Evaluation of Plasma Urea and Plasma Creatinine Concentrations in Asthmatic Patients under Steroids Therapy in Alshaab hospital and Bahri Hospital

تقويم تركيز البولين والكرياتينين في مصل المرضى المصابين بالربو الخاضعين للعلاج بالاستيرويدات في مستشفى الشعب وبحري

A dissertation Submitted in Partial fulfillment for the Requirements of B.Sc in Clinical chemistry (Honor)

Prepared by:
May Adam Alshreef
Nahid Abubaker Elisiddig

Supervisor:
Dr. Mohamed Abd Alrahim Abd Allah
Asso. Prof in Biochemistry

August 2014
قال تعالى:

وَلَنُبْلُوْنَكُم بِشَيْءٍ مِّنَ الْخَوْفِ وَالْجُوعِ وَنَفْصٍ مِّنَ الأَمْوَالِ وَالنَّفْسِ وَالثُّمَرَاتِ وَبَشَّرَ الصَّابِرِينَ * الَّذِينَ إِذَا أُصِبَّهُمْ مُصَيَّبَةٌ قَالُوا إِنَّا لِلَّهِ وَإِنَّا إِلَيْهِ رَاجِعُونَ * أُوْلَئِكَ عَلَيْهِمْ صَلَواتٌ مِّن رَّبِّهِمْ وَرَحْمَةٌ وَأُوْلَئِكَ هُمُ المُهْتَدُونَ

صدق الله العظيم

سورة البقرة آية (155_157)
Dedication

We would like to dedicate this study to fountain of love and tenderness who sacrificed her life for us

To our mothers

To the source of our inspiration and strength who still supports and encourages us

To our fathers

To all our family and friends who supported us and stands beside during the hard times

To our teachers and colleagues

To everyone from whom we have learned and benefited
Acknowledgements

We would like to give appreciated thanks to Allah.
And with deep sadness we would like to express our sincere gratitude and appreciation to the soul of our supervisor Dr. Mohammed Abd Alrahim who inspired us and helped us to finish this study.
And we are very grateful to all teachers specially our teacher Altaf Suliman for her encouragement, continuous guidance, invaluable support and patience throughout this work.
At last we wish to thank who ever lent a helping hand to us and who’s assistance, by way or another enable us to complete this work.

Thanks
Abstract

Asthma is a chronic inflammatory disease of airways and it is a very common disease with immense social impact. The treatment of asthma affects the concentration of plasma urea and creatinine.

This is descriptive analytical study conducted during the period from the February to July 2014 to evaluate plasma concentration of urea and creatinine in asthmatic patients under steroid therapy, included 30 patients with hypertensive and non-hypertensive Asthmatic patients as case group, and 20 healthy individual as control group. Samples were collected to measure the concentration of urea and creatinine by using colorimetric methods. The data analyzed by SPSS program.

The result found significant increase in the mean of creatinine between asthmatic patients and non-asthmatics (P-value = 0.001), and no significant difference between mean of urea in case and control (P-value = 0.06). Also there is no significant difference between mean of creatinine and urea in hypertensive and non-hypertensive asthmatic patients (P-value = 0.198, 0.797) respectively.

This study shows that there is significant negative moderate correlation between creatinine and concentration treatment duration and insignificant weak negative correlation between urea and concentration treatment duration.

The present study showed that the asthma treatment has effect on creatinine concentration with the duration of treatment resulting in lowering creatinine concentration, and no effect on urea concentration.
مستخلص الدراسة

أجرت هذه الدراسة في الفترة ما بين فبراير و يوليو 2014 بغرض تقييم مستويات البولينيا والكيراتينين في مصل المرضى المصابة بالربو الذين يخضعون للعلاج بالاستيرويدات.

تضمنت هذه الدراسة 30 مريضًا ربو من المصابين وغير المصابين بضغط الدم المتم مقارنته بنتائج 20 شخصًا من الأصحاء اختيروا عشوائيًا. ولقد أخذت عينات الدم من مجموعتي المرضى والأصحاء للحصول على المصل لقياس تركيز البولينيا والكيراتينين باستخدام مجهر الكاروميتر وتم تحليل النتائج إحصائيًا.

وأثبتت النتائج وجود ارتفاع في تركيز الكيراتينين بين مرضى الربو مقارنة بالأصحاء، وعدم وجود فروق ذات دلالات إحصائية معنوية في تركيز البولينيا بين مرضى الربو والأصحاء.

وبعد وجود فروق ذات دلالات إحصائية في تركيز البولينيا والكيراتينين بين مرضى الربو المصابين بضغط الدم مقارنة بمرضى الربو غير المصابين بضغط الدم، وجود علاقة معنوية سالبة بين تركيز الكيراتينين ومدة العلاج، وأيضا علاقة غير معنوية بين تركيز البولينيا ومدة العلاج.

أثبتت هذه الدراسة أن علاج الربو يقلل من تركيز الكيراتينين مع الزمن وليس له تأثير على تركيز البولينيا.
### List of Contents

<table>
<thead>
<tr>
<th>Contents</th>
<th>Page No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapter One</td>
<td></td>
</tr>
<tr>
<td>Introduction and Literature Review</td>
<td></td>
</tr>
<tr>
<td>1.1 Asthma</td>
<td></td>
</tr>
<tr>
<td>1.1.1 Definition</td>
<td>1</td>
</tr>
<tr>
<td>1.1.2 Sign and symptoms</td>
<td>1</td>
</tr>
<tr>
<td>1.1.3 Epidemiology</td>
<td>1</td>
</tr>
<tr>
<td>1.1.4 Causes</td>
<td>2</td>
</tr>
<tr>
<td>1.1.4.1 Genetic</td>
<td>2</td>
</tr>
<tr>
<td>1.1.4.2 Allergens</td>
<td>2</td>
</tr>
<tr>
<td>1.1.4.3 Pharmacologic stimuli</td>
<td>2</td>
</tr>
<tr>
<td>1.1.4.4 Environmental and air pollution</td>
<td>2</td>
</tr>
<tr>
<td>1.1.4.5 Occupational factors</td>
<td>3</td>
</tr>
<tr>
<td>1.1.4.6 Infections</td>
<td>3</td>
</tr>
<tr>
<td>1.1.4.7 Exercise</td>
<td>3</td>
</tr>
<tr>
<td>1.1.4.8 Emotional stress</td>
<td>3</td>
</tr>
<tr>
<td>1.1.4.9 Obesity</td>
<td>3</td>
</tr>
</tbody>
</table>

### Summary

- Dedication: 1
- Acknowledgements: 2
- Abstract: 3
- List of Contents: 4
- List of Tables: VIII
- List of Figures: IX
- List of Abbreviations: X
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1.5 Classification</td>
<td>3</td>
</tr>
<tr>
<td>1.1.6 Medications</td>
<td>4</td>
</tr>
<tr>
<td>1.1.6.1 Fast acting</td>
<td>4</td>
</tr>
<tr>
<td>1.1.6.2 Long term control</td>
<td>4</td>
</tr>
<tr>
<td>1.2 Hypertension</td>
<td>6</td>
</tr>
<tr>
<td>1.2.1 Classification</td>
<td>6</td>
</tr>
<tr>
<td>1.2.1.1 Primary essential hypertension</td>
<td>6</td>
</tr>
<tr>
<td>1.2.1.2 Secondary hypertension</td>
<td>6</td>
</tr>
<tr>
<td>1.2.2 Identifiable causes of secondary hypertension</td>
<td>6</td>
</tr>
<tr>
<td>1.3 Urea</td>
<td>7</td>
</tr>
<tr>
<td>1.3.1 Biochemistry and physiology</td>
<td>7</td>
</tr>
<tr>
<td>1.3.2 Abnormal plasma urea</td>
<td>7</td>
</tr>
<tr>
<td>1.3.2.1 Increase plasma urea</td>
<td>7</td>
</tr>
<tr>
<td>1.3.2.1.1 Pre-renal</td>
<td>7</td>
</tr>
<tr>
<td>1.3.2.1.2 Renal</td>
<td>8</td>
</tr>
<tr>
<td>1.3.2.1.3 Post-renal</td>
<td>8</td>
</tr>
<tr>
<td>1.3.2.2 Decrease plasma urea</td>
<td>8</td>
</tr>
<tr>
<td>1.4 Creatinine</td>
<td>9</td>
</tr>
<tr>
<td>1.4.1 Biochemistry and physiology</td>
<td>9</td>
</tr>
<tr>
<td>1.4.2 Abnormal plasma creatinine</td>
<td>10</td>
</tr>
<tr>
<td>1.4.2.1 Increases plasma creatinine</td>
<td>10</td>
</tr>
<tr>
<td>1.4.2.2 Decreases plasma creatinine</td>
<td>10</td>
</tr>
<tr>
<td>1.5 Rationale</td>
<td>11</td>
</tr>
<tr>
<td>1.6 Objectives</td>
<td>12</td>
</tr>
<tr>
<td>1.6.1 General objective</td>
<td>12</td>
</tr>
<tr>
<td>1.6.2 Specific objectives</td>
<td>12</td>
</tr>
</tbody>
</table>

Chapter Two

Materials and Methods
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1 Materials</td>
<td>13</td>
</tr>
<tr>
<td>2.2 Methods</td>
<td>14</td>
</tr>
<tr>
<td>Chapter Three</td>
<td></td>
</tr>
<tr>
<td>Results</td>
<td></td>
</tr>
<tr>
<td>3 Results</td>
<td>17</td>
</tr>
<tr>
<td>Chapter Four</td>
<td></td>
</tr>
<tr>
<td>Discussion, Conclusions and Recommendations</td>
<td></td>
</tr>
<tr>
<td>4.1 Discussion</td>
<td>23</td>
</tr>
<tr>
<td>4.2 Conclusions</td>
<td>24</td>
</tr>
<tr>
<td>4.3 Recommendations</td>
<td>25</td>
</tr>
<tr>
<td>References</td>
<td>26</td>
</tr>
<tr>
<td>Appendices</td>
<td></td>
</tr>
</tbody>
</table>
## List of Tables

<table>
<thead>
<tr>
<th>Table No</th>
<th>Content</th>
<th>Page No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table (3.1)</td>
<td>Comparison of the concentration of urea and creatinine in reference to control.</td>
<td>18</td>
</tr>
<tr>
<td>Table (3.2)</td>
<td>Comparison of the concentration of urea and creatinine between male and female in asthmatic patients</td>
<td>19</td>
</tr>
<tr>
<td>Table (3.3)</td>
<td>Comparison of the concentration of urea and creatinine between hypertensive and non-hypertensive asthmatic patients.</td>
<td>20</td>
</tr>
</tbody>
</table>
## List of Figures

<table>
<thead>
<tr>
<th>Figure No</th>
<th>Content</th>
<th>Page No</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Figure (3.1)</strong></td>
<td>Scatter blot dots the correlation between treatment duration and urea concentration in asthmatic patients.</td>
<td>21</td>
</tr>
<tr>
<td><strong>Figure (3.2)</strong></td>
<td>Scatter blot dots the correlation between treatment duration and creatinine concentration in asthmatic patients.</td>
<td>22</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Word</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>-------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>BUN</td>
<td>blood urea nitrogen</td>
<td></td>
</tr>
<tr>
<td>ESRD</td>
<td>End stage renal disease</td>
<td></td>
</tr>
<tr>
<td>FEV\textsubscript{1}</td>
<td>forced expiratory volume in one second</td>
<td></td>
</tr>
<tr>
<td>GFR</td>
<td>Glomerular filtration rate</td>
<td></td>
</tr>
<tr>
<td>LABA</td>
<td>Long-acting beta-adrenoceptor agonists</td>
<td></td>
</tr>
<tr>
<td>SABA</td>
<td>Short-acting beta\textsubscript{2}-adrenoceptor agonists</td>
<td></td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical package of social science</td>
<td></td>
</tr>
</tbody>
</table>
Chapter One

Introduction and Literature Review
1. Introduction and Literature Review

1.1 Asthma

1.1.1 Definition

Asthma is a common chronic disorder of the airways that is complex and characterized by variable and recurring symptoms, airflow obstruction, bronchial hyper responsiveness, and an underlying inflammation (NHLBI, 2007)

1.1.2 Signs and symptoms

The symptoms of asthma consist of a triad of dyspnea, cough, and wheezing, in its most typical form, all three symptoms coexist. At the onset of an attack, patients experience a sense of constriction in the chest, often with a nonproductive cough. Respiration becomes audibly harsh; wheezing in both phases of respiration becomes prominent expiration becomes prolonged; and patients frequently have tachypnea, tachycardia, and mild systolic hypertension (Kasper et al, 2005).

1.1.3 Epidemiology

As of 2011, 235–330 million people worldwide are affected by asthma (Blackhall et al, 2012), and approximately 250,000 people die per year from the disease. It is more common in developed than developing countries (GINA, 2014). One thus sees lower rates in Asia, Eastern Europe and Africa (Arshad, 2010) Within developed countries it is more common in those who are economically disadvantaged while in contrast in developing countries it is more common in the affluent (GINA, 2014). The reason for these differences is not well known. Asthma affects 4-5% of adults and 10% of children. Onset usually occurs in children and young adults (Henderson, 2006).
Bronchial asthma occurs at all ages but predominantly in early life. About one-half of cases develop before age 10, and another third occur before age 40. In childhood, there is a 2:1 male/female preponderance, but the sex ratio equalizes by age 30 (Kasper et al, 2005).

1.1.4 Causes

The stimuli that incite acute episodes of asthma can be grouped into major categories: genetic, allergenic, pharmacologic, environmental, occupational, infectious, exercise-related, obesity and emotional (Kasper et al, 2005).

1.1.4.1 Genetic

There is no doubt that there is a familial tendency with inheritance more obvious through the maternal line (Kasper et al, 2005).

1.1.4.2 Allergens

Allergic asthma is dependent on an IgE response controlled by T and B lymphocytes and activated by the interaction of antigen with mast cell-bound IgE molecule. (Kasper et al, 2005)

1.1.4.3 Pharmacologic Stimuli

The drugs most commonly associated with the induction of acute episodes of asthma are aspirin, coloring agents such as tartrazine, β-adrenergic antagonists, and sulfiting agents. (Kasper et al, 2005)

1.1.4.4 Environment and Air Pollution

Environmental causes of asthma are usually related to climatic conditions that promote the concentration of atmospheric pollutants and antigens (Kasper et al, 2005).
1.1.4.5 Occupational Factors:

Occupation related asthma is a significant health problem, and acute and chronic airway obstruction have been reported to follow exposure to a large number of compounds used in many types of industrial processes e.g. wood and vegetable dust, pharmaceutical agents and biological enzymes (Kasper et al, 2005).

1.1.4.6 Infections

Respiratory infections are the most common of the stimuli that evoke acute exacerbations of asthma e.g. para influenza virus, in older children and adults, rhinovirus and influenza virus predominate as pathogens (Kasper et al, 2005).

1.1.4.7 Exercise

Exercise is a very common precipitant of acute episodes of asthma, it does not evoke any long-term sequelae, nor does it increase airway reactivity. Typically the attacks follow exertion and do not occur during it (Kasper et al, 2005).

1.1.4.8 Emotional Stress

Psychological factors can worsen or ameliorate asthma. Changes in airway caliber seem to be mediated through modification of vagal efferent activity, but endorphins may also play a role (Kasper et al, 2005).

1.1.4.9 Obesity

There is a correlation between obesity and the risk of asthma with both having increased in recent years (Halapi et al, 2009; Rapini et al 2007) Several factors may be at play including decreased respiratory function due
to a buildup of fat and the fact that adipose tissue leads to a pro-inflammatory state (GINA, 2013).

1.1.5 Classification

Asthma is clinically classified according to the frequency of symptoms, forced expiratory volume in one second (FEV₁), and peak expiratory flow rate (Yawn, 2008). Asthma may also be classified as atopic (extrinsic) or non-atopic (intrinsic), based on whether symptoms are precipitated by allergens (atopic) or not (non-atopic) (Kumar et al, 2010) While asthma is classified based on severity, at the moment there is no clear method for classifying different subgroups of asthma beyond this system. Finding ways to identify subgroups that respond well to different types of treatments is a current critical goal of asthma research (Welsh et al, 2010).

1.1.6 Medications

Medications used to treat asthma are divided into two general classes: quick-relief medications used to treat acute symptoms; and long-term control medications used to prevent further exacerbation (Chen et al, 2010).

1.1.6.1 Fast acting

A. Short-acting beta₂-adrenoceptor agonists (SABA), such as salbutamol (albuterol USAN) are the first line treatment for asthma symptoms (NHLBI, 2007) They are recommended before exercise in those with exercise induced symptoms (NHLBI, 2007). Anticholinergic medications, such as ipratropium bromide, provide additional benefit when used in combination with SABA in those with moderate or severe symptoms (NHLBI, 2007). Anticholinergic bronchodilators can also be used if a person cannot tolerate a SABA (NHLBI, 2007).
Older, less selective adrenergic agonists, such as inhaled epinephrine, have similar efficacy to SABAs. They are however not recommended due to concerns regarding excessive cardiac stimulation (NHLBI, 2007).

1.1.6.2 Long–term control

A. Corticosteroids are generally considered the most effective treatment available for long-term control (Chen et al, 2010). Inhaled forms such as beclomethasone are usually used except in the case of severe persistent disease, in which oral corticosteroids may be needed (Chen et al, 2010). It is usually recommended that inhaled formulations be used once or twice daily, depending on the severity of symptoms (Thomson et al, 2005).

B. Long-acting beta-adrenoceptor agonists (LABA) such as salmeterol and formoterol can improve asthma control, at least in adults, when given in combination with inhaled corticosteroids in children this benefit is uncertain (Stapleton et al, 2011; Been, 2014). When used without steroids they increase the risk of severe side-effects (Chandratilleke et al, 2013), and even with corticosteroids they may slightly increase the risk. (Parson et al, 2013; Rodrigo et al, 2006)

C. Leukotriene antagonists (such as montelukast and zafirlukast) may be used in addition to inhaled corticosteroids, typically also in conjunction with LABA (Chen et al, 2010). Evidence is insufficient to support use in acute exacerbations (Rodrigo et al, 2006; NHLBI, 2007). In children they appear to be of little benefit when added to inhaled steroids In those under five years of age, they were the preferred add-on therapy after inhaled corticosteroids by the British Thoracic Society in 2009 (Ducharme et al, 2010).

D. Mast cell stabilizers (such as cromolyn sodium) are another non-preferred alternative to corticosteroids (Chen et al, 2010).
1.2 Hypertension

Is defined as 140 to 159 mm Hg systolic, 90 to 99 mm Hg diastolic (stage1) and ≥160 mm Hg systolic, ≥100 mm Hg diastolic (stage 2) (Goljan, 2014)

1.2.1 Classification

1.2.1.1 Primary essential Hypertension

Essential hypertension is the term applied to the 95% of hypertensive patients in which elevated blood pressure results from complex interactions between multiple genetic and environmental factors. The proportion regarded as “essential” will diminish with improved detection of clearly defined secondary causes and with better understanding of pathophysiology. The onset is usually between ages 25 and 55 years; it is uncommon before age 20 years. It’s due to endogenous (sympathetic nervous system hyperactivity, abnormal cardiovascular or renal development, renin–angiotensin system activity, defect in natriuresis, Intracellular sodium and calcium, exacerbating factors) and environmental determinants (Papadakis et al, 2013).

1.2.1.2 Secondary Hypertension

Approximately 5% of patients with hypertension have identifiable specific causes. Secondary hypertension should be suspected in patients in whom hypertension develops at an early age, those who first exhibit hypertension when over age 50 years, or those previously well controlled who become refractory to treatment (Papadakis et al, 2013).

1.2.2 Identifiable causes of secondary hypertension

Sleep apnea, Drug-induced or drug-related-chronic kidney disease, Primary aldosteronism,Reno vascular disease, Long-term corticosteroid therapy and
Cushing syndrome, Pheochromocytoma, Coarctation of the aorta, Thyroid or parathyroid disease (Papadakis et al, 2013).
1.3 Urea

Catabolism of proteins and amino acids results in the formation urea, which is predominantly cleared from the body by the kidney (Edward et al, 2001).

1.3.1 Biochemistry and physiology

Urea makes up the majority (>75%) of the non-protein waste products. It is filtered at glomeruli and about (40-60)% of filtered urea is reabsorbed in collecting ducts. Therefore, impaired glomerular filtration results in retention of urea and its concentration rises (Dinesh, 2006).

The biosynthesis of urea from amino acid nitrogen-derived ammonia is carried out exclusively by hepatic enzymes of the urea cycle. During the process of protein catabolism, amino acid nitrogen converted to urea in the liver by the action of the so called urea cycle enzymes.

More than 90% of urea is excreted through the kidneys, with losses through the gastrointestinal tract and skin accounting for most of the remaining minor fraction. Consequently, kidney disease is associated with accumulation of urea in blood. An increase in plasma urea concentration characterizes the uremic state. Urea is neither actively reabsorbed nor secreted by the tubules but is filtered freely by the glomeruli (Edward et al, 2001).

1.3.2 Abnormal plasma urea

1.3.2.1 Increase plasma urea

Condition causing increase plasma urea are classified according to the cause into three main categories:-
1.3.2.1.1 Pre renal

Congestive heart failure, Shock, Hemorrhage, Dehydration, Amount of protein metabolism, Hypovolemia, High protein diet or high protein catabolism.

1.3.2.1.2 Renal

Acute and chronic renal failure, Reduced GFR

1.3.2.1.3 Post renal

Obstruction of urinary tract by renal calculi, Malignancy (Beckett et al, 2010).

1.3.2.2 Decrease plasma urea

Low protein intake, Sever liver disease, Starvation (Beckett et al, 2010).
1.4 Creatinine

Is the cyclic anhydride of creatine that is produced as the final product of decomposition of phosphocreatine (Edward et al, 2001)

Creatinine is small compound readily filtered by the glomerulus, and unlike urea, is not reabsorbed by the tubules and collecting ducts. Elevated serum creatinine concentration is more sensitive indicator of glomerular damage than serum urea (Dinesh, 2006).

1.4.1 Biochemistry and physiology

Creatine is synthesized in the liver, kidney, and pancreas by two enzymatically mediated reactions. In the first transamidation of arginine and glycine forms guanidinoacetic acid. In second reaction, methylation of guanidinoacetic acid occur with S-adenosylmethionine as the methyl donor.

Creatine is then transported in blood to other organ as muscle and brain, where it is phosphorylated to phosphocreatine, a high energy compound.

Interconversion of phosphocreatine and creatine is a particular feature of the metabolic processes of muscle contraction. A proportion of the free creatine in muscle (thought to be between 1% and 2% day) spontaneously and irreversibly converts to it anhydride waste product creatinine. Thus the amount of creatinine produced each day is relatively constant and is related to the muscle mass. In health, the concentration of creatinine in the bloodstream also is relatively constant.

However, depending on the individual meat intake, diet may influence the value. Creatinine present in all body fluids and secretion, and is freely filtered by the glomerulus. Although it is not reabsorbed to any great extent by the renal tubules, there is a small but significant tubular
secretion. Creatinine production also decrease as the circulating level of creatinine increases, several mechanism for this have been proposed including feedback inhibition of production of creatine, reconversion of creatinine to creatine and conversion to other metabolisms.

The elderly and young children normally have lower creatinine level as result of reduced muscle mass. This may potentially mask renal disease in patients of these age group (Edward et al, 2001).

1.4.2 Abnormal plasma creatinine

1.4.2.1 Increase plasma creatinine

Large muscle mass, High meat intake, Vigorous exercise, High concentration of acetoacetate or cephalosporin antibiotic, Reduce GFR, Impaired renal perfusion, Acute and chronic glomerulonephritis, Urinary tract obstruction due to prostatic enlargement (Beckett et al, 2010).

1.4.2.2 Decrease creatinine level

Debilitation, Decreased muscle mass, Starvation, Wasting disease, Patient treated with corticosteroids, Pregnancy (Beckett et al, 2010)
1.5 Rationale

Asthma is common and serious potentially chronic disease that imposes a substantial burden on patient, their families and communities. It causes respiratory symptoms, limitation of activity, and flare-ups (attacks) that require urgent health care and can be fatal. Researcher found that Asthmatic treatment affects plasma urea and creatinine level.

Some studies have demonstrated normal level of urea and creatinine in asthmatic patients to the best of our knowledge, in Sudan; there are no studies on the impact of asthma treatment on level of plasma urea and creatinine. Accordingly the present study conducted to evaluate urea and creatinine concentrations in asthmatic patients under steroid treatment in Khartoum state.
1.6 Objectives

1.6.1 General objective

To evaluate plasma concentration of urea and creatinine in asthmatic patients under steroid therapy in Alshaab specialized hospital and Bahri teaching hospital.

1.6.2 Specific objectives

1- To measure plasma concentration of urea and creatinine in asthmatic patients in reference to control (asthma VS control).

2- To assess plasma concentration of urea and creatinine in asthmatic patients according to their sex.

3- To assess plasma concentration of urea and creatinine in hypertensive in comparison to non-hypertensive asthmatic patients.

4- To correlate the plasma concentration of urea and creatinine with duration treatment of asthma.
Chapter Two

Materials and Methods
2. Materials and Methods

2.1 Materials

2.1.1 Study design

This is a cross sectional descriptive study. Carried out during the period from February to July 2014.

2.1.2 Study area

This study was conducted in Alshaab Specialized Teaching Hospital and Bahri teaching hospital.

2.1.3 Study population

Thirty asthmatic patients were collected for this study as case group, and twenty healthy individual as control group.

2.1.4 Inclusion criteria

Asthmatic hypertensive and non-hypertensive individuals were included in this study as test group.

2.1.5 Exclusion criteria

Any person who has sense to affect urea and creatinine concentrations e.g. Patients with renal diseases, heart diseases were excluded.

2.1.6 Sample process

About 2.5ml of venous blood were taken from individual of study group, then sample were collected in heparin containers using sterile syringe.
All blood samples were centrifuged at 3000 RPM for 5 minutes to obtain plasma, which then stored at -20 °C till the time of analysis.

2.1.7 Ethical considerations

Patients who voluntary accepted to participate in the study were included.
2.2 Methods

2.2.1 Estimation of urea

2.2.1.1 Principle

Urea in the sample originate by mean of coupled reaction described below, a colored complex formed that can be measured by colorimeter

\[
\text{Urea} + \text{H}_2\text{O} \xrightarrow{\text{ureases}} 2\text{NH}_4^+ + \text{CO}_2
\]

\[
\text{NH}_4^+ + \text{Salicylate} + \text{NaClO} \xrightarrow{\text{nitroprusside}} \text{indophenols} \quad \text{(Bishop et al., 2010)}.
\]

2.2.1.2 Reagent preparation

Reagent B (Sodium hypochlorite, sodium hydroxide) and standard (S) were provided ready to use.

Reagent (A): \( r = -0.413, P\text{-value} = 0.023 \). The content of one reagent A2 was transferred (Urease) vial into reagent A1 (Sodium salisylate, sodium nitroprusside, phosphate buffer, pH 6.9) bottle.

Mixed thoroughly.
2.2.1.3 Procedure

The reagents were brought to room temperature.

Pipetted into labeled test tubes:

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea standard</td>
<td>_</td>
<td>10µl</td>
<td>_</td>
</tr>
<tr>
<td>50mg/dl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td>_</td>
<td>_</td>
<td>10µl</td>
</tr>
<tr>
<td>Reagent A</td>
<td>1.0ml</td>
<td>1.0ml</td>
<td>1.0ml</td>
</tr>
</tbody>
</table>

Mixed thoroughly and incubated for 10 minutes at room temperature.

Pipetted:

| Reagent B | 1.0 ml | 1.0 ml | 1.0 ml |

Mixed thoroughly and incubated for 10 minutes at room temperature.

The absorbance (A) of the standard and sample read at 600 nm against the blank.

2.2.1.4 Calculations

The urea concentration in the sample was calculated using the following general formula:

\[
\frac{A_{\text{sample}}}{A_{\text{standard}}} \times \text{Standard concentration} \times \text{sample dilution} = \text{sample concentration}
\]
2.2.2 Estimation of creatinine

2.2.2.1 Principle

Creatinine in the sample reacts with picrate in alkaline media forming colored complex measured by colorimeter at 490nm (Bishop et al, 2010).

2.2.2.2 Reagent preparation

Working reagent: equal volume of reagent A was mixed (sodium hydroxide, detergent) and reagent B (picric acid).

Mixed thoroughly

2.2.2.3 Procedure

1- The working reagent were mixed.

2- The working reagent were brought to room temperature.

3- into tube

<table>
<thead>
<tr>
<th>Working reagent</th>
<th>1.0 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample /standard</td>
<td>100 µl</td>
</tr>
</tbody>
</table>

Mixed well, read against distilled water.

4- The absorbance was recorded at 490nm after 30 seconds (A1) and after 90 seconds (A2).

2.2.2.4 Calculations

The creatinine concentration in the sample was calculated using the following general formula:
\[
\frac{(A_2 - A_1)_{sample}}{A_{standard}} \times \text{Standard concentration} \times \text{sample dilution factor} = \text{sample concentration}
\]

2.2.3 Quality control

It is recommended to use biochemistry control serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) to verify the performance of the measurement procedure.

2.2.4 Statistical analysis

Data was analyzed by using the SPSS computer program and the means and standard deviations of plasma urea and creatinine were detected and independent t-test was used for comparison (P-value < 0.05 is considered to be significant). Linear regression analysis was used to assess correlation between duration of treatment of asthma, and plasma urea and creatinine concentrations.
Chapter Three

Results
3. Results

This study was done during the period from February to June 2014 in Alshaab specializing hospital and Bahri teaching hospital include 30 samples which collected from asthmatic patients and 20 sample from non-asthmatic patients.

Table (3.1) shows the urea and creatinine concentration (mg/dl) in case and control the result expressed as (mean ± SD) with (P-value = 0.06, 0.001) respectively.

Table (3.2) shows urea and creatinine concentration in male and female in asthmatic patients the result expressed as (mean ± SD) with (P- value = 0.449, 0.554) respectively.

Table (3.3) shows urea and creatinine concentration in hypertensive and non-hypertensive asthmatic patients the result expressed as (mean ± SD) with (P- value = 0.797, 0.198) respectively.

Figure (3.1) scatter blot shows the correlation between treatment duration and urea concentration in asthmatic patients (r= -0.105, P- value = 0.58).

Figure (3.2) scatter blot show the correlation between treatment duration and creatinine concentration in asthmatic patients (r= -0.413, P- value = 0.023).
Table (3.1) Comparison of the concentration of urea and creatinine in reference to control.

<table>
<thead>
<tr>
<th></th>
<th>Asthmatic patient (case) NO= 30</th>
<th>Asthmatic patient (Control) NO= 20</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dl)</td>
<td>29.90 ± 11.9</td>
<td>25 ±5.3</td>
<td>0.06</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.25 ±0.79</td>
<td>0.70 ±0.25</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Table (3.2) Comparison of the concentration of urea and creatinine between male and female in asthmatic patients.

<table>
<thead>
<tr>
<th></th>
<th>Male NO= 18</th>
<th>Female NO= 12</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dl)</td>
<td>31.28 ± 13.73</td>
<td>27.83 ± 8.75</td>
<td>0.449</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.17 ±0.55</td>
<td>1.35 ± 1.08</td>
<td>0.554</td>
</tr>
</tbody>
</table>
Table (3.3) Comparison of the concentration of urea and creatinine between hypertensive and non-hypertensive asthmatic patients.

<table>
<thead>
<tr>
<th></th>
<th>Hypertensive NO= 8</th>
<th>Non-Hypertensive NO= 22</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Urea (mg/dl)</strong></td>
<td>30.75 ± 9.86</td>
<td>29.59 ± 12.81</td>
<td>0.797</td>
</tr>
<tr>
<td><strong>Creatinine (mg/dl)</strong></td>
<td>1.75 ± 1.34</td>
<td>1.06 ± 0.38</td>
<td>0.198</td>
</tr>
</tbody>
</table>
Figure (3.1) scatter blot dots the correlation between treatment duration and urea concentration in asthmatic patients ($r = -0.105$, $P$-value = 0.58).
Figure (3.2) scatter blot dots the correlation between treatment duration and creatinine concentration in asthmatic patients ($r = -0.413$, $P$-value $= 0.023$).
Chapter Four

Discussion, Conclusions and Recommendations
4. Discussion, Conclusions and Recommendations

4.1 Discussion

Asthma is a chronic inflammatory disease of airways that is characterized by increased responsiveness of the tracheobronchial tree to a multiplicity of stimuli and it is a very common disease with immense social impact.

The results of the present study found that there is significant increase in the mean of creatinine between asthmatic patients (case) and non-asthmatics (control) ($P$-value = 0.001), and this results disagreed with Dawson who found that the mean of creatinine concentration in asthmatics when compared to non-asthmatics was normal (Dawson et al, 1983). In contrast there is no significant difference between mean of urea in case and control ($P$-value = 0.06), and this result agreed with Dawson who found that the mean of urea concentration was normal in asthmatics when compared to non-asthmatics (Dawson et al, 1983). And this may be due to variation of diseases duration in randomly selected population. The result found no significant difference between the mean of plasma urea in male and female in asthmatic patients ($P$-value = 0.449), and mean of plasma creatinine in male and female in asthmatic patients ($P$-value = 0.554). In addition to this results there is no significant difference between mean of creatinine in hypertensive and non-hypertensive asthmatic patients ($P$-value = 0.198), and mean of urea in hypertensive and non-hypertensive asthmatic patients ($P$-value = 0.797).

This study shows that there is significant negative moderate correlation between creatinine level and treatment duration ($r = -0.413$, $P$-value = 0.023).

Also there is insignificant negative weak correlation between urea level and treatment duration ($r = -0.105$, $P$-value = 0.58).
4.2 Conclusions

The study concluded the following:

1. The plasma creatinine concentration increases in asthmatic patients, and it decreases with the duration of disease and treatment.

2. The plasma urea concentration is normal in asthmatic patients.

3. The plasma urea and creatinine concentrations are normal in hypertensive asthmatic patients in reference to non-hypertensive asthmatic patients.
4.3 Recommendations

The study recommended the following:

1. The dose of steroid treatment is put in consideration.

2. Increase the study population, to achieve more accurate results.

3. Classify patients with asthma according to type of treatment used, to study the effect of each treatment specifically.
References
References


Chandratilleke. MG, Carson. KV, Picot. J, Brinn. MP,


GINA,(2011).


Appendices
PRINCIPLE OF THE METHOD

Urea in the sample is converted, by means of the enzyme urease-catalyzed reaction, into carbon dioxide and ammonia which is measured by spectrophotometry. 

CONTENT

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea+NaOH</td>
<td>3.0 mL</td>
</tr>
<tr>
<td>pH 7.0</td>
<td>0.4 mL</td>
</tr>
<tr>
<td>distilled water</td>
<td>2.6 mL</td>
</tr>
</tbody>
</table>

A1. Reagent
A2. Reagent
B. Reagent

COMPOSITION

A. Reagent: Sodium thiosulfate (62 mmol), sodium hydroxide (3.4 mmol), phosphate buffer (29 mmol), pH 8.0
B. Reagent: Solution urea (300 U/L)
C. Reagent: Solution thermostated (37°C) sodium hydroxide (100 mmol), instant 0.005% solution to 2 hours after dilution in deionized water.
D. Glucose oxidase/peroxidase standard, Glucose 100 mmol, pH 6.0, 450 mmol, pH 6.0, 600 mmol, pH 6.0
E. Glucose oxidase/peroxidase standard, 50 mmol, pH 6.0, 250 mmol, pH 6.0
F. Glucose oxidase/peroxidase standard, 50 mmol, pH 6.0, 250 mmol, pH 6.0

STORAGE

Store at 4°C.

Reagent and standard are stable until the expiry date shown on the label when stored tightly closed at room temperature and protected from light.

PREPARATION

A1. Reagent: Dissolve 0.1 g of sodium thiosulfate in 100 mL of water.
B. Reagent: Dissolve 1 g of sodium hydroxide in 100 mL of water.
C. Reagent: Dissolve 0.5 g of glucose oxidase/peroxidase standard in 10 mL of water.
D. Reagent: Dissolve 0.5 g of glucose oxidase/peroxidase standard in 10 mL of water.

ADDITIONAL EQUIPMENT

- Thermostatted water bath at 37°C
- Analyser: spectrophotometer or colorimeter to read at 560±5 nm

SAMPLES

Urea, plasma or urine collected by standard procedure. Citrate urine was diluted with water before measurement.

Urea in plasma or urine is stable for 7 days at 27°C.

Procedure

1. Urea Reagent A is placed in the centrifuge and spun at 3000 rpm for 30 min.
2. Reagent B is placed in the centrifuge and spun at 3000 rpm for 30 min.
3. A 50 mL conical flask is used for each test. Place 5 mL of reagent A in each flask and add 0.5 mL of reagent B.
4. Mix thoroughly and allow the solution to become clear at room temperature (15-25°C) or at room temperature for 5 min.
5. Place the flask in the spectrophotometer and read the absorbance at 560±5 nm.

CALCULATIONS

The area under the curve is calculated using the following general formula:

A × 1000 ÷ C

BioSystems S.A. Costa Brava 30, Barcelona (Spain)

QUALITY CONTROL

A positive control is required to be added to the samples for each analysis. The results are considered to be acceptable if the integral is in the range of 30-70% of the expected value.
P R I N C I P L E  O F  T H E  M E T H O D
Creatinine in the sample reacts with picric acid in alkaline medium forming a colored complex. The complex formation rate is measured in a short period to avoid interference[7].

C O N T E N T S
COD 11952  2 x 50 mL
COD 11952  4 x 50 mL
COD 11942  1 x 1 L

S T O R A G E
Closed up to 15°C.
Reagents and Standard are stable until the expiry date shown on the label when stored tightly closed. If contamination is prevented during their use.

I n d i c a t i o n s  o f  D e t e r m i n a t i o n:
- Reagent: Presence of particulate material, turbidity, obscuration of the blank over 0.300 at 540 nm (1 cm cuvette).
- Standard: Presence of particulate material, turbidity.

R E A G E N T  P R E P A R A T I O N
Standard (S) is provided ready to use.
Working Reagent: Mix equal volumes of Reagent A and Reagent B. Mix thoroughly. Stable for 1 month at 2-8°C.

A D D I T I O N A L  E Q U I P M E N T
- Thermometer with bath at 37°C.
- Reaction, spectrophotometer or photometer able to read at 540 ± 20 nm.

S A M P L E S
Serum, plasma or urine collected by standard procedures. Dilute fresh urine 1:50 with distilled water before measurement. Hemogloin, EDTA, and sulfates may be used as anticoagulants. Creatinine in specimen is stable for 24 hours at 2-8°C.

P R O C E D U R E
1. Bring the Working Reagent and the photometer to 37°C.
2. Pipette 1.0 mL of Sample onto a cuvette (Note 1).
4. Record the absorbance at 540 nm after 30 seconds (A0) and after 60 seconds (A).

C A L C U L A T I O N S
The creatinine concentration in the sample is calculated using the following general formula (Note 2):

\[
\text{Creatinine (mg/dL)} = \frac{A - A_0}{C_{\text{Creatinine}}} \times \frac{190}{1900} \times \text{Sample dilution factor} + C_{\text{Standard}}
\]

(Where [A - A0] is blank correction; C Creatinine is sample dilution factor; C Standard is standard creatinine concentration).

The sample dilution factor is based on dilutions of the standard creatinine solution.

R E F E R E N C E  V A L U E S
Serum and plasma:
- Men: 0.8 - 1.3 mg/dL
- Women: 0.7 - 1.1 mg/dL

Urine:
- Men: 140 - 200 mg/dL
- Women: 80 - 130 mg/dL

Q U A L I T Y  C O N T R O L
It is recommended to use the Biochemistry Control Serum level (cod. 19005, 19003 and 19004) and R (cod. 19007, 18158 and 19003) and the Biochemistry Control Urine (cod. 18004) to verify the performance of the measurement procedure.

Ea laboratory should establish its own internal Quality Control scheme and procedures for corrective action if results do not recover within the acceptable tolerances.

M E T E R O L O G I C A L  C H A R A C T E R I S T I C S
- Accuracy: 0.08 mg/dL creatinine, 2.05 μmol/L creatinine.
- Linearity: 20 mg/dL creatinine, 1782 μmol/L creatinine. For higher values dilute sample 1:2 with distilled water and repeat measurement.
- Repeatability (within run):
- Sensitivity: 31 mAU/mg/L, 0.551 μmol/L/μL
- Interference: Hemoglobin (10 μL), bilirubin (10 μg/mL), protein and icteric substances do not interfere. Uremia (20 μg/mL), uric acid (1 μg/mL) may interfere. High concentrations of reducing compounds may inhibit. Other drugs and substances may interfere.

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a different procedure is used.

D I A G N O S T I C  C H A R A C T E R I S T I C S
Creatinine is a catabolite end product of creatine (or phosphocreatine). The amount produced each day is related to the muscle mass. Creatinine is freely filtered by the glomeruli (small amounts are reabsorbed and are also secreted by the renal tubules). Creatinine measurement is used almost exclusively in the assessment of kidney function (important renal perfusion, loss of tubular excretion) and it is the preferred renal dialysis[8].

The determination of creatinine in serum and urine is a reliable method for the diagnosis of some clinical conditions.

N O T E S
1. The test may be used in general automatic analyzers. Limitations for many of them are available commercially.

B I B L I O G R A P H Y