lean 67.1% ($P = 0.97$)].

Thus, the present study showed the receptor concentration of IgG and IgA from colonocytes which were isolated from obese and lean stool samples. The average receptor concentration of IgA in lean was more than obese individuals and average receptor concentration of IgG in obese was more than lean. In our study, receptor concentration of TLR2 was significantly more in obese as compared with lean and TLR4 was less in obese as compared with lean.

However, further research is required for understanding the human gut microbiota and its interaction with TLR. It will be helpful for physicians and researchers to find biomarkers which can determine predisposition to the obesity and helps to use alternative therapeutics such as probiotics to maintain gut homeostasis to prevent obesity.

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Conflicts of interest

There are no conflicts of interest.

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