Observations on the Association of Serum histamine, Interleukins and Other Serum Biochemical Values with Severity of Pruritus in Chronic Hemodialysis Patients

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Abstract

Pruritus is one of the most bothersome symptoms in patients on maintenance hemodialysis (HD), however little progress is seen in our understanding of its pathogenesis and the association of different serum biochemical values with its severity. The aim of this study was to evaluate pruritus in HD patients in Benha hospital, Egypt, and to correlate its presence and severity with relevant clinical and laboratory parameters. Fifty Hemodialysis patients were randomly selected from a total of ninety nine patients receiving chronic hemodialysis. An elaborate survey, that included interviews, was conducted to identify patients with pruritus. Twenty one patients on maintenance HD have uremic pruritus (UP) out of fifty patients were enrolled in the study. Blood samples from hemodialysis patients and hemodialysis patients with pruritus were taken during the dialysis. Serum samples were kept frozen at -20°C until chemical and immunosassay were performed. Some relevant clinical and laboratory parameters including Na, K, IL-2, IL-6, cortisol and histamine were evaluated. In 21 patients (42%) pruritus intensified during dialysis. Serum samples were kept frozen at -20°C until chemical and immunosassay were performed. There were significant increase (P<0.05) in the serum levels of BUN, Cr, IL-2, IL-6, and there was a highly significant increase in histamine and cortisol. The results of this investigation showed that several serum biochemical values (cortisol, histamine, IL-2 and IL-6) had increased significantly in uremic patients compared to hemodialysis patients, and healthy patients. The results of this investigation suggest that many factors may have important role in pathogenesis and progression of uremic pruritus from mild to severe. The association of histamine, cortisol, IL-2 and IL-6 are evident; compromised immune function and electrolyte disturbances are related to impaired kidney function and the accumulation of uremic toxins. Further research is needed to elucidate the main causes of UP, and the predictive value of serum biochemical components. The effect of the dialyzing membrane should be factored in any such investigation. The results of our study point out to the potential important role of both IL-2 and IL-6 in UP. Further studies are needed to investigate the contribution of histamine, IL-2 and IL-6, monocytes, and basophils on the development and severity of uremic pruritus, and the possible role of anti-IL-2 and IL-6 receptor antibodies in treatment of severe pruritus. Further, research using a proteomic approach to identify itching-related uremic toxins, and the biomarkers that can be used for early detection of PU is urgently needed.

Keywords: Cortisol; Histamine; Antibodies; Immunosassay; Hemodialysis; Inflammatory; Toxins

Introduction

Uremic pruritus is a common and sometimes severe complication of chronic renal failure. Itch affects 50-90% of patients undergoing hemodialysis, and 25% of patients with pre-terminal chronic renal failure. The mechanism underlying uremic pruritus is poorly understood; although possibilities include histamine, proteases, interleukin-2 (IL-2) and TNF- produced by skin mast-cells, substance P, neuropathy and neurological changes, high levels of Ca, P, PTH, Al, Mg, divalent ion abnormalities, inflammation, or some combination of these. Two hypotheses for the underlying pathophysiological mechanisms of uremic pruritus have been proposed, the immuno-hypothosis, and opioid hypothesis [1]. The pruritus were strongly associated with diminished health-related quality of life in multiple domains, including mood, sleep, and social relations [2]. Cytokines are essential mediators of immune response and inflammatory reactions. Patients with chronic renal failure (CRF) commonly present with abnormalities of immune function related with impaired kidney function and the accumulation of uremic toxins in addition to bio-incompatibility of dialyzer membranes. During a hemodialysis (HD) session, cytokines are released mainly by monocytes activated by endotoxin-type compounds in dialyzer fluid, complement factors and direct contact with dialyzer membrane [3]. Cytokines are the essential mediators of immune response and the inflammatory reactions. They are released by immune cells in response to numerous antigens, bacterial polysaccharides and lectins [4]. Patients with chronic renal failure commonly present with abnormalities of immune function strictly correlated with abnormalities of immune cell reactivity, phenotype alternations of receptors and altered expression of cell surface receptors. These abnormalities are caused by impaired excretory function of kidneys and the accumulation of uremic toxins in addition to bio incompatibility of dialyzer membranes [5]. The present work aimed to evaluate the association of serum histamine, Interleukins and other Serum biochemical values with severity of pruritus in chronic hemodialysis Patients in Egypt.

Materials and Methods

Patients

Fifty Hemodialysis patients were randomly selected from a total...
of ninety nine patients receiving chronic hemodialysis through venous catheter at the Artificial Kidney Unit of Benha University’s hospital, Egypt. An elaborated survey, that included patient’s interviews, was conducted to identify patients with pruritus for this study. Blood samples were drawn during the first 15 minutes of dialysis from each of the twenty one (n = 21) patients who had confirmed pruritus. Blood samples were also obtained from the control group.

Study design

The patients under study were assigned to one of three groups:

1. Control one group included patients with no chronic renal failure.
2. Control two group, included chronic renal failure patients undergoing hemodialysis, but have no pruritus.
3. Pruritus group, included chronic renal failure patients undergoing hemodialysis, who have pruritus.

Further, the degree of pruritus was classified according to a visual analogous scale as mild, moderate or severe pruritus:

1. Mild pruritus patients (n=6) had stings of itching all over their body or in parts of their body when the patients were receiving blood transfusion.
2. Moderate pruritus patients (n=8) were those who had desire to scratch sporadically, and had lesions in one or two parts of the body.
3. Severe pruritus patients (n=7) had constant itching, and they indicated that this itching had been disturbing their sleep; and they appeared to have skin lesions all over their body.

Blood samples

Blood samples were drawn, during the first 15 minutes of dialysis, from each of the twenty one (n=21) patients who had confirmed pruritus. Blood samples were also obtained from six (n=6) chronic hemodialysis patients without pruritus (control). Blood samples were drawn from the arterial end of the vascular access into 3 ml sterile test tubes. Samples were allowed to coagulate at room temperature for 30 minutes, then centrifuged at 3000 revolutions per minute (RPM) for 10 minutes. The serum samples were separated and kept frozen at -20°C for chemical and immunoassays. Laboratory assays performed on each sample included the following parameters: blood urea nitrogen (BUN), creatinine (Cr), sodium (Na), potassium (K), IL-2, IL-6, Histamine and cortisol. Data on the levels of Urea (Ur), Creatinine (Cr), Sodium (Na) and Potassium (K) in each patient’s blood were obtained from the most recent records of routine analysis laboratory of the Artificial kidney Unit of Benha’s hospital. The procedure used for measuring the above mentioned serum biochemical constituents involved kinetic methods and reading of the samples using spectrophotometer as follows: Urea was measured by urea UV method described by Patton and Crouch [6]. On the other hand, creatinine was determined by the Jaffe Colorimetric End point method as outlined by Houort [7].

Reference ranges are presented as mean ± S.E. Means values with different superscript letters in the same row are significantly different (P<0.05)

Table 1: Serum urea and creatinine concentration in normal control and hemodialysis patients with and without pruritus.

### Table 1:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control normal group</th>
<th>Haemodialysis patients group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Without pruritus</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>20.5 ± 4.455</td>
<td>63.67 ± 21.675</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.74 ± 0.15</td>
<td>5.08 ± 1.693</td>
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</tbody>
</table>

Means values with different superscript letters in the same row are significantly different (P<0.05)

Figure 1: Comparisons of serum Urea concentration in normal control and hemodialysis patients with and without pruritus.

Statistical analysis

The Results were expressed as means +/- standard error of the mean ((X ± S.E) and were compared using the F-test analysis of variance (ANOVA) following the method described by Snedecor and Cochran [13].

Results

The results for BUN and creatinine (mg/dl) are presented in Table 1, and illustrated in Figures 1 and 2. Table 1 and Figures 1 and 2 show that BUN and creatinine increased significantly in serum obtained from both hemodialysis patients and pruritus patients compared to the control group. On the other hand, the severe pruritus patients had a highly significant increase in serum urea and creatinine compared with that of mild and moderate pruritus patients. It could be observed that hemodialysing patients with or without pruritus has higher BUN and creatinine than the control group with no renal failure.

On the other hand, data presented in Table 2 and Figure 3 revealed a significant (P<0.05) decrease in sodium levels (hyponatremia) in hemodialysis control patients and pruritus patients compared to control group that has no renal failure. At the meantime, mild pruritus group has the lowest sodium serum level (60.42 ± 16.54 mg/dl), while the moderate group has the highest (85.11 ± 11.96 mg/dl) sodium serum levels. The hemodialysis no pruritus group and the severe pruritus group had similar serum sodium levels. When serum potassium (mg/dl) of control group and hemodialysis with and without pruritus were compared in Table 2 and Figure 4, it was observed that the serum potassium levels significantly increased (hyperkalemia) in the hemodialysis group.
hemodialysis patients with and without pruritus.

Serum Sodium and potassium concentration in normal control and

Table 2:

Mean values with different superscripts in the same row are significantly different
Reference ranges are given as Mean ± S. E.

Figure 2: Comparisons of serum creatinine concentration in normal control and hemodialysis patients with and without pruritus.

The mild pruritus group. Pruritus patients had higher serum histamine

levels than the hemodialysis control and this increase became highly

significant (p<0.05) in severe pruritus patients compared to other
groups. On the other hand, the mild pruritus group had the lowest

serum histamine levels, which was not different from the hemodialysis
control. Therefore, it seems that serum histamine levels increase as the

severity of pruritus increases.

Serum cortisol level, as shown in Table 3 and Figure 5, increased

significantly (P<0.05) in both pruritus patients and their haemodialysis
control when compared to that in the non-renal failure control group.
Within the pruritus groups, those patients with moderate pruritus had
higher serum cortisol level than the other two pruritus groups which
are not different (P>0.05) from each other.

Table 4 presents the mean and S.E of serum IL-2 and IL-6 (IU/ ml)
in the non-renal failure control, hemodialysis patients with no
pruritus, and the pruritus patients; Figures 6-8 illustrates graphically
the data. It could be concluded that there was non- significant increase
in serum IL-2 levels in hemodialysis patients and pruritus patients
groups compared with the non-renal failure control. It was clear that, in
both renal failure and pruritus group IL-6 serum levels of the pruritus
patients groups, and their hemodialysis control were significantly
higher (P<0.05) when compared with that in non-renal failure control
patients. Moderate pruritus patients revealed high significant increase
in IL-6 compared to other groups.

Discussion

Uremic pruritus remains a serious problem that interferes with the
quality of life of dialysis patients. In addition, severe uremic pruritus
is associated with poor outcomes in chronic HD patients as suggested

the hemodialysis patients compared to the control group that has no
renal failure. Within the hemodialysis groups, the moderate and severe
pruritus patients had the highest potassium levels compared to their
hemodialysis control. The mild pruritus group had lower potassium in
their serum compared with their hemodialysis control.

From Table 3 and Figure 5, it could be observed that, compared
to the control group serum histamine nmol/ml increased significantly
(p<0.05) in hemodialysis patients and the pruritus patients, except for
the mild pruritus group. Pruritus patients had higher serum histamine

Table 3:

Serum histamine and cortisol concentrations in normal control and
hemodialysis patients with and without pruritus.

Reference ranges are given as Mean ± S. E.

Mean values with different superscripts in the same row are significantly different
at (P<0.05)
by Narita et al. [14]. Our results showed there was an increase in blood urea nitrogen, and serum creatinine levels in hemodialysing patients, may suggest renal damage, as reported by zahrani et al. who concluded that the significantly (P<0.05) increased serum creatinine levels indicate development of renal failure. Plasma levels of substances such as urea and creatinine start to show significant increases only after total GFR has decreased to 50%, when the renal reserve has been exhausted [15,16]. Many reports had suggested that the plasma creatinine value will approximately double with a 50% reduction in GFR. In addition, a rise in plasma creatinine from a baseline value of 0.6 mg/dL to 1.2 mg/dL in a patient. Although the underlying mechanism of this increase is not clear, some evidence suggests that this increase could be attributed to the basic functions of the kidneys as the site of fluid and electrolyte regulation, and waste-product elimination [17]. The results observed in this study are consistent with those reported in the literature.

The highly significant (P<0.05) increase in level of serum potassium (hyperkalemia) in pruritus patients and their hemodialysis control are in agreement with Giebisch and Wang [18] and Bleyer et al. [19] who noticed that potassium retention remains one of the major causes of death in the dialysis patients. Also Rose and Post [20] indicated that oliguric patients tend to develop hyperkalemia, especially if they are on a high potassium diet, had decreased aldosterone levels, or suffered from increased tissue breakdown. Therefore, the increased serum potassium could be attributed to the fact that renal failure has caused a loss of the function as organ that has a crucial role K+ homeostasis. Understandably, hyperkalemia can be expected with advanced renal failure [21]. On the other hand, the highly significant decrease in serum sodium levels (hyponatremia) in pruritus patients and their haemodialysis control group is in agreement with the results obtained by Rosa et al. [22] who recognized that, the lack of free water excretion in advanced renal failure can lead to the retention of water consumed by the patient with the concomitant development of hyponatremia. However, Szepietowski et al. [23] argued that the decreased serum sodium may be due to uremic toxins, which are bound to the sodium/potassium ATP, and this would eventually cause accumulation of sodium inside the cells and its loss.

Serum histamine levels significantly increased in haemodialysis patients, and a highly significant increase was observed in severe pruritus patients indicating a positive association between histamine levels and severity of pruritus. These results are supported by the findings of a similar study conducted by Dugas-Breit et al. [24] who concluded that histamine released by skin mast of uremic patients is an important mediator for itching. They indicated that mast cells in the skin and increased serum histamine levels were observed in uremic patients. On the other hand, similar results were reported by Zhang and Lindup [25] who concluded that histamine levels in the serum significantly increased in severe uremic pruritus patients with chronic renal failure. It is interesting to note we observed that not only severe pruritus group had the highest serum histamine but also the highest IgE levels of all groups (Unpublished data). Also in this study we found an association between the increase in production of blood histamine and the increased basophils count (results not shown) similar to that found by Mukai et al. [26]. Histamine is produced predominantly by mast cells, but also is elaborated by basophils as indicated by Sokol et al. [27]. Basophils have long been associated with allergy [28], however their role in development and progress of pruritus remains unclear. The increased blood histamine levels in uremic patients observed in this study are also similar to those found by Stähle-Bäckdahl et al. [29] who concluded that histamine concentrations are much higher in uremic patients with itch than in non-uremic or non-itching patients. Interestingly, and in contrast to our findings and recent reports in the literature, Urbonas et al. [30] argued that there was no correlation could be found between the severity of the itching and the plasma histamine levels. It worth mentioning here that an intradermal injection of histamine uremic patients suffering from itching causes more intense localized itch than in either non-itching uremic patients or healthy individuals, suggesting that an increased sensitivity to histamine. Nevertheless, despite the increased levels of histamine in pruritus patients, most studies have failed to show any correlation between histamine levels and uremic pruritus [31]. Furthermore, while some studies showed reduction of uremic pruritus following an antihistaminic treatment, investigations could not confirm the benefits of the treatment [32]. Some reports [33] concluded that histamine alone may not the sole factor responsible for

![Figure 5: Comparisons of serum histamine concentration in normal control and hemodialysis patients with and without pruritus.](image)

![Figure 6: Comparisons of serum Cortisol concentration in normal control and hemodialysis patients with and without pruritus.](image)
patients and their hemodialysis control compared to control observed excrete cortisol and its water soluble metabolites. As a result, the serum increased cortisol levels are attributed to the inability of the kidney to was released in response to stress. Fallahzadeh [37] suggested that the (hydrocortisone), a glucocorticoid produced by the adrenal gland, the stress factors as stated by Rosman [36] who proved that, cortisol causes pruritus. The observed increase of serum cortisol levels in hemodialysis patients and in pruritus patients could be attributed to the stress factors as stated by Rosman [36] who proved that, cortisol (hydrocortisone), a glucoctoid produced by the adrenal gland, was released in response to stress. Fallahzadeh [37] suggested that the increased cortisol levels are attributed to the inability of the kidney to excrete cortisol and its water soluble metabolites. As a result, the serum half-life of cortisol becomes prolonged in advanced renal failure.

The non-significant (P > 0.05) increase in serum IL-2 in pruritus patients and their hemodialysis control compared to control observed in this study confirmed the findings of Vink et al. [38] who stated that, serum levels of IL-2 have been demonstrated to be higher in hemodialysis patients with itch (uremic pruritus) compared to those without itch. As a proof, therapeutic measures that inhibit IL-2, such as Ultraviolet therapy, have been demonstrated to be effective in treatment of uremic pruritus. In addition, direct injection of IL-2 into skin of healthy subjects as well as those with atopic dermatitis has resulted in itching [38]. However, in this study there was a numerical increase in IL-2 in the moderate and severe pruritus group compared to their hemodialysis control. These differences between the hemodialysis and pruritus groups were not statistically significant. However, there is a 5-7 increase in IL-2 in the pruritus groups compared to the non-renal failure control, suggesting a response to inflammation and perhaps an immune reaction. Smith [39] indicated that the release of IL-1β, IL-6 and TNF-α stimulates lymphocytes to synthetize of IL-2. Due to above mentioned facts, Vink et al. [38] hypothesized that activation of central pruritus center in CNS and/or activation of central or peripheral opioid μ-receptors, elevated serum level of IL-2, and may result in the even distribution of IL-2 in UP in HD patients. On the other hand, Pereira and Dinarello [40] attributed the increase serum level of IL-2, which is normally produced by the body during an immune response, when environmental substances (molecules or microbes) gain access to the body. These substances that lead to increased level of IL-2 concentration could be due to the contact of blood with dialyzer membrane as suggested by Kimmel et al. [41]. Kimmel et al. [41] found that the contact of blood with dialyzer membrane leads to degranulation in leukocytes and release of inflammatory mediators. On the other hand, blood and dialyzer membrane interactions leads to the activation of monocytes, increased release of IL-1, IL-2 by the activated monocytes [42]. This constitutes a reasonable explanation of the results documented in our study.

This study observed a significant increase in of IL-6 in pruritus patients and their hemodialysis patients control compared with that in healthy patients. The results of this study are similar to those reported by Carrero et al. [42], who documented high levels of interleukin (IL-6) in HD patients with pruritus. Although the elevation of serum IL-6 is consistent with the results of Carrero [17] who contended that ‘Numerous research studies on the synthesis and the release of prionflammatory cytokines IL-1β, IL-2, IL-6, IL-8 and TNF-α in patients with chronic renal failure on maintenance hemodialysis provide contradictory data”, although some of these studies revealed increased serum levels of IL-6 prior to and in the course of hemodialysis. However, according to Memoli et al. [43], there was a spontaneous elevation of IL-6 and TNF-α by peripheral blood leukocytes in HD patients and there was an increased release of IL-6 in the course of HD session. Engelberts et al. [44] Increased IL-6 serum levels in HD patients at each of the studied time points during hemodialysis session. This supports the findings of this study in that the concentration of IL-6 in peripheral blood was increased in HD patients compared with non-renal failure patients or healthy individuals. The increased serum levels of IL-6 in pruritus patients observed in this study may have be attributed to in that ‘Serum levels of inflammatory biomarkers such as IL-6 and white blood cell counts are also increased in patients with uremic pruritus.

The results of the investigation suggest that many factors may have important role in pathogenesis and progression of uremic pruritus from mild to severe. The association of histamine, cortisol, IL-2 and IL-6 are evident. Further research is needed to elucidate the main causes of UP, and association of serum biochemical values with its severity. The effect of the dialyzing membrane should be factored in any such investigation. The results of our study point out to the potential important role of both IL-2 and IL-6 in UP. Further studies are needed.
to investigate the contribution of histamine, IL-2 and IL-6, monocytes, and basophils on the development and severity of uremic pruritus, and the possible role of anti-IL-2 and IL-6 receptor antibodies in treatment of severe pruritus.

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