Immunomodulatory Effects of Secondary Hyperparathyroidism on Circulating CD4$^+$ and CD8$^+$ T-Lymphocytes in Chronic Renal Failure Patients


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The immunomodulatory effects of parathyroid hormone (PTH) in patients with end stage renal disease (ESRD) is controversial. This study was carried out to investigate the effect of PTH levels on the circulating CD4$^+$, CD8$^+$ T cell counts (%) in patients with chronic renal failure (CRF) on regular hemodialysis ((HD). The study included 22 patients with serum levels of PTH < 300 pg/ml (group I), 18 patients with PTH > 300 pg/ml (group II) and 10 age and sex matched normal controls (group III). Chemiluminescence and flowcytometry assays were performed for determination of serum PTH levels and T cell subset counts respectively. The mean (%) of total lymphocyte, CD4$^+$, CD8$^+$ and CD4/CD8 ratio of group I were (81.68± 9.38), (52.00±6.24), (27.13 ± 6.31) and (1.99±0.42) respectively, as compared to (73.83±13.30), (46.05±8.59), (23.05±4.63) and (2.03±0.41) respectively in group II. Values of group I and II were significantly ($P<0.001$) lower than controls (88.50 ± 6.02), (63.30 ± 6.44), (36.80 ± 6.44) and (1.76±0.36) respectively. In group II, the reduction was significantly ($P<0.001$) prominent in patients with high PTH levels, with significant inverse correlations ($P<0.001$) between PTH and % of total lymphocyte (r= -0.93), CD4$^+$ (r= -0.74) and CD8$^+$ % (r=-0.69). In conclusion, increased level of PTH in CRF patients on hemodialysis is associated with lymphopenia and reduction in CD4$^+$ & CD8$^+$ subsets of T cells. Monitoring circulating PTH levels in such patients can restore their immune competence.

Chronic kidney disease is a worldwide public health issue. In Egypt, the incidence and prevalence of kidney failure are increasing, outcomes are poor, and the cost is high. It is difficult to give an exact incidence of renal failure in Egypt, however, 200 new cases/million inhabitants/ year is a reasonable estimate, based on analysis of mortalities according to official statistical reports (Barsoum, 2000).

Chronic kidney diseases (CKD) are serious public health problems. The number of persons with kidney failure who are treated with dialysis and transplantation is expected to nearly double, with a projected increase from 340,000 in 1999 to 651,000 in 2010. The poor outcomes of CKD are not restricted to kidney failure but include the morbidity and mortality related to decreased kidney functions and immunological impairment (Coresh et al., 2003).

Haemodialysis replacement therapy removes toxic products and corrects the electrolytes, water, and acid base abnormalities associated with renal failure. It does not correct the endocrine abnormalities nor prevent immunodeficiency and not prolong life (Levy et al., 2002).

Chronic renal failure is frequently associated with secondary hyperparathyroidism: Parathyroid hormone (PTH) has been shown to be a major uremic toxin underlying many of uremic syndromes (Massry, 1991). It is over secreted during the end stage renal disease (ESRD) in response to many factors 1) Hypocalcaemia, 2) Hyperphosphatemia, 3) Impaired calcemic response to PTH, 4) Altered vitamin D metabolism & resistance to calcitriol and 5) Altered degradation of PTH by the kidney (Coresh et al., 2003).
Chronic renal failure is accompanied by various immunologic abnormalities of innate and acquired immunity that are observed at an early stage of the disease; worsen with the progression of uremia (Cohen et al., 1997). Secondary immune failure in uremia is multifaceted and is influenced by uremic intoxication per se, by altered renal metabolism of immunological active proteins and by specific effects of renal replacement therapy (Vendetti et al., 2000). PTH seems to be one of the toxins incriminated in immune insufficiency that increases the rate of infections, mortality, and incidence of malignant tumors, and affects erythropoiesis and outcome of end-stage uremic patients (Sakellariou, 1999).

The clinical relevance of altered immune response and the possible relation with parathyroid hormone toxin is not clear. Some studies support the hypothesis that high levels of PTH may contribute to the impairment of cellular and humoral responses (Griveas et al., 2005). On the other hand, other researches have reported a stimulatory effect of PTH on T lymphocytes (Alexiewich et al., 2003). In general, several studies support the hypothesis that PTH has an immunomodulatory effect; however the data and the conclusions remain controversial (Lewin et al., 2000). This study was carried out to assess circulating T-lymphocyte subsets (CD4+ and CD8+) in chronic renal failure patients, who are under regular hemodialysis, in relation to their circulating parathyroid hormone levels; in a trial to answer the question of possible immunomodulatory effect of parathyroid hormone on the cellular immunity of those patients.

Materials and Methods

Forty patients with chronic renal failure diagnosed as end stage renal disease (stage 5) were studied. They were on regular hemodialysis therapy, attending the dialysis units of El-Doaa Hospital, Italian Hospital, Cairo Medical Center or Al-Zahraa University Hospital. They were under regular hemodialysis, three times a week, 3-4 hours/ session, using polysulfone membrane and bicarbonate dialysate. The duration of hemodialysis ranged from 10-96 months. Drug therapy included calcium carbonate as a phosphorus binder, vitamin D therapy; and drugs that control the hypertension in patients with hypertensive nephropathy.

According to serum levels of PTH, patients were classified as follows:

Group I: included patients with PTH level < 300 pg/ml.
Group II: included patients with PTH level >300 pg/ml.

A cut off value for PTH level of 300 pg/ml is chosen as recommended by National Kidney Foundation (NKF) (2004).

A third group that included ten age and sex matched healthy controls, with normal kidney functions and PTH levels were also studied. They were randomly selected from healthy blood donors and designated Group III.

Individuals on steroid, and/or cytotoxic drugs were excluded. Those with autoimmune disorders, e.g. diabetes mellitus; collagen vascular disease; cancer and acute or chronic infections were also excluded from the study.

Informed consents were taken from all subjects.

All patients and controls were subjected to:

1) Clinical history {Age, sex, causes of chronic renal failure, dialysis (duration, number/week, and type of dialysate fluid and membrane used), calcium, vitamin D3 therapy and other therapies}. (2) Laboratory investigations (a complete blood picture, kidney function tests, serum calcium and phosphorus).

3) Measurements of serum levels of intact PTH.

4) Determination of the percentage of lymphocyte subsets (CD4+, CD8+ and CD4+/CD8+ ratio).

Morning serum samples after an overnight fast and before dialysis session were taken for measurements of PTH levels and kept at -20°C till the time of use. Peripheral blood samples were collected aseptically into sterile K2-EDTA vacutainers for determination of CD4+ and CD8+ T-cell counts and their ratio.

Methods

I- Determination of parathyroid hormone concentration

Two-site chemiluminescent enzyme-labeled immunometric assay was performed utilizing a solid phase immulite 2000 analyzer, used for the quantitative measurements of intact parathyroid hormone in serum
according to the instructions of the company. Intact PTH coated bead, alkaline phosphatase conjugated to affinity-purified goat polyclonal anti-PTH, and chemiluminescent substrate were used. Results were automatically read by the device reader. The instrument setting was daily optimized using laboratory reference samples with low and high PTH levels according to the manufacturer's instructions.

II- Determination of CD4⁺ and CD8⁺ T cell counts by Flowcytometry

Phenotypic analysis of peripheral blood leukocytes was carried out using two-color Flowcytometry (FACSCaliber Becton Dickinson (BD), San Jose, California, USA), and monoclonal antibodies directed to the T cell surface markers in whole blood samples; FITC-Labeled CD4, PE-labeled CD8, FITC-conjugated LeucoGATE reagent (CD45/CD14) for lymphocytes gating, isotype control IgG1-FITC/IgG2a-PE for negative control to monitor the non specific staining or FC binding. All monoclonal antibodies were from (BD), (California, USA). (Fig. 1).

LeucoGATE reagent (CD45/CD14) was used for gating on lymphocytes utilizing the Simulset software and Dot plot programs version 2.5 for data acquisition and analysis (Nicholson et al, 1996). The forward and side scattered can gate CD45 lymphocyte population in the bottom of the dot plot.

Results were reported as the percentage of positive cells per lymphocytes population (differential) or as the number of positive cells per microliter of blood (absolute count).

Statistical Analysis

Statistical presentation and analysis was conducted, using the mean, standard deviation (SD) and linear correlation coefficient. Testing the correlation between two variables was done using Pearson’s correlation coefficient test. Linear Correlation coefficient was used for detection of correlation between two quantitative variables in one group. The statistical analysis of the results was computed on IBM PC micro processor. The computing was done by means of statistical software package SPSS (statistical package for the social sciences), version (11) 2001 (SPSS incorporation USA).

Results

All 40 chronic renal failure patients had end stage renal disease; (stage 5). They were under regular hemodialysis therapy.

Table (1) demonstrates the classification and demographic data of the cohort population.

Group I: included 22 patients with PTH level <300 pg/ml. The level of PTH ranged from 26.8 to 227 (pg/ml) and the mean±SD was 103.5 ± 54.1(pg/ml). They were 15 males
and 7 females, the mean±SD of their age was (54.9 ± 10.79) years, the hemodialysis duration ranged from 11-72 months with a mean±SD of (34.77 ± 27.09) months.

Group II: included 18 patients with PTH level >300 pg/ml. The level of PTH ranged from 305 to 858 (pg/ml) and the mean±SD was 609.8 ± 196.8 (pg/ml). They were 11 males and 7 females, their age ranged from 25-70 years with a mean±SD of (52.00 ± 13.00) years, and the hemodialysis duration ranged from 10-96 months with a mean±SD of (51.33±28.03) months.

Group III: included 10 age and sex matched healthy control subjects with normal kidney function tests. The level of PTH ranged from 12 to 27 (pg/ml) and the mean±SD was 18.7 ± 4.37 (pg/ml). They were 5 males and 5 females, their age ranged from 35-65 years with a mean± SD of (49.70 ± 12.41) years.

There was insignificant difference between the three groups regarding the age, sex; and between group I and II regarding the duration of hemodialysis (P>0.05). (Table 1).

Table 1. Demographic data of patients with chronic renal failure and controls

<table>
<thead>
<tr>
<th></th>
<th>Patients with chronic renal failure</th>
<th>Normal controls</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Group I (n = 22)</td>
<td>Group II (n =18)</td>
</tr>
<tr>
<td>PTH levels (pg/ml)</td>
<td>&lt; 300</td>
<td>&gt; 300</td>
</tr>
<tr>
<td>Range</td>
<td>26.80 - 227.00</td>
<td>305 – 858</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>103.55 ± 54.10</td>
<td>609.80 ± 196.80</td>
</tr>
<tr>
<td>Sex: M/F</td>
<td>15/7</td>
<td>11/7</td>
</tr>
<tr>
<td>Age (Years)</td>
<td>32 – 73</td>
<td>25 - 70</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>54.90 ±10.79</td>
<td>52 ± 13</td>
</tr>
<tr>
<td>Duration of *HD (month)</td>
<td>11 - 72</td>
<td>10 - 96</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>34.77 ±27.09</td>
<td>51.33 ± 28.03</td>
</tr>
</tbody>
</table>

Insignificant (P>0.05) difference between the three groups regarding age, sex; and between group I and II regarding the duration of hemodialysis). *HD: Hemodialysis.

Biochemical Laboratory Investigations of Chronic Renal Failure Patients and Normal Controls

Table (2) demonstrates the mean±SD of serum urea, creatinine, phosphorus, calcium and hemoglobin in the studied groups.

There were marked increases in serum urea and creatinine levels in group I and II versus group III (P & P<0.001) and insignificant increases between group I and II (P> 0.05). There were marked increases in serum phosphorus level (P & P<0.001) while marked decreases in serum calcium level (P<0.05) (P<0.001) in group I and II versus group III and also between group I and II (P2 < 0.001).

There was a marked decrease in serum hemoglobin level in group I and II versus group III, (P & P<0.001) and also in group I versus group II (P2 <0.001).
The Immunological Parameters (WBC count, Total lymphocyte%, CD4⁺%, CD8⁺% and CD4⁺/CD8⁺ ratio) in Chronic Renal Failure Patients and Controls

Table (3) demonstrate the mean±SD of WBC count (cell/mm³), total lymphocyte %, CD4⁺%, CD8⁺% and CD4⁺/CD8⁺ ratio. They were (6.99±1.84), (81.68±9.38), (52.00±6.24), (27.13 ± 6.31) and (1.99 ± 0.42) respectively in group I, while they were (7.00 ± 0.66), (73.83 ± 13.30), (46.05±8.59), (23.05 ± 4.63) and (2.03 ± 0.41) respectively in group II, and (9.30 ± 0.95), (88.50 ± 6.02), (63.30 ± 6.44), (36.80 ± 6.44), (1.76±0.36) respectively in group III.

Group I and II showed marked decreases in WBC count than group III ($P_1<0.001$), while there was an insignificant difference between group I and II ($P_2>0.05$).

There was a significant decrease in total lymphocyte % in group I versus group III ($P<0.05$), while there was a marked decrease in group II versus group III ($P<0.001$) and a significant decrease in group II than group I ($P_2<0.05$).

CD4⁺ % in group I and II showed marked decreases than group III ($P & P_1<0.001$), and a significant decrease in group II than group I ($P_2<0.05$).

There were marked decreases in CD8⁺ % in group I and II versus group III ($P & P_1<0.001$) and a significant decrease in group II than group I ($P_2<0.05$), Table (3).

Correlation between Immunological Parameters (Total lymphocyte %, CD4⁺%, CD8⁺% and CD4⁺/CD8⁺ ratio) and Parathyroid Hormone Levels in Chronic Renal Failure Patients

In group I, a significant negative correlation ($r=-0.57, P<0.05$) was detected between PTH levels and CD8⁺%, (Figure 2). On the other hand, there were insignificant negative correlations ($P > 0.05$) between PTH and the other immunological parameters.

In group II, marked negative correlations ($P<0.001$) were detected between PTH levels and total lymphocyte % ($r=-0.74$), CD4⁺% ($r=-0.74$) and CD8⁺% ($r=-0.69$), (Figures 3, 4, 5), while there were insignificant negative correlations ($P > 0.05$) between PTH levels and WBC count ($r = -0.05$) and CD4⁺/CD8⁺ ratio ($r=-0.09$).

Table 2. Biochemical Findings in chronic renal failure patients and controls:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I PTH &lt; 300 n=22</th>
<th>Group II PTH &gt;300 n=18</th>
<th>Group III Controls n=10</th>
<th>$P$- value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum urea (mg/dl) Mean ± SD</td>
<td>132.04 ±33.28</td>
<td>151.88 ±39.16</td>
<td>33.10 ± 6.63</td>
<td>$P&lt;0.001$</td>
</tr>
<tr>
<td></td>
<td>$P_1&lt;0.001$</td>
<td>$P_1&lt;0.001$</td>
<td>$P_2&gt;0.05$</td>
<td></td>
</tr>
<tr>
<td>Serum creatinine (mg/dl) Mean ± SD</td>
<td>8.34 ± 3.26</td>
<td>9.33 ± 3.99</td>
<td>0.98 ± 0.16</td>
<td>$P&lt;0.001$</td>
</tr>
<tr>
<td></td>
<td>$P_1&lt;0.001$</td>
<td>$P_1&lt;0.001$</td>
<td>$P_2&gt;0.05$</td>
<td></td>
</tr>
<tr>
<td>Serum phosphorus (mg/dl) Mean ± SD</td>
<td>5.52 ± 0.82</td>
<td>6.74 ± 1.46</td>
<td>4.18 ± 0.76</td>
<td>$P&lt;0.001$</td>
</tr>
<tr>
<td></td>
<td>$P_1&lt;0.001$</td>
<td>$P_1&lt;0.001$</td>
<td>$P_2&lt;0.001$</td>
<td></td>
</tr>
<tr>
<td>Serum calcium (mg/dl) Mean ± SD</td>
<td>8.59 ± 0.79</td>
<td>7.472 ± 0.73</td>
<td>9.39 ± 0.69</td>
<td>$P&lt;0.05$</td>
</tr>
<tr>
<td></td>
<td>$P_1&lt;0.001$</td>
<td>$P_1&lt;0.001$</td>
<td>$P_2&lt;0.001$</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dl) Mean ± SD</td>
<td>9.25 ± 1.13</td>
<td>7.84 ± 1.21</td>
<td>13.12 ± 1.32</td>
<td>$P&lt;0.001$</td>
</tr>
<tr>
<td></td>
<td>$P_1&lt;0.001$</td>
<td>$P_1&lt;0.001$</td>
<td>$P_2&lt;0.001$</td>
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</tbody>
</table>

$P$: Significance between group I & III, $P_1$: Significance between group II & III, $P_2$: Significance between group I & II.
### Table 3. T lymphocyte % subpopulations in patients with chronic renal failure and controls

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I PTH &lt; 300 (pg/ml) n=22</th>
<th>Group II PTH &gt; 300 (pg/ml) n=18</th>
<th>Group III Normal controls n=10</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>*WBCs (cell/mm$^3$) Mean ± SD</td>
<td>6.99 ± 1.84</td>
<td>7.00 ± 0.66</td>
<td>9.30 ± 0.95</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Total Lymphocyte (%) Mean ± SD</td>
<td>81.68 ± 9.38</td>
<td>73.83 ± 13.30</td>
<td>88.50 ± 6.02</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>CD4$^+$ (%) Mean ± SD</td>
<td>52.00 ± 6.24</td>
<td>46.05 ± 8.59</td>
<td>63.30 ± 6.44</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>CD8$^+$ (%) Mean ± SD</td>
<td>27.13 ± 6.31</td>
<td>23.05 ± 4.63</td>
<td>36.80 ± 6.44</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>CD4$^+$/CD8$^+$ Ratio Mean ± SD</td>
<td>1.99 ± 0.42</td>
<td>2.03 ± 0.41</td>
<td>1.76 ± 0.36</td>
<td>P&gt;0.05</td>
</tr>
</tbody>
</table>

Groups I, II are patients with chronic renal failure, *WBCs: white blood cells. 
P: significance between group I & III, $P_1$: significance between group II & III, $P_2$: significance between group I & II.

**Figure 2.** Correlation between PTH levels and CD8$^+$ lymphocyte (%) in group I (chronic renal failure patients with serum PTH levels <300 pg/ml); A significant negative correlation was detected between PTH levels and CD8$^+$ % ($r=-0.57, P < 0.05$)
Figure 3. Correlation between PTH levels and total lymphocyte (%) in group II (chronic renal failure patients with serum PTH level >300 pg/ml); A marked negative correlation between PTH levels and total lymphocyte (%) ($r = -0.93$, $P < 0.001$)

Figure 4. Correlation between PTH levels and CD4$^+$ lymphocyte (%) in group II (chronic renal failure patients with serum PTH level >300 pg/ml); A marked negative correlation between PTH levels and CD4$^+$ % ($r = -0.74$, $P < 0.001$)

Figure 5. Correlation between PTH levels and CD8$^+$ lymphocyte (%) in group II (patients with chronic renal failure patients with serum PTH level >300 pg/ml); A marked negative correlation was revealed between PTH levels and CD8$^+$ % ($r = -0.69$, $P < 0.001$)
Discussion

Secondary hyperparathyroidism is a prominent hormonal abnormality in CRF. The parathyroid hormone has been shown to be a major uremic toxin, underlying the uremic syndrome (Slatopolsky & Delmez, 1994). Several pieces of information raised the possibility that it also plays a role in the pathogenesis of impaired immune responsiveness. PTH induces many abnormalities in leukocyte and platelet functions, which contribute to mortality and morbidity of the patients (Perna et al., 1990).

This study was carried out to assess the effect of secondary hyperparathyroidism on the number of peripheral blood T-cell subsets (CD4⁺ helper, CD8⁺ cytotoxic and CD4⁺/CD8⁺ ratio) in patients with chronic renal failure under regular hemodialysis. The study included forty patients with chronic renal failure on regular hemodialysis and ten age and sex matched healthy controls. The selection of PTH level of 300 pg/ml was chosen relying on the recommendation of National Kidney Foundation (2004) that PTH level should be two to three folds above the upper value of the normal in dialysis patients. Thus a cut off value of 300 pg/ml for PTH level was chosen to analyze whether there are differences in the percentage of total lymphocyte, CD4⁺ and CD8⁺ in hemodialysis patients with serum levels of PTH more than 300 pg/ml compared to patients with PTH levels less than 300 pg/ml to help the nephrologists to guide the immune status of their patients through the regular follow up and measurement of serum PTH level.

Patients were chosen to be cross matched as regard the duration of hemodialysis to exclude the probability of a statistical difference in this condition as a variable which may affect the immunological status of those patients.

The cohort population in this study was age matched, thus the difference in age was statistically insignificant (P>0.05). However, the study of Santagostino et al., (1999) on lymphocyte subpopulations has shown that age does not affect the degree of immunodeficiency in CRF patients.

In the current study, the biochemical laboratory investigations revealed that there is an impairment in the kidney function tests of both groups of patients with CRF as compared to controls. All patients had higher serum urea and creatinine levels than those of controls (P<0.001). These findings were explained by the study of Messa et al. (1994) who reported that in patients with progressive renal failure, PTH level increases as renal function decreases. The insignificant differences (P>0.05) in kidney function tests between the two groups of patients might be due to administration of calcium and phosphorus chelating agents and HD. This finding is supported by the study of Martinez et al., (1997) who found that PTH levels increased progressively as kidney function declined in all untreated patients who reached ESRD (stage 5) or under regular HD. Secondary hyperparathyroidism develops as a compensatory response to declining kidney function.

The present study revealed that in spite of administration of calcium therapy and phosphorus chelating agents, there is a significant increase in serum phosphorus levels and a decrease in serum calcium levels in patients as compared to controls. However, there are significant differences in serum phosphorus and calcium levels (p<0.001) in CRF patients with PTH levels > 300 pg/ml (Group II) when compared with those with PTH levels < 300 pg/ml (Group I) which may point out to the effect of high PTH level.

These findings are in agreement with Salvatore and Vincenzo, (1995) who reported that the failure of hydroxylation of vitamin D in the kidney of CRF patients leads to low vitamin D activity, which is associated with hypocalcaemia (caused by low intestinal
calcium absorption) and increased phosphate concentration (caused by insufficient renal excretion) and contribute to the stimulation of parathormon secretion. In addition Bover (1999) reported that as the disease progresses, there is a decrease in the number of vitamin D receptors and calcium receptors, which makes the parathyroid glands more resistant to calcitriol and calcium. On the other hand phosphorus induces hyperplasia of the parathyroid glands independent of calcium and calcitriol, and this induces increase PTH synthesis and secretion.

The current study also demonstrated that both groups of CRF patients suffer from anemia and their hemoglobin level is significantly lower than the control group \((P < 0.001)\). In addition, group II showed lower levels \((P<0.001)\) than group I. These findings might be explained by suppression of erythropoiesis by the bone marrow fibrosis (Zingraff \textit{et al.}, 1988) or interfere with the endogenous erythropoietin production (Urena \textit{et al.}, 1991). Wus \textit{et al.}, (1998) added that there is a direct relationship between blood levels of intact PTH and RBCs fragility in patients with CRF on dialysis.

The present study revealed that there is lymphopenia with reduction of \((\text{CD}^4\% \text{ helper} \& \text{CD}^8\% \text{ cytotoxic})\) in the peripheral blood of both group of patients when compared with controls. Several hypotheses have been put award to explain the immunosuppression, which is associated with chronic uremia (Tzanno-Matins \textit{et al.}, 2000; Alvarez-lara \textit{et al.}, 2004). They reported that reduction of T cells in the peripheral blood of uremic patients may be due to either increase apoptosis or accelerated activation and differentiation of T-cells into effector memory T-cell subsets, the latter is plausible as dialysis dependent patients are commonly subjected to repetitive exposure to microbial products and other antigenic stimulations that may lead to accelerated turn over and exhaustion of the T-cell. Cohen \textit{et al.} (1997) demonstrated that the accelerated T cell activation in CRF patients is manifested by increased numbers of cells expressing IL-2 receptors, increased levels of soluble IL-2 receptors in the circulation, as well as increased expression of HLA-DR.

In contrast, Greenfield \textit{et al.}, (1998) demonstrated that a diminished T-cell activation occurs in patients with CRF under hemodialysis because T cell becomes anergic to the particular antigen due to absence of B7 signals. Revital \textit{et al.}, (1995) demonstrated that the T cell is a target for parathyroid hormone action.

In this study, we demonstrated a significant lower number of circulating total lymphocytes, \text{CD}^4^+ and \text{CD}^8^+ in CRF patients with PTH level > 300 (pg/ml) than CRF patients with PTH< 300 (pg/ml) \((P<0.05)\), while \text{CD}^4^\%/\text{CD}^8^\% ratio is not changed. These findings are in agreement with those reported by Moser \textit{et al.}, (2003) and Kurs \textit{et al.} (2004) who explained the immunosuppression associated with chronic uremia including an intrinsic defect of uremic effector T lymphocytes and/or the presence of an abnormal immunoregulation mediated by regulatory lymphocytes (such as helper and suppressor T cells). Additionally, Ioannis \textit{et al.}, (2005) demonstrated that there is an increase in T lymphocyte apoptotic cells in end stage renal disease. This decline in T cells affects both \text{CD}^4^+ and \text{CD}^8^+ and thus explains why \text{CD}^4^\%/\text{CD}^8^\% ratio are not significantly influenced.

Correlation analysis in this study revealed a significant negative correlation \((p<0.001)\) between serum levels of PTH and of total lymphocyte \%, \text{CD}^4^\% \% and \text{CD}^8^\% \% in patients with PTH more than 300 (pg/ml) (group II). This data is corroborated with several studies that reported a decrease in lymphocyte counts in CRF patients (Descamps-Latscha, 1993; Haag-weber & Horl, 1994)
Previously, Nassberger et al. (1992) investigated the hemodialyzed patients, and reported that the abnormal T-cell preactivation in those patients may be due to the high plasma concentrations of IL-2R, which can bind IL-2 and inhibit IL-2-dependent proliferation of T and B cells and may be responsible for lymphopenia. The increase in plasma levels of IL-2R is an index of impaired T-cell function and this increase is greater in patients with hyperparathyroidism since there is a linear correlation with plasma levels of PTH.

Unfortunately, determination of the number of circulating lymphocytes and their subsets does not elucidate the actual deficiency in the immune system and the respective role of T lymphocytes. It is therefore important to evaluate the T lymphocyte function among these patients to confirm the relationship between PTH and immunodeficiency. Other research has documented that PTH has suppressor effects on lymphocyte counts, as well as their functions. This possible effect of PTH may be directly on peripheral blood lymphocyte functions; since both B and T cells have receptors for the hormone or possibly it acts via cyclic adenosine monophosphate stimulation in a way resembling its mode of action on other systems (Fadda et al., 1990).

Gaciong et al., (1991) also documented that PTH enhances entry of calcium into many cells and the chronic exposure to PTH is associated with increased calcium content of many tissues, in addition to elevation of the basal levels of (Ca^{2+}). Such increased cell burden of calcium is associated with dysfunction of many cells such as brain synaptosomes, pancreatic islets, PMNCs, platelets and B and T cells. These observations are supported by the study of Stojceva-Taneva et al., (1993) who reported that CRF is a state of cellular calcium intoxication, mediated by excess parathyroid hormone. Another hypothesis for the mechanism of defective T-cell response is that antigen presenting cells could be affected and resulted in impaired antigen processing or altered antigen presentation to the T-cell. At the T-cell receptor level a down regulation of the TCR/CD3 complex by the uremic milieu has been postulated. Incubation of T-cells with uremic serum lowered TCR/CD3 receptor density on normal and uremic CD4^+ cells (Stachowski et al., 1991).

In conclusion, high levels of PTH are associated with lymphopenia and reduction of (CD4^+ % & CD8^+ %) in the peripheral blood of CRF patients on regular haemodialysis. Secondary hyperparathyroidism is an important risk factor in the impairment of T cell-mediated immunity in uremic patients.

Further studies are recommended to (1) assess T cell functions in CRF patients, (2) evaluate possible mechanisms of interaction between PTH and abnormalities of T-cell function and (3) analyze possible causes of immunodeficiency in uremic patients. Such studies will probably contribute to more efficient and preventive strategies that may be used as a prognostic index of mortality, and pave the ways to promote and improve immune status of CRF patients.

References


