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Convergence of innate immunity and insulin resistance as evidenced by increased nucleotide oligomerization domain (NOD) expression and signaling in monocytes from patients with type 2 diabetes

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ABSTRACT

Despite the well known role of nucleotide oligomerization domain (NOD) receptor proteins in innate immunity, their association with diabetes is less explored. Here we report the transcriptional level of NODs and their downstream molecular signatures in $CD14^+$ monocytes from subjects with different grades of glucose tolerance. NOD1 and NOD2 mRNA expression were significantly up-regulated in monocytes from patients with type 2 diabetes (T2DM) and positively correlated with HOMA-IR and poor glycemic control. Patients with T2DM also exhibited increased monocyte activation markers (CD11b and CD36) and proinflammatory signals downstream of NOD (RIPK2 and NFκB) along with the increased circulatory levels of TNF-α and IL-6. *In vitro* stimulation of monocytes with NOD specific ligands-i-EDAP and MDP significantly up regulated the mRNA expression of NOD1 and NOD2 respectively in T2DM. Our study exposes up regulation of NODs in monocytes as an important component of inflammation and insulin resistance in patients with T2DM.

1. Introduction

Increasing evidences suggest that chronic low grade inflammation play an important role in the development of type 2 diabetes (T2DM). Though multiple links between metabolic and immune responses have emerged as a critical factor in the progression of insulin resistance, evidences are not adequate to delineate the specific immune elements which mediate the metabolic aberrations. Therefore it is important to decipher the intricate cross talk between innate immunity and metabolic signaling to identify new drug targets to combat insulin resistance and T2DM. A major step forward in this direction was the identification of pattern recognition receptors (PRRs) such as the toll like receptors (TLRs) in the association of inflammation with insulin resistance [1,2]. Though TLR4 has been shown to play a role in high-fat-diet-induced insulin resistance in mice [3], it is not the sole pathway by which fatty acids could affect insulin sensitivity [4]. In this regard, nucleotide binding oligomerization domain (NOD) like receptors (NLRs), the cytosolic proteins of the innate immunity are known to propagate inflammatory signals in response to peptidoglycan (PGN) [5,6]. NOD1 detects the peptidoglycan component, D-glutamyl-meso-diaminopimelic acid (meso-DAP) structure unique to gram-negative bacteria, whereas NOD2 detects muramyl dipeptide (MDP) found in both gram-negative and gram-positive strains [7,8]. NODs recognize these structures through the leucine rich repeat domain at the carboxy terminus [9,10] and activate common NFκB pathways similar to TLR4 [11,12]. However, polyubiquitination of receptor interacting protein kinase 2 (RIPK2, also called RIP2) is required for NFκB activation induced through NOD but not TLRs [13,14]. Mice deficient in NOD1/2 were shown to be protected against obesity induced inflammation and insulin resistance [15] and this has been substantiated by *in vitro* studies [16]. NLRP3 inflammasome has been shown to be a key element in the obesity induced inflammation in adipose tissue [17]. However, there is inadequate clinical evidence on the involvement of specific

Abbreviations: HbA1C, glycated hemoglobin; HOMA, homeostasis assessment model; iEDAP, γ-D-glutamyl-meso diaminopimelic acid; IGT, impaired glucose tolerance; IL-6, interleukin-6; MDP, muramyl dipeptide; NFκβ, nuclear factor kappa beta; NGT, normal glucose tolerance; NOD, nucleotide binding oligomerization domain; OR, odds ratio; PGN, peptidoglycan; PRR, pattern recognition receptor; RIPK2, receptor interacting protein kinase 2; T2DM, type 2 diabetes mellitus; TLR, toll like receptor; TNF-α, tumor necrosis factor – alpha.

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inflammasomes in relation to inflammation in T2DM. Therefore this study was carried out to analyze the level of NODs expression in monocytes from patients with T2DM and to investigate its association with glycemic status and insulin resistance.

2. Research design and methods

2.1. Study groups

Study subjects were recruited from the on-going epidemiology cohorts and from Dr. Mohan's Diabetes Specialities Centre (DMDSC), a large tertiary diabetes care center at Chennai in southern India. Study group (n = 25 each) comprised of (a) subjects with normal glucose tolerance (NGT), (b) subjects with impaired glucose tolerance (IGT) and (c) patients with T2DM. Anthropometric measurements including weight, height, and waist, were obtained using standardized techniques as detailed elsewhere [18]. Subjects with fasting plasma glucose <5.5 mmol/l (100 mg/dl) and 2 h post glucose value <7.8 mmol/l (140 mg/dl) were considered as normal glucose tolerance (NGT) individuals. Those with 2 h post-glucose value of $\geqslant$7.8 mmol/L (140 mg/dL) and $\leqslant$11.1 mmol/L (200 mg/dL) were diagnosed as IGT. Subjects who were confirmed by oral glucose tolerance test (OGTT) to have 2 h plasma glucose value $\geqslant$11.1 mmol/l [200 mg/dl] based on WHO consulting group criteria [19] were diagnosed as T2DM patients. Of the diabetic patients, 90% were on oral hypoglycemic agents (OHA) and 10% were on OHA plus additional insulin therapy for control of hyperglycemia. Institutional ethical committee approval was obtained for the study and informed consent was obtained from all the study subjects. Fasting plasma glucose (hexokinase method) was measured on Hitachi 912 Auto analyzer (Hitachi, Mannheim, Germany) using kits supplied by Roche Diagnostics (Mannheim, Germany). Glycated hemoglobin (HbA1C) was estimated by high-pressure liquid chromatography using the Variant machine (Bio-Rad, Hercules, Calif., USA). Serum cholesterol (cholesterol oxidase–peroxidase-amidopyrine method), serum triglycerides (glycerol phosphate oxidase–peroxidase-amidopyrine method) and HDL cholesterol (direct method-polyethylene glycol-pretreated enzymes) were measured using Hitachi-912 Autoanalyser (Hitachi, Mannheim, Germany). Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula. The intra and inter assay co-efficient of variation for the biochemical assays ranged between 3.1% and 7.6%. The laboratory is certified by the College of American Pathologists (CAP) and the National Accreditation Board for Testing and Calibration of Laboratories (NABL).

2.2. Monocyte isolation

Mononuclear cells were isolated from fasting heparinized blood by magnetic separation using Dynabeads® FlowComp™ Human CD14 monocyte isolation (Invitrogen, CA, USA) according to kit instructions. The purity of monocytes (Mean + SD) from the magnetic bead-based isolation as determined by flow cytometry was 95 ± 3.4%. Isolated monocytes were studied before and after activation for 16 h with endotoxin-free NOD1 (c12-iEDAP, Tri-DAP; 1 μg/ml) or NOD2 ligands (L18-MDP; 1 μg/ml) (Invivogen; San diego, USA) and lipopolysaccharide (LPS) (*Escherichia coli*, 100 ng/ml; Sigma Chemicals, St. Louis, MO).

2.3. THP-1 cell culture

The human monocyte cell line THP-1 was obtained from the National Centre for Cell Science (NCCS, Pune). THP-1 cells were maintained in endotoxin free RPMI-1640 medium containing 10% heat-inactivated fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 2.5 μg/l amphotericin B, and 10 mM HEPES, pH 7.0–7.4, under a humidified condition of 5% CO2 at 37 °C. For *in vitro* experiments, 1×10^6 cells were subjected to either 5.5 mM or 30 mM glucose treatment for 16 h in the absence or presence of iEDAP or MDP or LPS. *In vitro* measurements were done independently at least three times.

2.4. Real time PCR

RNA was extracted from monocytes using RNAspin mini RNA isolation kit (GE healthcare, Amersham, UK) according to the manufacturer's instructions. 500 ng of RNA was reverse transcribed with M-MuLV reverse transcriptase (NEB) for quantitative real-time PCR analysis in an ABI 7000 RT-PCR system (Applied Biosystems) with appropriate cycle conditions. Negative controls (no template) were run as well to ensure the absence of contamination. Analysis was performed using 2^{-ddCt} method and normalized to endogenous 18S ribosomal RNA control.

2.5. Cell surface expression of CD36 and CD11b

Monocyte activation was examined by analyzing the surface expression of activation markers CD11b and CD36 by flow cytometry. In brief, 3 ml of whole blood (n = 10 each)collected in EDTA tubes was lysed with 1:10 diluted BD FACS lysing solution (BD Biosciences, San Jose, CA) in 1× PBS. Subsequently the cells were fixed in 4% paraformaldehyde and staining was done with previously titrated volumes of FITC and PE conjugated antibodies. The antibodies used in the study were as follows: PE labeled antihuman CD14; FITC labeled antihuman CD11b and CD36 (eBioscience, San Diego, CA). Fluorescence was measured on a FACS Calibur (BD Biosciences, San Jose, CA) using 30,000 gated cells. The percentage of CD14+ cells for a particular molecule was calculated using Flow JO software (Treestar Inc., Ashland, OR, USA).

2.6. Measurement of TNF-α and IL-6

Circulating levels of TNF-α and IL-6 in the serum or culture supernatants were determined by quantitative sandwich Enzyme-linked immunosorbent assay (ELISA) (eBioscience, San Diego, CA). In brief, monoclonal antibody specific for TNF-α or IL-6 was coated over night onto a microplate. Standards or samples were added to the wells and an enzyme-linked polyclonal antibody specific for TNF-α or IL-6 was added to the wells after washing away any unbound substances. Following the addition of a substrate solution, the color development was read at 450 nm. The intra and inter assay coefficients of variation were <5 and <10%, respectively.

2.7. Statistical analysis

Comparison between groups was performed using one-way ANOVA (with Tukey honestly significant difference test). Pearson correlation analysis was carried out to determine the relation of NODs with other risk variables. Regression analysis was done to determine the association of NOD with clinical parameters. All analyses were done using Windows-based SPSS statistical package (Version 10.0, Chicago, IL), and $P < 0.05$ were taken as significant.

3. Results

Clinical and biochemical characteristics of the study subjects are illustrated in Table 1. There were no significant differences in

Table 1
Clinical characteristics of the study subjects.

Parameters	NGT (n = 25)	IGT (n = 25)	T2DM (n = 25)
Age (years)	43 ± 9	44 ± 9	49 ± 8
BMI (kg/m^2)	27.1 ± 4.5	28.5 ± 4.2	28.6 ± 4.3
Waist circumference	92.8 ± 11	93.9 ± 12	100 ± 12.4
Fasting plasma glucose (mg/dl)	94.3 ± 8.7	101.3 ± 15*	155.3 ± 42*,#
Post prandial plasma glucose (mg/dl)	110.6 ± 24	164 ± 17*	272.1 ± 73*,#
HbA1c (%)	5.7 ± 0.3	6.2 ± 0.5	8.8 ± 2*,#
HOMA-IR	1.6 ± 0.9	1.92 ± 1.0	4.6 ± 2.8*,#
Serum cholesterol (mg/dl)	178.5 ± 31	176.2 ± 25	181 ± 36
Serum triglycerides (mg/dl)	135.2 ± 75	137.1 ± 59	145.6 ± 56
LDL Cholesterol (mg/dl)	109 ± 30	111.3 ± 19	112 ± 30
HDL Cholesterol (mg/dl)	41.8 ± 8.3	37.5 ± 6	40.9 ± 7.2

* $p < 0.05$ Compared to NGT.
\# $p < 0.05$ Compared to IGT.

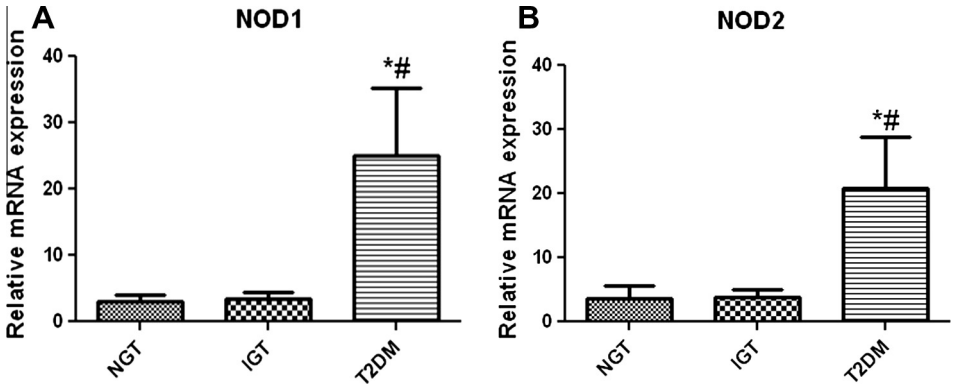

Fig. 1. NOD1 and NOD2 mRNA expression. Realtime quantitative PCR analysis on the expression of NOD1 (A) and NOD2 (B) mRNA in CD14+ monocytes from subjects with normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and type 2 diabetes (T2DM). mRNA level was quantified after normalizing with endogenous 18S ribosomal RNA control. Data are expressed as Mean ± SE. *$p < 0.05$ compared to NGT, #$p < 0.05$ compared to IGT.

age and BMI among the groups. Fasting as well as post prandial glucose levels were significantly higher in patients with T2DM and subjects with IGT compared to NGT ($p < 0.001$). HbA1c ($p < 0.001$) and insulin resistance ($p < 0.001$) (as evident by HOMA-IR) values were significantly higher in T2DM. There were no significant differences in the lipid profile among the study groups.

3.1. Increased expression of NOD1 and NOD2 in circulating monocytes from T2DM

In monocytes from patients with T2DM, there was a significant ($p < 0.05$) transcriptional upregulation of NOD1 and NOD2 compared to monocytes from subjects with IGT and NGT (Fig. 1). mRNA expression of NODs showed a significant positive correlation with HOMA-IR, HbA1c and fasting as well as post plasma glucose levels ($p \leqslant 0.05$) (Table 2). NOD1 and NOD2 expression was also positively correlated with age ($p < 0.01$) and waist circumference ($p < 0.05$). Multiple logistic regression analysis was done using type 2 diabetes as the dependent variable and NODs as independent variables. NOD1 [Odds Ratio [OR] 1.782; 95% CI: 1.177–2.698; $p < 0.006$] and NOD2 Odds Ratio [OR] 1.239; 95% CI: 1.054–1.456; $p < 0.009$] showed a significant association with T2DM even after adjusting for age and waist circumference. Interestingly when adjusted for insulin resistance, this association was lost implying that the link between NODs and diabetes originates from proinflammation that underlie the insulin resistant state.

3.2. Increased expression of RIPK2 and proinflammatory transcription factors in monocytes from T2DM

Since RIPK2 is a critical mediator of NOD1/2 signaling and an important trigger for NFκB-mediated proinflammatory responses, we then probed the mRNA expression of RIPK2 and NFκB, downstream of NODs. Both mRNA expression of RIPK2 (Fig. 2A) and NFκB (Fig. 2B) were significantly ($p < 0.05$) up regulated in monocytes from T2DM compared to other groups. IGT group showed a trend in increased expression of RIPK2 and NFκB albeit it was not statistically significant.

3.3. Increased monocyte activation in patients with T2DM

We further analyzed the expression levels of monocyte activation markers in the study subjects by flow cytometry. The expres-

Table 2
Pearson correlation analysis of NOD1/2 with other risk variables.

Variables	NOD1		NOD2	
	r	p	r	p
Age	0.293	0.028	0.196	0.046
Waist circumference	0.345	0.016	0.119	0.031
BMI	0.131	0.365	0.025	0.875
Fasting plasma glucose	0.353	0.008	0.428	0.003
Post prandial plasma glucose	0.534	0.002	0.542	0.001
HbA1c	0.37	0.006	0.443	0.003
HOMA-IR	0.245	0.050	0.267	0.045

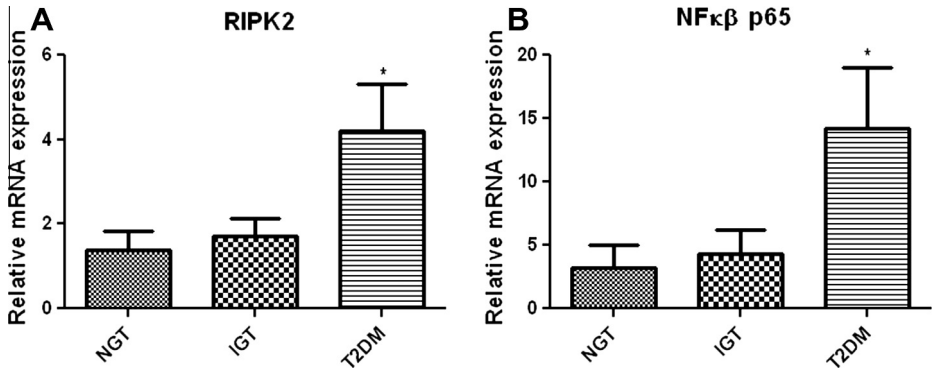

Fig. 2. mRNA expression of inflammatory mediators. Real time PCR analysis showing the increased mRNA expression of RIPK2 (A) and NFκβ (B) in monocytes from the study subjects. mRNA level was quantified after normalizing with endogenous 18S ribosomal RNA control. Data are expressed as Mean ± SE.*$p < 0.05$ compared to NGT.

sion of CD11b was significantly higher in monocytes from T2DM ($p < 0.05$) when compared to NGT and IGT (Fig. 3A). The expression of scavenger receptor CD36 in monocytes from T2DM and IGT were significantly ($p < 0.05$) and progressively higher when compared to NGT (Fig. 3B).

3.4. High glucose induced NOD expression in human monocytes

The differential activation of NODs among the subjects with different grades of glucose tolerance was determined after *in vitro* stimulation of circulatory monocytes with NOD specific ligands. When monocytes were challenged with either iEDAP or MDP, they exhibited high mRNA expression of NOD1/2 in patients with T2DM compared to other two groups ($p < 0.05$). Similar to NOD specific ligands, LPS also induced the expression of NODs (Fig. 4A and B).

As there was an up regulated expression of NODs in monocytes from patients with T2DM, next we analyzed whether high glucose activates NOD expression. Interestingly, when freshly isolated monocytes from the healthy volunteers were treated with high glucose for 16 h, it showed significant ($p < 0.05$) up regulation of both NOD1/2 mRNA expression (Fig. 5A and B). Moreover, high glucose augmented the NOD-ligand mediated mRNA expression of both NOD1 and NOD2 in monocytes ($p < 0.05$). Data was further verified *in vitro* on THP-1 monocyte cell line, where high glucose not only increased the NOD expression, but it also augmented the NOD-ligand mediated increases in NOD1/2 mRNA expression ($p < 0.05$) (Fig. 5C).

3.5. Serum and cell supernatant TNF-α and IL 6 levels among the study groups

Next we have analyzed the serum samples for circulatory levels of inflammatory cytokines, TNF-α and IL-6 which is the most sensitive systemic indicators of inflammation often considered to be

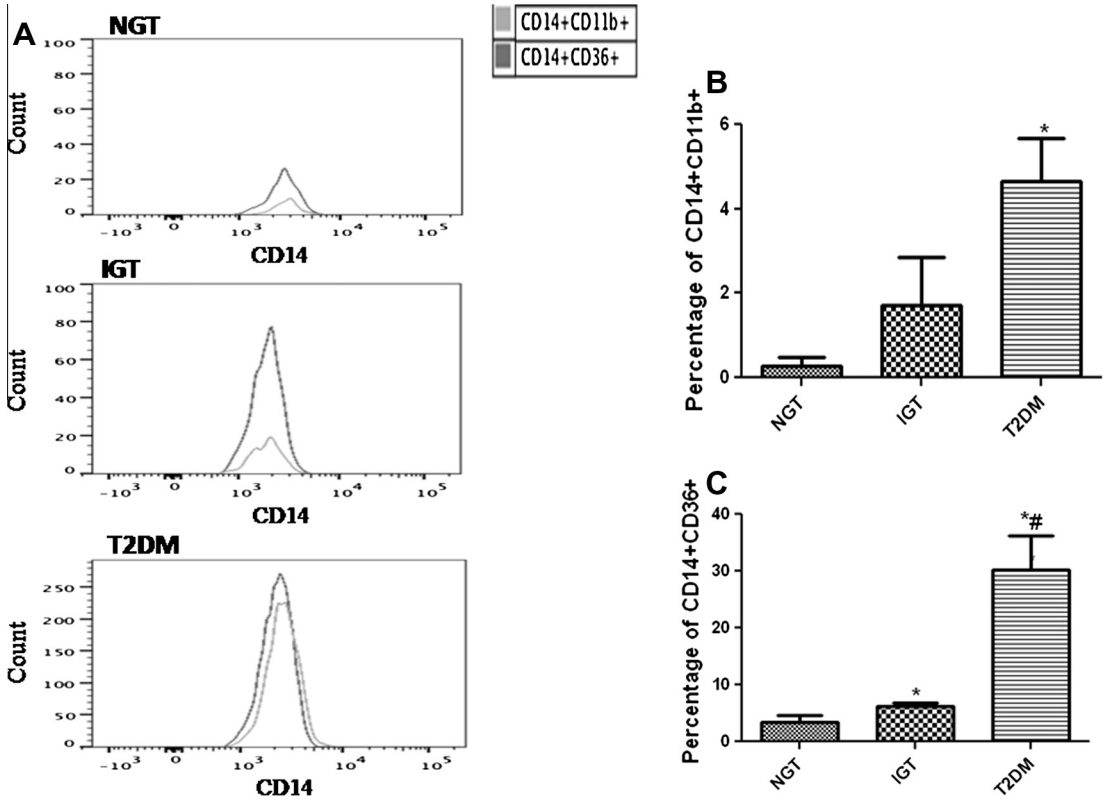

Fig. 3. CD11b and CD36 expression on monocytes. (A) Representative flow cytometry analysis showing the expression of CD11b and CD36 as an overlaying histogram. Cumulative data on the percentage of CD14+ monocytes expressing CD11b (B) and CD36 (C) in the study subjects ($n = 10$ each). Data are represented as the Mean ± SE. *$p < 0.05$ compared to NGT, #$p < 0.05$ compared to IGT.

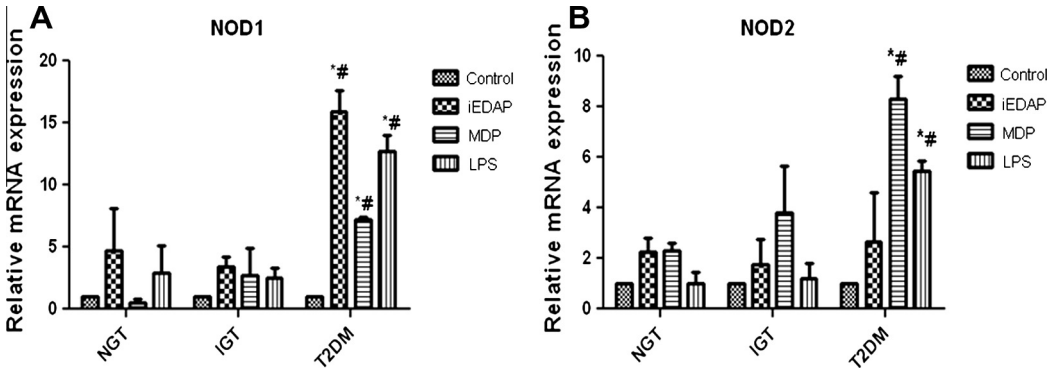

Fig. 4. *In vitro* analysis showing the expression of NOD1 and NOD2 mRNA in monocytes. Bars represent the mRNA expression of NOD1 (A) and NOD2 (B) in monocytes untreated or treated with iEDAP, MDP and LPS for 16 h. mRNA level was quantified after normalizing with endogenous 18S ribosomal RNA control. Data are represented as Mean ± SE ($n = 5$ each). $^{*}p < 0.01$ compared to NGT, $^{\#}p < 0.01$ compared to IGT.

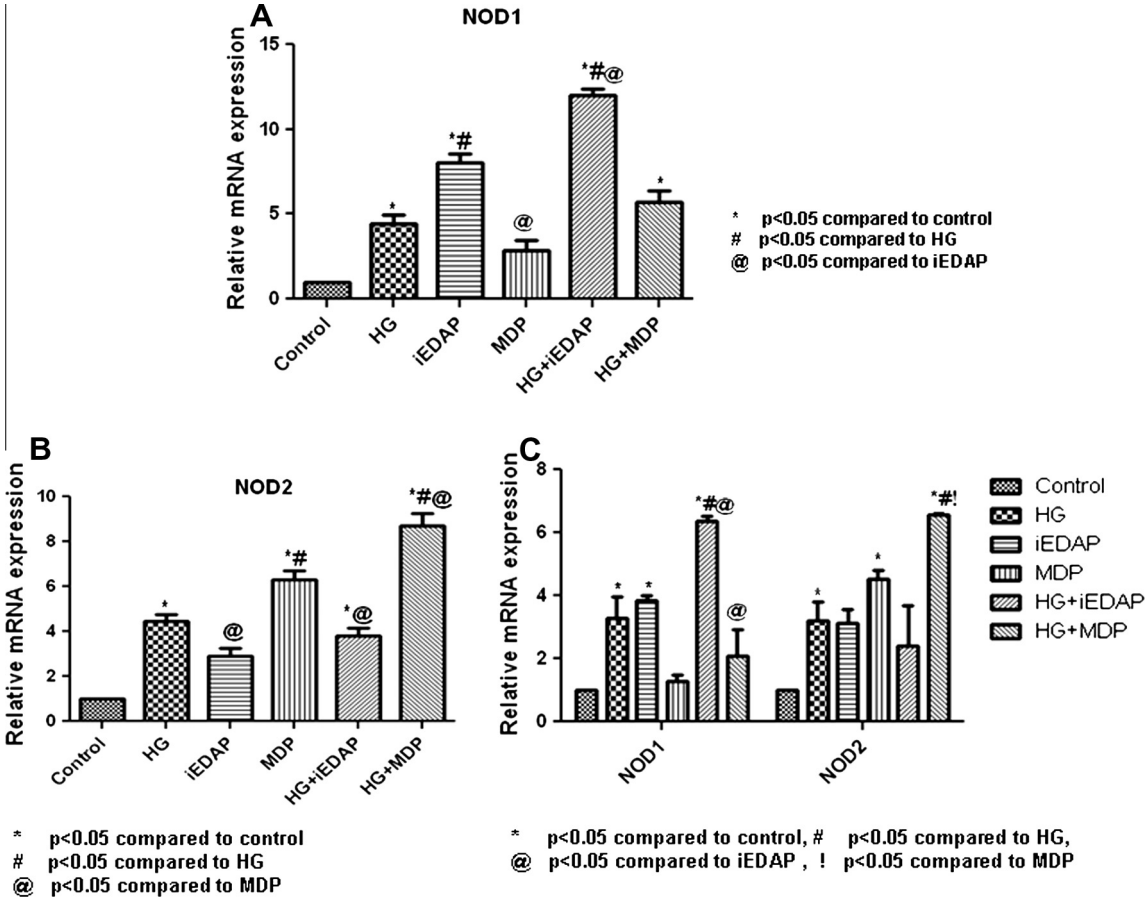

Fig. 5. High glucose stimulates the mRNA expression of NOD1 and NOD2. High glucose (30 mM) increased the mRNA expression of NOD1/2 and significantly augmented the iEDAP mediated expression of NOD1 (A) MDP mediated expression of NOD2 (B) in monocytes from healthy volunteers ($n = 5$). The effect was further verified by in vitro analysis on THP-1 monocytes (C). Data represents the mean ± SEM.

implicated in the genesis of diabetes and its complications. Levels of both TNF-α (Fig. 6A) and IL-6 (Fig. 6B) were significantly higher in the serum samples of T2DM (TNF: 27 ± 10.9; IL-6: 30 ± 23.55) compared to IGT (TNF: 20.63 ± 13.97; IL-6: 19.16 ± 14.8) and NGT (TNF: 12.9 ± 9.08; IL-6: 12.3 ± 3.6).

TNF-α and IL-6 levels in the culture supernatants of *in vitro* treated monocytes were higher in patients with T2DM (iEDAP stimulated; TNF-α 360.03 ± 47.3, IL-6 4792.3 ± 157.9: MDP stimulated; TNF-α 405.9 ± 32.3, IL-6-7773.6 ± 123.6) compared to NGT (iEDAP stimulated; TNF-α 82.11 ± 26.3, IL-6–2177.7 ± 85.9: MDP stimulated; TNF-α 76.35 ± 13.02, IL-6-2568.7 ± 102.3) and IGT which showed a significantly increased level of TNF-α (iEDAP stimulated 174.15 ± 3.08 MDP stimulated 184.95 ± 27.75) and IL-6 (iEDAP stimulated 3403.63 ± 78.56 MDP stimulated 5108.7 ± 89.5) when compared to NGT (Fig. 6C and D).

4. Discussion

Although the mechanisms by which T2DM develop are still not clearly understood, there is growing evidence that chronic, low-grade inflammation may play a role in promoting the development of this disease. While recent studies imply a role for aberrant acti-

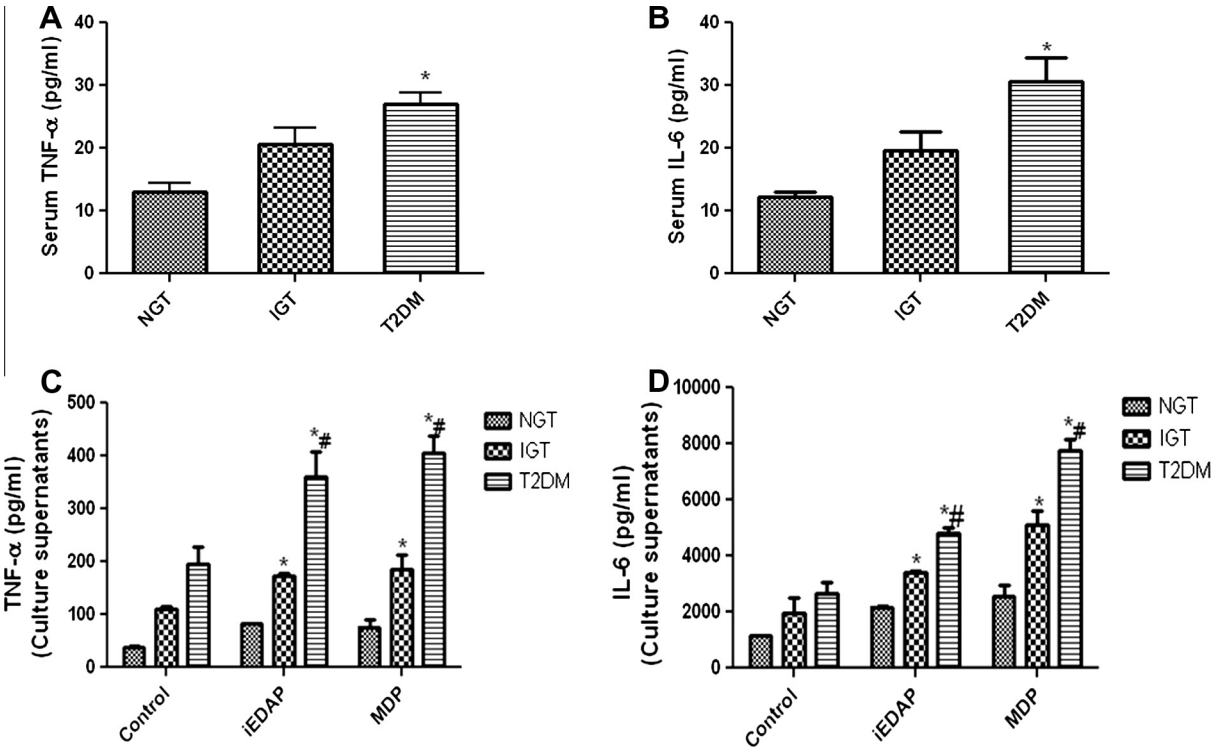

Fig. 6. TNF-α, IL-6 levels in the serum and culture supernatants. ELISA showing the increased levels of serum TNF-α and IL-6 in the study subjects (A and B). Levels of TNF-α and IL-6 in the culture supernatants when monocytes from the study subjects were stimulated with NOD ligands (C and D). Data are expressed as Mean ± SD. $^{*}p < 0.05$ compared to NGT; $^{\#}p < 0.05$ compared to IGT.

vation of the innate immune system in metabolic disorders such as T2DM, there is scant clinical evidence on the involvement of specific inflammasomes. In this context, our study attains significance for the following reasons: (1) This is the first clinical demonstration of elevated NOD1 and NOD2 expression in monocytes from patients with T2DM; (2) The downstream target of NODs viz., RIPK and NFκB were also upregulated in patients with type diabetes. (3) Elevated levels of NODs were positively correlated to poor glycemic control and insulin resistance.

Available literature evidences suggest that insulin resistance in the context of T2DM can be associated with chronic low-level inflammation, with innate immunity constituents such as pattern recognition receptors (PRRs) implicated in this pathophysiology. However, there is paucity of data examining the role of NODs in diabetes. Our study demonstrated elevated mRNA expression of NODs in monocytes from T2DM which has been positively correlated to both insulin resistance and poor glycemic control. NOD1 ligand DAP has been shown to activate NFκB leading to IL-6 secretion in isolated human preadipocytes [20]. Schertzer et al. [15] have identified NLR family proteins as critical factors involved in HFD (high-fat diet)-induced inflammation and insulin resistance. More importantly, NOD1/2-double-KO mice were shown protected from HFD-induced insulin resistance. Treatment of 3T3-L1 adipocytes with DAP impaired insulin signaling and glucose uptake and this phenotype was specifically reverted by using NOD1 specific siRNAs (small interfering RNAs) [21]. Recent studies also suggest that activation of NLRP3 inflammasome leads to the maturation and secretion of interleukin IL-1β and is involved in the pathogenic mechanisms of obesity-induced inflammation, insulin resistance, and T2DM development [22,23]. Similarly, addition of NOD2 ligand MDP suppressed basal and insulin-stimulated glucose uptake in L6-GLUT4-*myc* myotubes [24]. Very recently, Lee et al. [25] have reported that monocytes derived from type 2 diabetic patients display elevated levels of both mRNA and protein expression of NLRP3. Taken together, these results imply a possible role for NODs in inflammation and insulin resistance.

In the present study, enhanced expression of immune receptor NOD1/2 is paralleled by activation of peripheral blood monocytes in patients with T2DM. Higher expression of CD11b (integrins) in T2DM substantiate the enhanced monocyte activation since it is the most important marker involved in monocyte activation. Resting monocytes constitutively express integrins which are the important signal transducers for virtually all monocyte functions and new copies of CD11b are translocated to the cell surface from granules upon activation [26]. Increased CD11b expression on monocytes have been reported previously in patients with type 1 diabetes and leukocytes of T2DM [27,28]. In addition to CD11b, T2DM patients showed elevated expression of CD36, which is a scavenger receptor that binds to oxidized LDL to induce proinflammation through NFκB. Elevated expression of CD36 has been reported previously in T2DM [29] and it has been reported as a biomarker for monocyte activation in T2DM [30].

In addition to the elevated expression of NOD1/2, our study also demonstrated increased expression of downstream signaling mediators RIPK2 and NFκB in monocytes from patients with T2DM. Stimulation of NOD1/2 by their specific bacterial ligands causes the recruitment of RIPK2, a caspase recruitment domain containing kinase (CARD), which then directly binds to Iκβ kinase (IKK-γ) to activate NFκB for pro-inflammation [11,12]. In embryoblast derived from mice deficient in RIPK2, transfection of plasmids expressing NOD1/2 could not activate NFκB, suggesting that RIPK2 is critical for signaling via NOD1/2 [31]. Moreover, RIPK2 participates in innate immunity specifically by mediating NOD1/2 signaling but not TLR mediated immune responses [14].

Another intriguing observation from our study is the augmentation of NOD1/2 mRNA expression by high glucose in monocytes from healthy volunteers. Glucose showed a synergistic effect with NOD ligands by augmenting ligand-mediated expression of NOD1/

2. The data was further validated *in vitro* on THP-1 cells, which is a well characterized human monocyte cell line. Although it is not clear how glucose could activate NOD expression, non bacterial molecules like lauric acid [32] and DMXAA [33] have been shown to activate NOD. In this context, glucose has been shown to induce TLR2/4 expression via PKC-α and PKC-δ, respectively by stimulating NADPH oxidase in human monocytes [34]. Although the interplay between NADPH oxidase and NOD is unclear, it is possible that high glucose could modulate ROS (reactive oxygen species) production and thereby trigger NOD mediated inflammation. In pancreatic islets it has been shown that glucose activates NOD via ROS – sensitive TXNIP (Thioredoxin interacting protein) [35]. We have also demonstrated earlier that lymphocytes from patients with T2DM and subjects with prediabetes exhibited increased TXNIP mRNA expression which was positively associated with protein oxidation and gene expression patterns of IL-6, TNF-α, and NADPH oxidase [36]. Therefore, glucose-mediated cellular triggers could be the denominators that differentially regulate PRRs including NODs and even contribute to the cross-talk among the different receptors involved in proinflammatory responses. Since NODs are also the key regulators linking microbiota to intestinal mucosal immunity [37], aberrant NOD alterations might result in microbiota dysbiosis which is emerging as a contributing factor in the development of metabolic diseases including T2DM [38].

To conclude, our demonstration of increased NOD1 and NOD2 expression in monocytes from patients with type 2 diabetes assumes clinical significance as this could also explain the underlying pro-inflammation and higher insulin resistance status in Asian Indians. Future research to identify explicit molecular mechanism of immune-metabolic interactions that lead to organ dysfunction from chronic inflammatory damage and development of specific NOD modulators may pave the way for novel strategies to manage type 2 diabetes and its complications.

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