C-Reactive Protein in Inflammation with Special Reference to Chronic Kidney Disease.

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ABSTRACT
The serum levels of C-reactive protein were determined on 30 patients (22 males and 8 females) with chronic kidney disease who had not commenced haemodialysis and 30 apparently normal healthy individuals (16 males and 14 females) used as control. CRP was measured semi-quantitatively using modified Latex agglutination technique. The levels were determined on pre and post dialysis of the first session of dialysis and the fifth successive session on same patients. The results obtained showed that the mean and the standard deviation were 73 ± 24.1 and 75 ± 21.9 for pre and post dialysis of the first session respectively, 74 ± 22.3 and 74 ± 22.6 for pre and post dialysis of fifth successive session respectively and 7 ± 5.0 for control group. The results obtained showed significant differences between pre, post dialysis and control p < 0.01. However, no significant difference was obtained between pre and post dialysis (P>0.05). Similar statistical result was obtained for the fifth successive dialysis. Positive correlation was seen between pre and post dialysis of first session (r = 0.99). The results suggest that the CRP level is not useful for management of chronic kidney disease. However, with the finding of elevated levels of CRP in apparently healthy individuals, it is recommended that the estimation of CRP should be included in standing order test so that sub-clinical diseases could be discovered and treated before they become manifest.

C-REACTIVE PROTEIN IN PATIENTS WITH END-STAGE RENAL DISEASE

INTRODUCTION
Raised C-reactive protein (CRP) is a strong predictor of cardiovascular events and all cause mortality in end-stage renal disease (ESRD)¹. Several studies have confirmed elevated CRP as an independent cardiovascular risk factor in general population² and ESRD patients in predialysis³ as well as in those undergoing haemodialysis⁴ or peritoneal dialysis⁵.

C-reactive protein is a major acute phase response protein synthesized mainly in the liver in response to elaboration of acute phase response cytokines largely interleukin-6 (IL-6)⁶. During inflammatory reactions, CRP can rise up to a 1000 folds above normal levels⁷. The acute phase response usually lasts only a few days but in cases of chronic or recurring inflammation, an aberrant continuation of some aspects of acute phase response may contribute to the underlying tissue damages that are associated with the ESRD and subsequent complications⁸. Stevinkel et al have noted that the prevalence of inflammation is high in uremic patients⁹. Various causative factors have been implicated for these infections, viz. vascular access surgery, the dialysis procedure, heart failure and renal or systemic inflammatory diseases. It is not yet resolved if CRP is just a sensitive marker of systemic inflammation or if it indeed contributes actively to development and progression of atherosclerotic lesions. But several reports have shown that CRP may be directly involved in active inflammatory processes in atherosclerotic lesions¹⁰. These studies have located CRP inside the atherosclerosis intima in the aorta¹¹, coronary and carotid arteries. Atherosclerosis which until recently was considered a degenerative disease is now thought to be a chronic inflammatory process. The role of inflammation from start of atherosclerosis through its progression and complications has

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Ugonabo Mc et al. “C-Reactive Protein in Inflammation with Special Reference to Chronic Kidney Disease”

been documented\textsuperscript{12}. Studies have documented that the use of ingitors off CRP (such drugs as statins, aspirin, ACE-1) can reduce its levels\textsuperscript{13}. Lovastatin therapy has been recently shown to reduce CRP level by 14.8\% and decrease cardiac events in patients in a 5 – year randomized trial of lovastatin for primary prevention of coronary events. This implies therefore that identification of patients with elevated CRP and early inclusion of appropriate treatment regime may prevent deleterious cardiovascular complications.

Though 8 – 10\% of patients admitted in hospitals in Nigeria have chronic renal failure, very few studies have been done to document cardiovascular events in this population of patients\textsuperscript{14}. Indeed to our knowledge no study has been done till date to document prevalence of CRP in ESRD patients in Nigeria. This present study is intended to determine the level of CRP in ESRD and to document the correlation if any between the CRP level pre and post-dialysis.

PATIENT SELECTION
The studies were done at the departments of Medicine and Chemical Pathology, University of Nigeria Teaching Hospital (UNTH), Enugu, Nigeria. The UNTH is 760 bedded hospital in South–east of Nigeria and serves about a third of the country. Thirty consecutive patients who fulfilled the inclusion criteria were selected and thirty subjects who did not have CRF, diabetes mellitus, hypertension, infections or any disease that would cause an increase in CRP were selected at random and used as control.

**INCLUSION CRITERIA**
1. Patients with end-stage renal disease 18 years and above with no evidence of infections i.e. no fever or raised white cell count (WBC).
2. Patients who had not commenced dialysis.

**EXCLUSION CRITERIA**
1. Evidence of infection (upper or lower respiratory tract infection, urinary tract infection etc) viz. fever, raised WBC.
2. Unstable patients, patients who have malignancies and patients on immunosuppressant.

**SPECIMEN COLLECTION**
One ml of whole blood was collected from each patient before and after each session of dialysis. The blood was transferred to a plain bottle and allowed to clot at room temperature. The blood specimen was centrifuged at 3,000rpm for ten minutes and separated serum stored at 20\textdegree C with added sodium aside.

**REAGENTS**
CRP kits purchased from DARLEZ were used for semi quantitative method of LATEX agglutination which is a modified method of Chetona (1996). This is based on the principle of latex particles coated with goat IgG anti-human CRP. These were agglutinated when mixed with sample containing CRP.

**PROCEDURE**
0.05ml of patients serum samples, positive and negative controls were placed in separate circles on the disposable slide using automatic micropipette with disposable plastic tips. All specimens together with positive and negative controls were assayed neat in the first instance. Reactive specimens were further diluted with normal saline by doubling dilution. Neat samples, diluted samples together with positive and negative controls were mixed with 0.05ml of the latex reagent on the slide and tilted back and forth for 2 minutes. The tests were observed for visible agglutination and the high-test dilution showing a positive result was taken as the titer.

**CALCULATIONS**
Specimens with no agglutination were assumed to contain less than 6mg/l of CRP (\leq 6MG/l). Agglutination in the diluted specimens was multiples of 6.

**RESULTS**
The mean and standard deviation of CRP for pre-, post-dialysis and positive control were 73 \pm 24.1, 5 \pm 21.9 and 7 \pm 5.0mg/l respectively. This shows slight increase in the mean plus standard deviation of post dialysis when compared with that of pre-dialysis.
Ugonabo Mc et al. “C-Reactive Protein in Inflammation with Special Reference to Chronic Kidney Disease”

**Table1. Mean and standard deviation of pre and post dialysis c-reactive protein of 30 srp patients on the fifth successive dialysis.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MEAN mg/l</th>
<th>STANDARD DEVIATION mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-dialysis</td>
<td>74</td>
<td>± 22.3</td>
</tr>
<tr>
<td>Post-dialysis</td>
<td>74</td>
<td>± 22.6</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>± 5.0</td>
</tr>
</tbody>
</table>

The concentration of mean and standard deviation of pre- and post-dialysis C-reactive protein of 30 chronic renal failure patients and control were 74.0 ± 22.3 mg/l, 174.0 ± 23.6 mg/l and 7.0 ± 5.0 mg/l respectively.

The statistical comparison of the serum levels of initial CRP level of pre-dialysis and control showed a significant difference (P<0.01). The statistical comparison of serum CRP levels of post dialysis and control also showed a significant difference (P<0.01) while statistical comparison of pre- and post-dialysis showed no significant difference (P > 0.5).

**Table2. Statistical comparison of crp levels of first, pre-, post-dialysis and control.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>TEST OF SIGNIFICANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Pre-dialysis and Control</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>1st Post-dialysis and Control</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>1st Pre-dialysis and 1st Post-dialysis</td>
<td>P&gt;0.05</td>
</tr>
</tbody>
</table>

The statistical comparison of mean and standard deviation obtained for pre- and post-dialysis and control were similar for the results calculated for the first pre- and post-dialysis and control. Statistical comparison of CRP levels of pre-initial dialysis session and post fifth successive dialysis session showed no significant difference (P>0.5).

A positive correlation analysis between pre- and post-initial dialysis (r=0.99) was observed. A positive pre- and post-dialysis of fifth successive session was observed (r=0.99).

**Table3. Correlation analysis**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>First-dialysis Pre and Post</td>
<td>0.99</td>
</tr>
<tr>
<td>Fifth dialysis Pre and Post</td>
<td>0.99</td>
</tr>
</tbody>
</table>

**DISCUSSION**

C-reactive protein is an acute phase response protein. It is a highly specific and sensitive marker of inflammation. Nowadays many diseases like cardiovascular diseases, diabetes mellitus, uremia and peritoneal diseases have been suggested to have a low grade inflammatory origin. Injury to tissue such as infection and multiplication of micro-organisms elicits an inflammatory response and CRP being a highly specific marker of inflammation is increased during such response. It has been postulated that CRP is increased in pre- and post-dialysis. Some workers have recorded an increase in CRP level in transplant patients and patients on dialysis.

Although report shows that the level of CRP is increased in CKF, there are still some controversies as in the level of CRP in pre- and post-dialysis. Dahaba and Rehak, (2003) reported that there was no significant difference between CRP plasma concentrations before and after three successive sessions of haemodialysis. The levels of CRP in post-dialysis patients are higher than the level of pre-dialysis patients. This difference was attributed to the process of haemodialysis which was seen as a stimulus for inflammation. Results of this study show that CRP is increased in both pre- and post-dialysis. Significant differences were also observed between the post dialysis and control (P<0.01) of the first dialysis session and pre-dialysis and control (P<0.01) of fifth successive sessions of dialysis. Significant differences were also observed between the post-dialysis and control (P<0.01) of the first dialysis session and post dialysis and control (P<0.01) of fifth successive session of dialysis. The mean result of this study showed that the level of post-dialysis of first session was higher than the level of pre-dialysis but no significant difference was observed (P>0.05). The slight increase in the mean level of post-dialysis of the first session (though not significant, P>0.05) might correspond to Reyes et al’s proposal that sees haemodialysis as a stimulus to inflammation. This increase cannot be strongly accepted because both the pre- and post-dialysis of the first session showed significant difference when compared together (P>0.05).
Ugonabo Mc et al. “C-Reactive Protein in Inflammation with Special Reference to Chronic Kidney Disease”

The mean results obtained in fifth session showed no change between the pre and post dialysis and no significant difference was observed. This implies that the CRP level was not affected by dialysis. Positive correlation was observed between CRP level of pre-dialysis and post-dialysis of first session (r=0.99). Positive correlation was also observed between CRP level of pre-dialysis and post-dialysis of the fifth successive session (r=0.99). This implies that as the level of the pre-dialysis is increasing, the post-dialysis is also increasing. The results of the frequency distribution showed that a greater number of CKF patients showed increased CRP level in pre- and post-dialysis of both first and fifth successive sessions of dialysis. However, the levels of CRP of some individuals used as controls were raised. This implied that such individuals have low grade inflammatory reactions without knowing and in effect they are more prone to develop some disease like cardiovascular diseases.

In view of the result of this study, the increased level of CRP in pre and post dialysis has made CRP very useful as a tool in the treatment and management of CRF patients’ C-reactive protein level is increased in pre and post dialysis. The level is also increased after successive sessions of dialysis. CRP being highly specific and sensitive for inflammatory reactions maintained highly related values between the level of the pre and post dialysis of the first session and the pre and post of the fifty successive sessions.

The increased and highly related values can be attributed to the irreversible nature of chronic renal failure. So long as the kidney function cannot be reversed, the inflammatory responses are chronic and the CRP is persistently being produced.

CONCLUSION

Dialysis has no effect on the CRP level of chronic kidney failure patients. Moreover, some individuals have high CRP level without knowing. It is suggested that every individual should know his/her CRP level and CRP should be included in standing order test so that diseases can be predicted and prevented before the actual disease manifests. This will go a long way in improving the general medical practice.

REFERENCES

Ugonabo Mc et al. “C-Reactive Protein in Inflammation with Special Reference to Chronic Kidney Disease”


