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Soluble CD36 in plasma and urine: A plausible prognostic marker for diabetic nephropathy

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ABSTRACT

Aims: This study was designed to analyze the level of soluble CD36 (sCD36) in both plasma and urine of type 2 diabetic patients with and without microalbuminuria/macroalbuminuria.
Methods: Study subjects (n = 20 each) comprised of those with normal glucose tolerance, type 2 diabetes (T2DM) with normoalbuminiria, T2DM with microalbuminuria and T2DM with macroalbuminuria. The biochemical parameters were analyzed using auto-analyzer, and the level of sCD36 was estimated using an in-house Sandwich ELISA.
Results: The presence of sCD36 has been identified for the first time in the urine sample. Significant increase in the level of sCD36 was observed in both plasma and urine of diabetic patients with microalbuminuria ($P < 0.01$) and macroalbuminuria ($P < 0.001$). Positive correlation of sCD36 with the kidney markers such as urea, creatinine and eGFR confirmed the association of sCD36 with kidney damage in diabetic patients. Microalbuminuria, which is clinically used as a biomarker for nephropathy showed a strong positive correlation with urine sCD36 ($r = 0.642$; $P < 0.001$) and plasma sCD36 ($r = 0.498$; $P < 0.001$) in Pearson correlation analysis, which was further substantiated in stepwise multiple regression analysis.
Conclusions: Our study implies a plausible prognostic/adjuvant biomarker role of soluble CD36 for diabetic nephropathy.

1. Introduction

Diabetic nephropathy (DNP) is a chronic kidney disease caused by diabetes mellitus with detrimental effects in both kidney morphology and function. Approximately 40% of patients with type 1 diabetes and 15% with type 2 diabetes eventually develop nephropathy over the period of 10–15 years from the onset of diabetes (Parving et al., 1988). The progression of DNP could be retarded by preventing the risk factors such as hyperglycemia, hypertension and dyslipidemia and by blocking the renin angiotensin aldosterone system (RAAS) (Cooper, Jandeleit-Dahm, & Thomas, 2005). However, in order to prevent the risk factors, the disease has to be diagnosed at an early stage of progression. The marker that is clinically used for the diagnosis of DNP is albuminuria along with glomerular filtration rate (Perkins et al., 2007). However, there are some drawbacks associated with albuminuria as a biomarker for DNP. Low grade albuminuria i.e. microalbuminuria is highly non-specific (Caramori, Fioretto, & Mauer, 2000), and it has been proposed as a biomarker for cardiovascular risk in diabetic patients (Mancia et al., 2009). Further, high grade albuminuria i.e. macroalbuminuria develops at a stage when it is too late to prevent the progression to end stage renal disease (Perkins et al., 2007). Therefore, there is an imperative need to look for specific biomarkers that could help diagnose or predict the development of diabetic nephropathy at an early stage.

Cluster of differentiation 36 (CD36) is a multifunctional protein present in diverse cell types such as epithelial cells, adipocytes, monocytes, platelets and skeletal muscle cells (Febbraio, Hajjar, & Silverstein, 2001). It acts as a scavenger receptor with an ability to bind with ligands such as advanced glycation end products (AGEs), oxidized low-density lipoprotein (Ox-LDL), thrombospondin and apoptotic neutrophils (Ohgami et al., 2002). Though CD36 was known as a membrane glycoprotein, a soluble form of CD36 (sCD36) has been identified in plasma (Handberg, Levin, Hojlund, & Beck-Neilson, 2006). An elevated level of plasma sCD36 has been reported in different pathological states such as liver injury (Fernandez-Real et al., 2009; Takashi et al., 2013), obesity, insulin resistance (Handberg et al., 2006), steatosis (Garcia-Monzon et al., 2014), atherosclerosis, plaque instability, monocytes activation and inflammation (Handberg et al., 2008). Combining the human, animal and in vitro cell culture work, Susztak, Ciccone, McCue, Sharma, and

Conflict of Interest: All authors have made substantial contribution to the conception and design of study, or analysis and interpretation of data, drafting the article or revising it for the intellectual content. There is no conflict of interest for any of the authors, and all authors approved the final version of the article to be published.

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Bottinger (2005) have elucidated a pathological role for CD36 in DNP. They reported that in the hyperglycemic condition, there is formation of AGEs which could bind with the receptor protein CD36. The binding of ligand to CD36 activates the serine-threonine kinases Lyn and Fyn, which in turn activates the protein kinase C and MAP kinase pathway. This ultimately results in the activation of caspases, which leads to the apoptosis of proximal tubular epithelial cells (PTEC) (Susztak et al., 2005). Although, CD36 has been claimed to be involved in the pathological mechanism of DNP and its ligands such as ox-LDL and AGEs were shown elevated in diabetic complications, there are few studies that have looked at the role of sCD36 in the clinical context of diabetic nephropathy. Therefore, this study was designed to analyze the levels of sCD36 in both plasma and urine of diabetic patients with different stages of DNP along with other conventional clinical parameters so as to demonstrate its potential as a biomarker of DNP.

2. Materials and methods

2.1. Study design

Study subjects were recruited from Dr. Mohan's Diabetes Specialties Centre, a tertiary-care diabetes centre at Chennai, India. Patients with reported history of kidney disease other than diabetic nephropathy, cardiovascular disease, cancer, hematuria, hypothyroidism and history of any known infection or inflammatory diseases were excluded from the study. The study was approved by the Institutional Ethics Committee (IEC) of the Madras Diabetes Research Foundation, Chennai (IRB No. IRB00002640) and University Human Ethical Committee (UHEC) at VIT University (VIT/UHEC-4/NO.1). It was performed in accordance to the Helsinki Declaration. Informed written consent was obtained from volunteers involved in the study.

Study subjects (n = 20 each) comprised of subjects with normal glucose tolerance, type 2 diabetic patients with normoalbuminuria, diabetic patients with microalbuminuria and diabetic patients with macroalbuminuria. According to the World Health Organization consulting group criteria, subjects with a 2 h plasma glucose value >11.1 mmol/l or higher (>200 mg/dl) and with absence of ketosis and adequate beta cell reserve as shown by C-peptide assay were diagnosed to have type 2 diabetes (T2DM); and those with a 2 h plasma glucose value of 7.8 mmol/L or lower (<140 mg/dl) as normal glucose tolerant (NGT) individuals. T2DM patients were categorized as normoalbuminuria (<30 mg/dl), microalbuminuria (≥30 mg/dl) and macroalbuminuria (≥300 mg/dl) based on the urine albumin levels.

2.2. Anthropometric measurements

Anthropometric measurements such as height, weight, hip size and waist circumference were observed using standard methods (Deepa et al., 2003). BMI was calculated as weight/square of height (kg/m^2). Blood pressure was recorded from the right arm with the subject in the sitting position using mercury sphygmomanometer (Deluxe BP apparatus, Diamond®, India).

2.3. Biochemical analysis

Urine and serum samples were collected using standard procedures as discussed in Chennai Urban Rural Epidemiological Study (CURES 32) (Surendar et al., 2009). The biochemical analyses were performed immediately after the sample collection, and the samples were stored at −80 °C for further analyses. Glycated hemoglobin content was measured by high performance liquid chromatography using Variant Chromatograph (Bio-Rad, Hercules, USA) and was performed using standard procedures. Fasting plasma glucose (hexokinase method), serum cholesterol (cholesterol oxidase-peroxidase-amidopyrine method), serum triglycerides (glycerol phosphate oxidase-peroxidase-amidopyrine method), high density lipoprotein cholesterol (HDL) (polyethylene glycol-pretreated enzymes), creatinine (Jaffe method), albumin (Bromocresol-green method), and urea nitrogen (Diacetyl monoxime-Thiosemicarbazide method) were measured using auto-analyzer (Hitachi 912Autoanalyser, Hitachi, Germany). Glomerular filtration rate was calculated using the study equation developed by the Modification of Diet in Renal Diseases, $eGFR = 186 \times \text{serum creatinine}^{(-1.1.54)} \times \text{age}^{(-0.203)} \times 0.742$ (if females) (ml/min/1.73 m^2) (Levey et al., 1999). Low-density lipoprotein cholesterol (LDL) was calculated using the Friedewald equation (Friedewald, Levy, & Fredrickson, 1972). The intra and inter-assay coefficients of variation for the glucose ranged from 0.78% to 1.68%. The intra and inter-assay coefficients of variation for the HbA1c ranged from 0.59% to 1.97%.

2.4. Sandwich ELISA for soluble CD36 in urine and plasma

Soluble CD36 level in urine was determined for the first time using an in-house Sandwich ELISA method as described by Handberg et al. (2006) with minor modifications. ELISA plates were coated with capture antibody against CD36 (rabbit polyclonal IgG provided at 200 μg/ml and the epitope corresponds to N-terminal region of CD36 of human origin, sc-5522, Santa Cruz Biotechnology, Santa Cruz, USA), and incubated overnight at 4 °C. The unbound sites were blocked by incubating with 4% BSA (analytical grade) for 2 h at room temperature. Urine samples from 30 different subjects were pooled, aliquoted and stored at −80 °C. This urinary pool served as the standard and was applied in increasing dilutions in triplicates. Urine sample from the patients were diluted 10 times and was used for the analysis. After the addition of samples, the plates were incubated at room temperature for 2 h. Anti-CD36, (rabbit polyclonal IgG provided at 200 μg/ml and the epitope corresponds to 1–300 amino acids within the extracellular domain of CD36 of human origin, sc-9154, Santa Cruz Biotechnology, Santa Cruz, USA) dissolved in PBS was used as the detection antibody and incubated at room temperature for 2 h. The secondary antibody (anti-rabbit IgG HRP, GeNei, Bangalore, India) dissolved in PBS was added, and the plates were incubated at room temperature for 2 h. After each incubation step, the wells were washed three times with PBST (PBS with 0.05% Tween20). Detection was carried out using TMB/H_2O_2 for ELISA (GeNei, Bangalore, India) and was incubated in dark at room temperature for 30 min. After the color development, the reaction was terminated using 2 M sulfuric acid, and the absorbance was measured at 450 nm using plate reader (ELx 800, Biotek India). The absorptions were calculated relative to the standard urine pool, and the values are corrected with the level of urine creatinine. The results computed were expressed as relative units.

For plasma sample analysis, EDTA-plasma pooled from 20 different subjects served as standard and was applied in increasing dilutions in triplicates. Since purified native human CD36 is not available as absolute standard, plasma pool was used as standard for calibration. Plasma samples from the patients were diluted 20 times and were added in triplicates. The dilutions that we used for capture antibody, detection antibody and secondary antibody were same as that we used for the urine analysis. The absorptions were calculated relative to the standard plasma pool, and the results were expressed as relative units. The intra-assay variability and the inter-assay variability were found to be 6% and 13% respectively based on high and low control samples.

2.5. Statistical methods

Statistical analyses were performed using Statistical Package for Social Sciences version 16.0 for windows. Shapiro–Wilk test was done for all the parameters to check whether the data follow a normal distribution. Those variables that followed normal distribution were expressed as mean ± S.D., and the non-Gaussian variables were represented as median with inter-quartile range. Those parameters that did not follow normal distribution were log transformed to improve the symmetry of the data for subsequent analyses. The

difference between the groups for all the parameters was assessed using one-way ANOVA followed by Dunnett's test. The association between the variables was observed by using Pearson correlation. Step-wise multivariate regression analysis was done to find the relation between CD36 and selected variables. The values were considered statistically significant if the P value ≤ 0.05.

3. Results

The ANOVA for the clinical characteristics and anthropometric measurements are shown in Table 1. For the study population, no significant difference was observed between the groups for the anthropometric measurements. Although not significant, increased waist-to-hip ratio (WHR) was observed in diabetic patients with microalbuminuria. Diastolic and systolic blood pressures were higher in diabetic patients with microalbuminuria and macroalbuminuria. Poor glycemic control was confirmed in patients with type 2 diabetes by the significant increase ($P < 0.001$) in the fasting plasma glucose (FBS) and HbA1c values (Table 1). Serum triglycerides were high ($P < 0.01$) in patients with macroalbuminuria with decreased HDL-cholesterol content compared to control subjects. However, there was no significant difference between the groups for other lipid parameters such as total cholesterol or LDL cholesterol.

The level of plasma sCD36 in the four groups is shown in Fig. 1. Increased level of sCD36 was observed in T2DM without microalbuminuria compared to control (NGT) subjects, though not statistically significant. There was a significant increase in the plasma level of sCD36 in T2DM with microalbuminuria ($P < 0.01$) and macroalbuminuria ($P < 0.001$). Similar to plasma sCD36, an increasing trend in the level of sCD36 was observed in the urine of diabetic patients without microalbuminuria which further increased in T2DM with microalbuminuria ($P < 0.01$) and macroalbuminuria ($P < 0.001$) (Fig. 2). Of the total study subjects (n = 80), only 25% of them were females (n = 20). The increasing trend in the level of soluble CD36 in plasma and urine from control subjects to macroalbuminuria is more or less the same as evident from our separate analysis.

The Pearson correlation of sCD36 with the selected variables is tabulated in Table 2. Waist to hip ratio was found to correlate positively with plasma sCD36 ($r = 0.394$; $P = 0.001$) and urine sCD36 ($r = 0.501$; $P = 0.001$). Plasma sCD36 showed a positive correlation with cholesterol ($r = 0.289$; $P = 0.01$) and a negative correlation with HDL ($r = -0.412$; $P = 0.001$). Urine sCD36 was found to be positively correlated with FBS ($r = 0.264$; $P = 0.019$) and PPBS ($r = 0.317$; $P = 0.004$). Glycated hemoglobin was also found to show positive correlation with both plasma sCD36 ($r = 0.262$; $P = 0.02$) and urine sCD36 ($r = 0.266$; $P = 0.018$). Plasma sCD36 showed positive correlation with the kidney markers such as creatinine ($r = 0.28$; $P = 0.012$), urea ($r = 0.295$; $P = 0.008$), eGFR ($r = 0.346$; $P = 0.000$) and albuminuria ($r = 0.498$; $P = 0.000$). Urine sCD36 level was also found to correlate positively with creatinine ($r = 0.356$; $P = 0.001$), eGFR ($r = 0.398$; $P = 0.000$) urea ($r = 0.295$; $P = 0.008$) and albuminuria ($r = 0.642$; $P = 0.000$) (Figs. 3, 4).

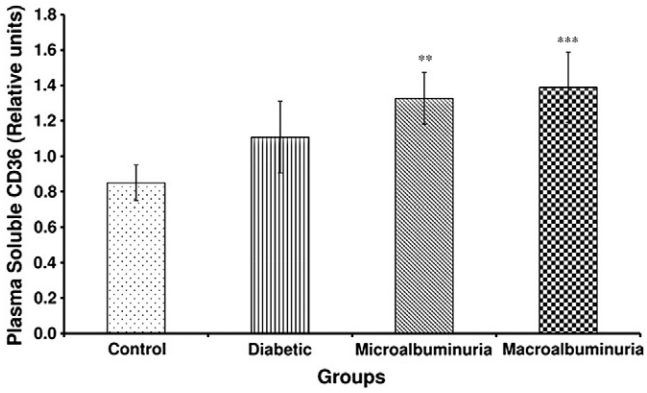

Fig. 1. Level of plasma sCD36. This figure shows the level of plasma sCD36 (relative units) in all the four groups. Significant difference between control (NGT) and T2DM with normoalbuminuria, microalbuminuria, macroalbuminuria are represented as *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

Stepwise-multiple linear regression was done to find the contribution of different parameters to CD36, and the results are shown in Table 3. In the study subjects, urea and creatinine contributed for 29.5% and 28% respectively for the variance of sCD36 in plasma. Further, it was found that urea and creatinine accounted 23% and 27% respectively for the variance of urinary sCD36. Further, microalbuminuria which is considered as the gold standard biomarker for DNP was found to contribute for 49.8% and 62.3% of the variance in plasma sCD36 and urine sCD36, respectively.

4. Discussion

To our knowledge, this is the first study that holistically evaluated the level of soluble CD36 in plasma and urine of T2DM with and without diabetic nephropathy. The study marks the following points: First, the levels of sCD36 in both plasma and urine were significantly

Table 1
Anthropometric and biochemical parameters of study subjects.

Parameters	Control	Diabetic	Microalbuminuria	Macroalbuminuria
N	20	20	20	20
Male/Female	13/7	17/3	13/7	17/3
Age	41 ± 5	44 ± 8	50 ± 7	51 ± 6
BMI	25.4 ± 2.4	25.9 ± 3.9	25.5 ± 2.9	25 ± 3.4
Waist/Hip ratio	0.73 ± 0.32	0.86 ± 0.22	0.96 ± 0.08	0.85 ± 0.37
Diastolic pressure	120 ± 14	129 ± 9	134 ± 13	130 ± 15
Systolic pressure	78 ± 12	80 ± 8	83 ± 7	81 ± 8
Fasting plasma glucose (mg/dl)[#]	84.5 (80.0 – 89.0)	115 (104 – 145)	128 (99–236)***	166 (134–222)***
HbA1C (%)[#]	5.6 (5.4 – 5.8)	7.2 (6.3–8.4)*	8.4(7.2–10.4)***	8.4 (7.0–11.0)***
Serum cholesterol (mg/dl)	178.5 ± 27.7	177 ± 34	170 ± 42.2	177 ± 67
Serum triglycerides (mg/dl)[#]	120.5 (97.2 – 200)	137 (102–187)	139 (112–270)	182 (106–238)
HDL cholesterol (mg/dl)	40.4 ± 8.8	40.7 ± 7.9	36.9 ± 6.4	37.7 ± 11.7
LDL cholesterol (mg/dl)	108.7 ± 21.4	108 ± 31	97.1 ± 34.8	104 ± 63.7
Blood urea [#]	21.0 (18.0 – 23.0)	17 (15–21)	21.5 (16.3–34.8)	23(17–27)**
Serum creatinine [#]	0.8 (0.6 – 0.87)	0.8 (0.7–0.8)	0.8 (0.7–1.1)	1.1 (0.8–1.6)**
eGFR (ml/min per 1.73 m^2)	115.27	115.96	91.77*	76.14***
Urine albumin [#]	4.5 (2.3 – 8.8)	7 (3–15)	78 (49–162)***	350 (320–360)***

Values represent mean ± S.D. of the samples (n = 20) for Gaussian variables.
Significant difference observed between control and diabetic, microalbuminuria, macroalbuminuria are represented as ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.
[#] Values represent median with interquartile range for non-Gaussian variables. ANOVA used for statistics.

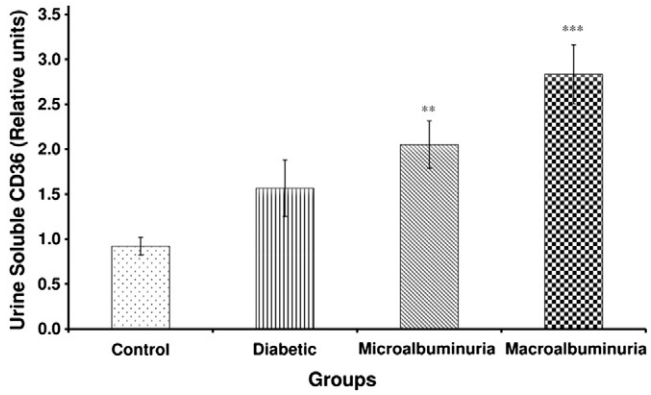

Fig. 2. Level of urine sCD36. This figure shows the level of plasma sCD36 (relative units) in all the four groups. Significant difference between control (NGT) and T2DM with normoalbuminuria, microalbuminuria, macroalbuminuria are represented as *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

increased in diabetic patients with micro and macroalbuminuria and showed positive association with the kidney markers such as urea, creatinine and albuminuria. Secondly, compared to control subjects, patients with T2DM have progressively increasing trend in both plasma and urine levels of sCD36 with increasing severity of nephropathy.

Although elevated plasma levels of sCD36 has been reported in different pathological states (Fernandez-Real et al., 2009; Garcia-Monzon et al., 2014; Handberg et al., 2008; Takashi et al., 2013) and is found to be associated with cardiovascular risk factors (Ramos-Arellano et al., 2014; Zhang et al., 2014), our study is probably the first to report a significant increase in the level of sCD36 in plasma and urine of diabetic patients with nephropathy. It was also found that there was association of both urine and plasma sCD36 with the kidney markers such as urea, creatinine and albuminuria. This implies a relationship of early kidney damage and increased level of sCD36 in plasma and urine in diabetic patients. Combining the human, animal and in vitro cell culture work, Susztak et al. (2005) have elucidated a pathological role of CD36 in DNP. It is inferred from the study that several modified products of AGEs/ox-LDL are up-taken by the scavenger receptor CD36 present in PTEC of kidney. This activates the PKC-MAPK signaling cascade which ultimately leads to the apoptosis of PTEC. Thus CD36 was found to mediate tubular epithelial degeneration, which is a hallmark in the progression of DNP (Susztak et al., 2005). While there is an increased formation of AGEs and Ox-LDL under hyperglycemia (Chen, Yang, Loux, Georgeson, & Harmon, 2006), it has also been shown in vitro and in vivo studies that there is increased expression of CD36 with the increase in the ligands such as AGEs and Ox-LDL (Koonen, Jacobs, Febbraio, et al., 2007). Thus, these converging mechanisms could mediate the genesis of diabetic nephrophathy. Iwao et al. (2008) have also shown in vitro that CD36 is an important receptor for promoting renal tubular injury via advance oxidation protein products (AOPP). Further, via a CD36 mediated and redox dependent pathway, AOPP activates the intrarenal renin-angiotensin system causing detrimental effect on the progression of chronic kidney diseases (Cao et al., 2013). In our study, microalbuminuria, which is considered as a gold standard diagnostic marker for DNP showed a strong association with sCD36 and was found to contribute for 49.8% and 62.3% of the variance in plasma and urine sCD36 respectively. This suggests that sCD36 in plasma and urine could possibly be considered as a prognostic marker for DNP.

Table 2
Pearson correlation between soluble CD36 and selected variables.

Variables	log Plasma CD36	log Urine CD36
Cholesterol	0.289 (0.010)	−0.023 (0.840)
HDL	−0.412 (0.000)	−0.078 (0.493)
LDL	0.216 (0.061)	0.006 (0.961)
Insulin	0.450 (0.003)	0.320 (0.039)
BMI	0.028 (0.806)	0.050 (0.659)
WHR	0.394 (0.001)	0.501 (0.000)
eGFR	0.346 (0.000)	0.398 (0.000)
log FBS	0.123 (0.279)	0.264 (0.019)
log PPBS	0.148 (0.192)	0.317 (0.004)
log HbA1C	0.262 (0.02)	0.266 (0.018)
log Triglycerides	0.051 (0.657)	0.101 (0.374)
log Urea	0.295 (0.008)	0.295 (0.008)
log Creatinine	0.280 (0.012)	0.356 (0.001)
log Albuminuria	0.498 (0.000)	0.642 (0.000)
log Plasma CD36	1	0.576 (0.000
log Urine CD36		1

This table shows the correlation coefficient and *P*-value for the selected variables with plasma and urine soluble CD36. HDL: high density lipoprotein, LDL: low density lipoprotein, BMI: body mass index, WHR: waist to hip ratio, FBS: fasting blood sugar, PPBS: postprandial blood sugar, HbA1C: glycated hemoglobin, eGFR: estimated glomerular filtration rate.

The basal level of sCD36 observed in control subjects could have been originated from muscles and other tissues that express CD36 (Handberg et al., 2009). It has been reported that even in glucose tolerant subjects, fat free mass is found to contribute for sCD36. But in glucose intolerant patients, because of the abdominal fat accumulation there is infiltration of macrophage that contributes for the increased level of sCD36 (Liang et al., 2004). In accordance with this finding, an increased expression of CD36 has been reported on the surface of monocytes in type 2 diabetic patients. Further, sCD36 in plasma was reported to have a strong positive correlation with the pro-inflammatory cytokine IL-6 (Handberg et al., 2009). Combining all these findings, sCD36 was identified as a surrogate marker for macrophage activation and inflammation (Sun et al., 2010). In a recent finding, the inflammatory role of CD36 in the proximal tubular cells of renal origin has been identified (Kennedy et al., 2013). This led to the speculation that low grade inflammation might have contributed for the increased release of CD36 from monocytes and macrophages in the diabetic condition. Soluble CD36 was reported to correlate positively with the liver injury markers such as alanine transaminase (ALT) and aspartate transaminase (AST) (Fernandez-Real et al., 2009). Similar to the release of ALT during liver cell destruction, there is a possibility for the release of sCD36 from the cells undergoing necrosis.

CD36 resides in the intracellular vesicles in the inactive form and is brought to the plasma membrane in the active form, when exposed to ligand (Bonen, Luiken, Arumugam, Glatz, & Tandon, 2000). In the hyperglycemic condition, because of the decreased intracellular transport of fatty acids into muscle and adipose tissue (Cai, Wang, Ji, Meyer, & Van der Westhuyzen, 2012), there is a possibility for shedding of the receptor protein CD36 in the soluble form from adipocytes and skeletal muscle cells (Alkhatatbeh, Enjeti, Acharya, Thorne, & Lincz, 2013). Increased concentration of cell derived microparticles has also been reported in the diabetic condition (Omoto et al., 2002). This led to the speculation that CD36 is present in the circulation as a peptide fraction or may shed as microparticles from platelets and macrophages when triggered by various stimuli. The presence of CD36 has been reported in mesangial cells and PTEC of kidney (Iwao et al., 2008). Whenever there is tubular damage, there is decreased protein reabsorption which in-turn accounts for the increased release of proteins in the urine. Since CD36 has been proven to mediate apoptosis in PTEC (Susztak et al., 2005), it led to the speculation that CD36 or a truncated form of it might have been released in urine during apoptosis, as a part of low grade inflammatory state. In chronic kidney disease, there is elevated homocysteine content which has also been proven to increase CD36 expression (Chmielewski, Bragfors-Helin, Stenvinkel, Linholm, & Anderstam, 2010). In our study, there was an increased trend (albeit not statistically significant) of urinary and plasma levels of sCD36 even in insulin-resistant patients with T2DM (without complications) compared to control subjects. This supports the earlier studies that claimed a relationship between insulin resistance and sCD36 levels (Handberg et al., 2006). Interestingly, Gautam, Pirabu, Agrawal, and Banerjee (2013) have recently reported CD36 gene variants and their

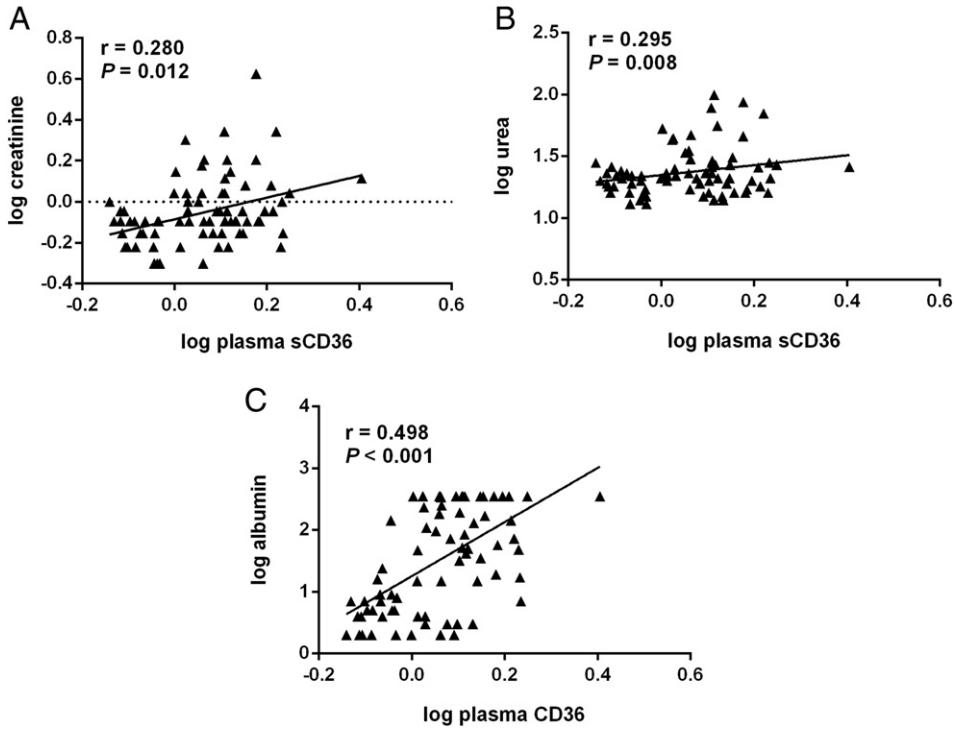

Fig. 3. Correlation of plasma sCD36 with the kidney markers. This figure shows the correlation of plasma sCD36 with the kidney markers such as creatinine (A), urea (B) and albumin (C). The Pearson correlation coefficient (r) and the level of significance (*P*) for each variable have been represented in the graph.

association with type 2 diabetes in an Indian population. One of the limitations of our study is that being a cross sectional one, no cause–effect relationship can be established. Therefore, future longitudinal studies should focus on whether sCD36 could be a predictive biomarker for both the genesis of diabetes in general and diabetic nephropathy in particular. Further, future studies will be focused in finding the specificity and sensitivity of soluble CD36 in predicting diabetic nephropathy.

5. Conclusions

This study has reported the presence of sCD36 in urine for the first time. The level of sCD36 in both plasma and urine are found to be associated with the kidney markers such as urea, creatinine and albuminuria. The results indicate that sCD36 in plasma and urine could possibly be considered as a prognostic marker for DNP. However this has

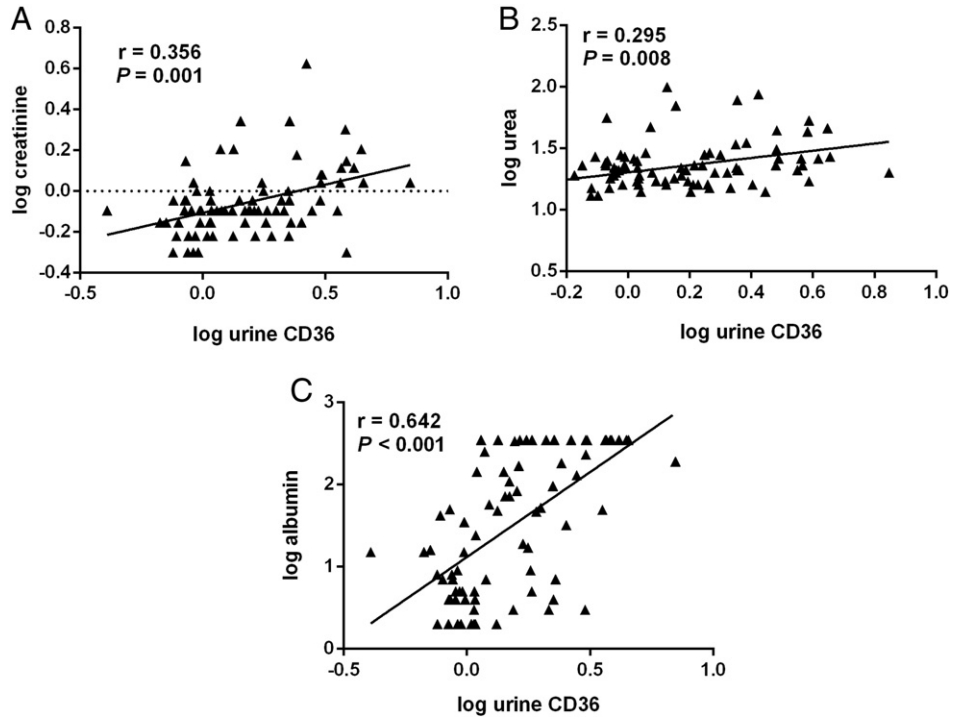

Fig. 4. Correlation of urine sCD36 with the kidney markers. This figure shows the correlation of plasma sCD36 with the kidney markers such as creatinine (A), urea (B) and albumin (C). The Pearson correlation coefficient (r) and the level of significance (*P*) for each variable have been represented in the graph.

Table 3
Stepwise multivariate linear regression of sCD36 with selected variables.

Variables	Plasma CD36											
	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*
log FBS	0.12	0.279	0.016	0.943	−0.13	0.568	−0.11	0.612	−0.08	0.707	−0.13	0.542
log PPBS			0.139	0.221	−0.03	0.891	−0.07	0.744	−0.11	0.639	−0.12	0.562
log HbA1C					0.378	0.03	0.334	0.05	0.333	0.05	0.192	0.239
log Urea							0.295	0.008	0.156	0.33	0.115	0.439
log Creatinine									0.28	0.012	0.011	0.942
log Albumin											0.498	0.000
	Urine CD36											
log FBS	0.26	0.02	−0.01	0.965	−0.04	0.84	−0.03	0.9	0.028	0.896	−0.03	0.861
log PPBS			0.32	0.131	0.283	0.263	0.245	0.263	0.179	0.414	0.159	0.389
log HbA1C					0.089	0.602	0.048	0.77	0.047	0.776	−0.15	0.295
log Urea							0.23	0.04	0.04	0.796	−0.02	0.897
log Creatinine									0.27	0.05	0.095	0.493
log Albumin											0.623	0.000

This table represents the standardized coefficient, β and *P*-value for the selected variables. FBS: fasting blood sugar, PPBS: postprandial blood sugar, HbA1C: glycated hemoglobin.

to be investigated further in larger sample population to confirm it as a reliable biomarker for DNP. Further, the significance of sCD36 in different stages of nephropathy could be elucidated in future in concurrence with the histological assessment of kidney biopsy samples from patients with DNP.

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