Correlation of Inflammatory markers C-Reactive Protein and Interleukin 6 with Visfatin in Chronic Kidney Disease Patients

Nosheen Mahmood1*, Rashid Awan2, Muhammad Saqib3, Humera Akhlaq4, Saima Amir1

1Lecturer Pathology, King Saud Bin Abdul Aziz University, Kingdom of Saudi Arabia.
2Internal Medicine, Ministry of Health Madina Al Munawara, Kingdom of Saudi Arabia.
3Scientific Assistant, Karachi Institute of Radiotherapy and Nuclear Medicine, Pakistan.
4Assistant Professor Oral Pathology, Jinnah Sind Medical University, Pakistan.

Abstract

Inflammation and oxidative stress have a significant contribution in pathogenesis of chronic kidney disease. Visfatin, an adipocytokine has been proposed as a pro inflammatory cytokine. In this study, we evaluated correlation of serum visfatin with inflammatory markers C - reactive protein (CRP) and IL-6 in chronic kidney disease patients. Serum visfatin, IL-6 and CRP levels were measured in 80 subjects including 30 healthy controls and 50 diagnosed patients of CKD. Subjects with a history of any autoimmune or chronic joint disease and fever within last 2 months were not included in this study. Visfatin concentration was significantly high in patients with CKD compared to controls (8.7 ± 4.7 vs 5.2± 3.3 p< 0.001). We found positive correlation of Visfatin with CRP (r=0.256, p<0.01) and IL-6 (r=0.756, p<0.01). Serum Visfatin correlated positively with CRP and IL-6 which suggests its association with inflammation in chronic kidney disease however; further studies at molecular level may clarify mechanism of inflammation in CKD.

Key Words: Visfatin, C - reactive protein, Inflammation, Chronic kidney disease.

1. Introduction

Research has brought a notable improvement in understanding of the complex mechanisms involved in pathogenesis of CKD. There is a shift to include inflammation and resulting oxidative stress and endothelial damage as important players in pathogenesis of atherosclerosis in CKD [1]. Pro inflammatory cytokines found in
unique milieu of uremia are shown to be linked to inflammation, endothelial damage and oxidative stress [2].

CKD results in a state of chronic, low-grade inflammation which is evident as a concomitant rise in pro inflammatory cytokines. Among such pro inflammatory cytokines C-reactive protein (CRP) and interleukin-6 (IL-6) are the most extensively studied [3].

Furthermore, adipose tissue is a metabolically active organ and factors produced by adipocytes; visfatin being one of them, may further link inflammation in chronic kidney disease. Visfatin is an adipocytokine released from visceral fat which was discovered by Fukuhara et al in 2004 [4] and has remained a subject of intense research since then. It has been observed that Visfatin levels are elevated in circulation of patients with a variety of inflammatory diseases. It functions as a cytokine, and induces the cellular expression of inflammatory cytokines such as TNF-alpha, IL-1β, and IL-6. In many acute and chronic inflammatory diseases including sepsis [5], acute lung injury, rheumatoid arthritis, inflammatory bowel disease levels are found to be elevated [6]. It is secreted by activated lymphocytes, monocytes and neutrophils and plays a key role in the persistence of inflammation through its capacity to increase neutrophil survival by inhibiting apoptosis [5]. It also acts as a Nicotinamide phosphoribosyl transferase and is thus involved in production of reactive oxygen species which may contribute to its pro inflammatory properties [6].

Jia and colleagues reported that Visfatin was an inflammatory cytokine since it was up regulated in neutrophils and acted to delay neutrophil apoptosis in experimental inflammation and sepsis [5]. It prevents apoptosis through caspases 3 and 8 and induces chemotaxis, production of IL1 β, TNF α, IL-6 and co stimulatory molecules by CD14+ monocytes [6]. The visfatin level correlates with CRP which is a well known marker of inflammation. This cross sectional study was carried out to evaluate the potential link of visfatin with CRP and IL-6 in a group of CKD patients.

2. Experimental

2.1. Patients and Methods

We determined serum CRP, IL-6 and Visfatin level in 80 subjects (40-60 years old) including 30 normal healthy controls and 50 patients of CKD. Diagnosed cases of CKD were recruited from outpatient department of Nephrology Ziauddin University and outpatient department of Nephrology, JPMC Karachi via non randomized purposive sampling.

Controls were drawn from same socioeconomic group and matched to cases by age. All cases and controls enrolled in the study provided informed consent as approved by Institutional Board of Advanced Studies and Research and Ethical Review Committee (10-2008. NM Patho). Data on case demographics and exposure to smoking were derived from administered questionnaire.

Criteria for inclusion of cases included diagnosed cases of CKD defined as an estimated GFR <60 ml/min/1.73 m² for more than 3 months according to the Kidney Disease Outcomes Quality Initiative (KQODI) guide lines [7]. None of the patients were receiving on renal replacement therapy.

Subjects with a history of any autoimmune or chronic joint disease and fever within last 2 months were not included in this study. Anthropometric and blood pressure measurement was done according to standard methods.
Samples from patients and controls were collected for quantitative analysis of serum visfatin, CRP and IL-6 after an overnight fast. Within 30 min of collection samples were centrifuged and separated serum was immediately frozen at -70 C. Serum Visfatin was measured using EIA kit from Phoenix Pharmaceuticals, Burlingame, CA, serum CRP and IL-6 was measured through commercially available kit from Randoux Laboratories UK and R&D systems UK according to manufacturer’s protocol.

2.3. Statistical Analysis
Statistical analysis was done using Statistical Package for Social Sciences, version.16 SPSS Inc, Chicago, Illinois, USA. To compare baseline characteristics between the CKD and control groups, we used the χ² tests for qualitative variables among different groups and students ‘t’ Test for quantitative differences between the groups. Pearson’s correlation was used to analyze linear correlation between continuous variables. ‘p’ value of <0.05 was viewed as significant.

3. Results
Blood samples of eighty subjects were analyzed including 30 controls and 50 CKD subjects. CKD was secondary to diabetic nephropathy in 22 patients, 10 patients had membranous glomerulonephritis, 6 had hypertensive nephropathy, 5 had focal segmental glomerulonephritis, 2 had chronic pyelonephritis and 5 had CKD secondary to unknown cause.

There were 42 men and 38 women including 24 men and 26 women among CKD subjects and 18 men and 12 women among control group. Mean serum visfatin was not significantly different between men and women (7.35±3.6 vs 7.8±5.4, p>0.05). Similarly mean CRP level was not different between men and women (4±5.5 vs 5.5±8.5 p>0.05).

Mean serum CRP and IL6 levels in controls were significantly lower than CKD subjects (6.8±2.6 vs 11.4±8.2 p<0.05) and (3.5±1.1 vs 6.9±2.5 p<0.01) as shown in Table 1. Among CKD subjects CRP level in diabetics and non diabetics was not significantly different (11±8.2 vs 11.9±8.4).

Upon sub grouping of CKD subjects according to CKD stages there were 10 cases of stage 3, 31 of stage 4 and 9 of stage 5. Mean CRP among stage 3 subjects was 5.4±5.6, stage 4 was 5.4±7.5 and stage 5 was 11±11.9. Serum CRP could not achieve statistically significant difference between any of the three groups.

A positive correlation was observed between serum visfatin and CRP (r=0.257, p<0.05, 0.022) (Figure 1) and between visfatin and IL6 (IL-6 (r=0.756, p<0.01) (Figure 2).

<table>
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<tr>
<th>Parameter</th>
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<th>'P' value</th>
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<tr>
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<td>50</td>
<td></td>
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<tr>
<td>Age</td>
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<td>6.4±8.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>IL6</td>
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<td>3.5±1.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>VISFATIN</td>
<td>5.2 ±3.2</td>
<td>8.8± 4.6</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

CKD: Chronic Kidney Disease; BMI: Body mass index; WC: Waist circumference; CRP: C - reactive protein.

Table 1. CRP, IL6 and Visfatin levels in study population.
Figure 1. Correlation of Visfatin and CRP.

Figure 2. Interleukin 6 level between groups.

Figure 3. Correlation of Visfatin with IL-6.
4. Discussion

We observed a positive correlation of visfatin with CRP and IL-6 suggesting its possible link to inflammation in CKD. This directs us to the fact that visfatin is primarily an inflammatory cytokine and the role of low-grade inflammation in CKD is no more a hidden fact [8].

Our findings are in concordance with Malyszko and coworkers, who also observed a positive correlation between visfatin and high sensitivity C-reactive protein (hsCRP) [9]. Their findings were based on kidney transplant recipients whereas none of our patients was on renal replacement therapy. Thus, it could be one of the inflammatory cytokine involved in pathogenesis of CKD.

Similarly, Seo and colleagues observed a significant positive correlation between visfatin levels and IL-6 \((r = 0.269)\), CRP \((r = 0.233)\) and percent body fat \((r = 0.206)\), in non-diabetic Korean women which was observed only for CRP on multiple step wise regression analysis [10].

In our concordance was the work carried out by Kanda and colleagues who studied plasma visfatin levels in 154 elderly bedridden patients from Osaka, Japan. They observed a positive relationship of plasma visfatin with diastolic blood pressure and CRP suggesting a possible pro inflammatory role of visfatin [11].

Adya and colleagues studied the effect of visfatin on nuclear factor-.B (NF-.B). Visfatin led to increased transcriptional activity of NF-.B in human vascular endothelial cells transfected with a plasmid containing 5 NF-.B. Moreover, addition of a NF-.B inhibitor reduced visfatin’s induction of MMP-2 and MMP-9 mRNA, protein levels and activity. This supports the role of visfatin in vascular inflammation [12].

Ognjanovic and colleagues found that visfatin mRNA levels were increased in fetal membranes from patients with severe infection-related pre-term labor as compared to controls and they also observed that increased visfatin expression in amniotic epithelial cells upon treatment with IL-6, TNF-a, and IL-1ß supporting cytokine-like properties for visfatin [13].

Oki and colleagues investigated the associations between serum visfatin and IL-6 and CRP in Japanese Americans and observed a positive correlation [14]. Similar findings were reported by Otero and colleagues who observed a positive correlation of visfatin with CRP in rheumatoid arthritis [15].

Zhang and colleagues investigated the potential link of visfatin with CRP in 630 healthy Italians subjects and observed a significant association of -948 T allele with plasma CRP and fibrinogen levels, suggesting a possible connection with low-grade inflammation [16].

To further validate the pro inflammatory role of visfatin studies were conducted at molecular level. Administration of Rheumatoid Arthritis related cytokines to fibroblasts led to increased expression of visfatin and similarly visfatin administration led to activation of cytokines and MMPs (Matrix Metalloproteinases) [17].

Administration of recombinant visfatin to monocytes and peripheral blood mononuclear cells led to increased level of MMP 9 in monocytes and Tumor Necrosis factor Alpha (TNF \(\alpha\)) and IL8 in peripheral blood mononuclear cells. Increased visfatin mRNA levels are seen in fetal membranes from patients with severe infection related preterm labor [18].

We observed a linear correlation of IL-6 and CRP with visfatin supporting the hypothesis that increasing visfatin level parallels with inflammatory status. From our observations in CKD patients, we can suggest that serum
visfatin correlates with inflammatory marker CRP. The same holds true for its association with IL-6 another inflammatory marker. We can conclude that visfatin may be taken as a new addition to the list of inflammatory markers involved in pathogenesis of CKD and may serve as an important target for drug therapy.

5. Ethical Consideration
All cases and controls enrolled in the study provided informed consent as approved by Institutional Board of Advanced Studies and Research and Ethical Review Committee (10-2008. NM Patho)

6. Conflicts of Interest
The author(s) report(s) no conflict(s) of interest(s). The author along are responsible for content and writing of the paper.

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8. References


