Sudan University of Science and Technology
College of Medical Laboratory Science
Clinical Chemistry Department

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

تقويم تركيز البولينا والكرياتينيين 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)

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الآية

قال تعالى:

(وَلَنَبْلُوَنَّكُمْ بِشَيْءٍ مِنَ الْخَوْفِ وَالْجُوعِ وَنَقْصٍ مِنَ الْأَمْوَالِ وَالْأَنْفُسِ وَالثَّمَرَاتِ وَبَشِّرِ الصَّابِرِينَ * الَّذِينَ إِذَا أَصَابَتْهُمْ مُصِيبَةٌ قَالُوا إِنَّا لِلَّهِ وَإِنَّا إِلَيْهِ رَاجِعُونَ * أُولَئِكَ عَلَيْهِمْ صَلَوَاتٌ مِنْ رَبِّهِمْ وَرَحْمَةٌ وَأُولَئِكَ هُمُ الْمُهْتَدُونَ)

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

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We would like to give appreciated thanks to Allah.

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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 2014to 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

أجريت هذه الدراسة في الفترة ما بين فبراير و يوليو 2014 بغرض تقدير مستويات البولينا والكرياتينين في مصل المرضى المصابين بالربو الذين يخضعون للعلاج بالاستيرويدات.

تضمنت هذه الدراسة 30 مريض ربو من المصابين وغير المصابين بضغط الدم لتتم مقارنتهم بنتائج 20 شخص من الأصحاء اختيروا عشوائيا. ولقد أخذت عينات الدم من مجموعتي المرضى والاصحاء للحصول على المصل لقياس تركيز البولينا و الكيرياتينين باستخدامطريقة الكلروميتر وتم تحليل النتائج احصائيا.

وأثبتت النتائج وجودارتفاع في تركيز الكيرياتينين بين مرضى الربو مقارنة بالأصحاء، وعدم وجود فروق ذات دلاله احصائيه معنويه في تركيز البولينا بين مرضى الربو والأصحاء.

وعدم وجود فروق ذات دلالة احصائية في تركيز البولينا والكيرياتينين بين مرضى الربو المصابين بضغط الدم مقارنة بمرضى الربو غير المصابين بضغط الدم

وجود علاقة معنوية سالبة بين تركيز الكيرياتينين ومدة العلاج، وأيضا علاقة غير معنوية بين تركيز البولينا ومدة العلاج.

أثبتت هذه الدراسة أن علاج الربو يقلل من تركيز الكيرياتينين مع الزمن وليس له تأثير على تركيز البولينا.

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List of Abbreviations

Abbreviation	Word
BUN	blood urea nitrogen
ESRD	End stage renal disease
FEV_1	forced expiratory volume in one second
GFR	Glomerular filtration rate
LABA	Long-acting beta-adrenoceptor agonists
SABA	Short-acting $beta_2$-adrenoceptor agonists
SPSS	Statistical package of social science

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 age10, 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 infuenza 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 (FEV_1), 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 $beta_2$-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 (Ducharm*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).

1.3.1Biochemistry 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%and2%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

1.4.2.1Increase 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 concentrationof 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 tonon-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

2.2.1.1Principle

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

$$\text{Urea} + H_2O \xrightarrow{\text{ureases}} 2NH_4^+ + CO_2$$

$$NH_4^+ + \text{Salicylate} + \text{NaClO} \xrightarrow{\text{nitroprusside}} \text{indophenols}$$ (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*- 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:

	Blank	Standard	Sample
Urea standard 50mg/dl	_	10µl	_
Sample	_	_	10µl
Reagent A	1.0ml	1.0ml	1.0ml

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:

$\frac{\text{A sampe}}{\text{A standard}}$ × Standard concentration × sample dilution = 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

Working reagent	1.0 ml
Sample /standard	100 µl

Mixed well, read against distilled water.

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

2.2.2.4 Calculations

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

$\frac{(A2-A1)sample}{Astandard}$ ×Standard $concentration$ × sample dilution factor=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.

	Asthmatic patient (case) NO= 30	Asthmatic patient (Control) NO= 20	*P*-value
Urea (mg/dl)	29.90 ± 11.9	25 ±5.3	0.06
Creatinine (mg/dl)	1.25 ±0.79	0.70 ±0.25	0.001

Table (3.2) Comparison of the concentration of urea and creatinine between male and female in asthmatic patients.

	Male NO= 18	Female NO= 12	*P*-value
Urea (mg/dl)	31.28 ± 13.73	27.83 ± 8.75	0.449
Creatinine (mg/dl)	1.17 ±0.55	1.35 ± 1.08	0.554

Table (3.3) Comparison of the concentration of urea and creatinine between hypertensive and non-hypertensive asthmatic patients.

	Hypertensive NO= 8	Non-Hypertensive NO= 22	*P*-value
Urea (mg/dl)	30.75 ± 9.86	29.59 ± 12.81	0.797
Creatinine (mg/dl)	1.75 ± 1.34	1.06 ± 0.38	0.198

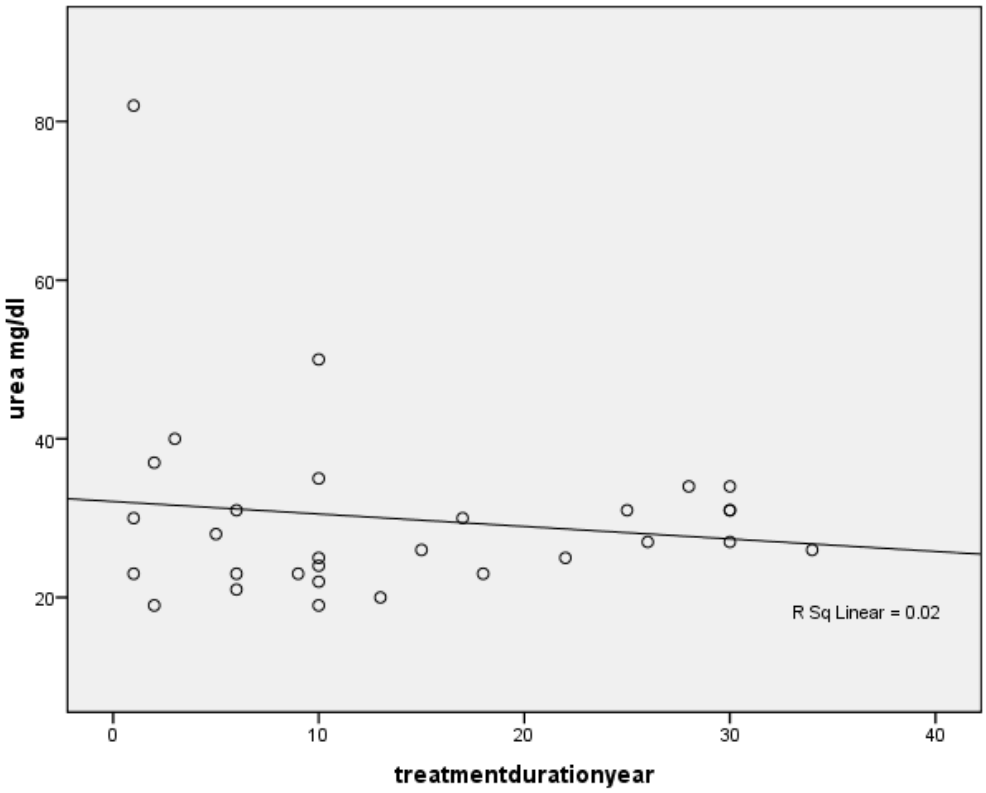

Figure (3.1) scatter blot dots the correlation between treatment duration and urea concentration in asthmatic patients (r = -0.105, *P*- value = 0.58).

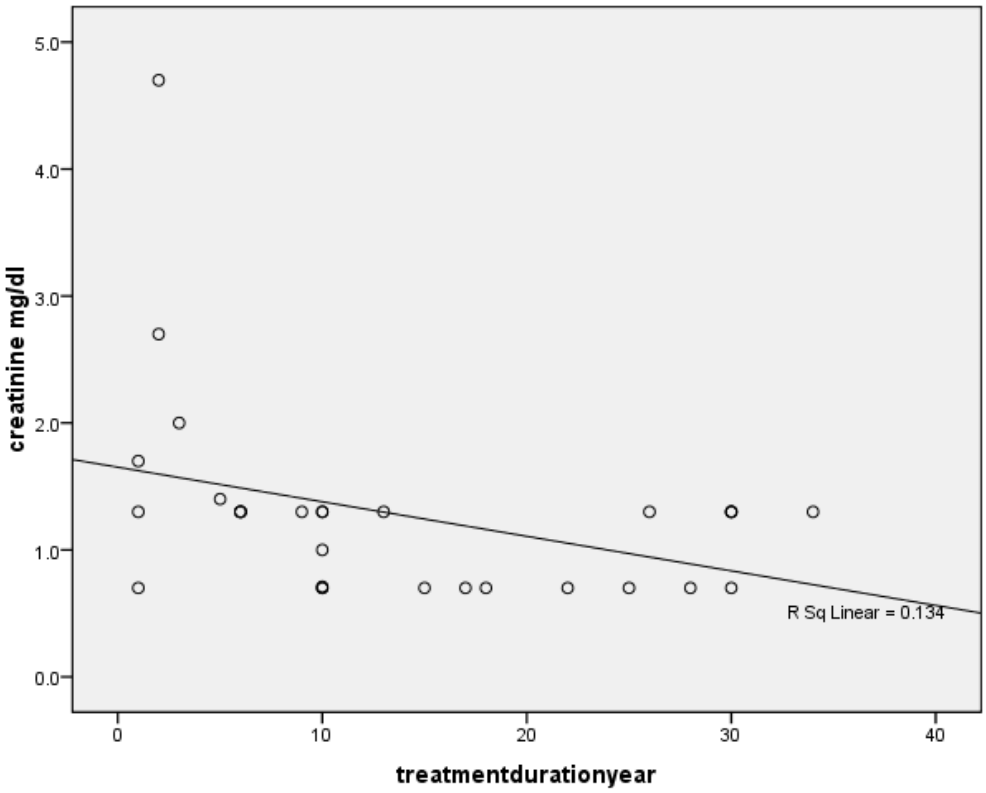

Figure (3.2) scatter blot dots the correlation between treatment duration and creatinine concentration in asthmatic patients (r = -0.413, *P*- value = 0.023).

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.

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Appendices

COD 11536 4 x 50 mL	COD 11537 2 x 250 mL
STORE AT 2-8°C	
Reagents for measurement of urea concentration Only for *in vitro* use in the clinical laboratory	

UREA/BUN - COLOR

CE

BIOSYSTEMS
REAGENTS & INSTRUMENTS

UREA/BUN - COLOR
UREASE/SALICYLATE

PRINCIPLE OF THE METHOD

Urea in the sample originates, by means of the coupled reactions described below, a coloured complex that can be measured by spectrophotometry[1,2,3].

$$\text{Urea} + H_2O \xrightarrow{\text{urease}} 2NH_4^+ + CO_2$$

$$NH_4^+ + \text{Salicylate} + NaClO \xrightarrow{\text{nitroprusside}} \text{Indophenol}$$

CONTENTS

	COD 11536	COD 11537
A1. Reagent	2 x 48 mL	1 x 240 mL
A2. Reagent	2 x 2 mL	1 x 10 mL
B. Reagent	2 x 50 mL	1 x 250 mL
S. Standard	1 x 5 mL	1 x 5 mL

COMPOSITION

A1. Reagent: Sodium salicylate 62 mmol/L, sodium nitroprusside 3.4 mmol/L, phosphate buffer 20 mmol/L, pH 6.9.

A2. Reagent: Urease > 500 U/mL.

B. Reagent Sodium hypochlorite 7 mmol/L, sodium hydroxide 150 mmol/L.
Irritant (Xi): R36/38: Irritating to eyes and skin. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S37/39: Wear suitable gloves and eye/face protection.

S. Glucose/Urea/Creatinine Standard. Glucose 100 mg/dL, urea 50 mg/dL (8.3 mmol/L, BUN 23.3 mg/dL), creatinine 2 mg/dL. Aqueous primary standard.

STORAGE

Store at 2-8°C.

Reagents and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

- Reagents: Presence of particulate material, turbidity, absorbance of the blank over 0.250 at 600 nm (1 cm cuvette).
- Standard: Presence of particulate material, turbidity.

REAGENT PREPARATION

Reagent (B) and Standard (S) are provided ready to use.
Reagent (A): Transfer the contents of one Reagent A2 vial into a Reagent A1 bottle (Note 1). Mix thoroughly. Other volumes can be prepared in the proportion: 1 ml. Reagent A2 + 24 mL Reagent A1. Stable for 2 months at 2-8°C (Note 2).

ADDITIONAL EQUIPMENT

- Thermostatic water bath at 37°C
- Analyzer, spectrophotometer or photometer able to read at 600 ± 20 nm.

SAMPLES

Serum, plasma or urine collected by standard procedures. Dilute urine 1/50 with distilled water before measurement.

Urea in serum or plasma is stable for 7 days at 2-8°C. Heparin is recommended as anticoagulant.

Urea in urine is stable for 3 days at room temperature if microbial growth is prevented.

PROCEDURE

1. Bring the Reagents to room temperature.
2. Pipette into labelled test tubes:

	Blank	Standard	Sample
Urea Standard (S)	—	10 µL	—
Sample	—	—	10 µL
Reagent (A)	1.0 mL	1.0 mL	1.0 mL

3. Mix thoroughly and incubate the tubes for 10 minutes at room temperature (16-25°C) or for 5 minutes at 37°C.
4. Pipette:

Reagent (B)	1.0 mL	1.0 mL	1.0 mL

5. Mix thoroughly and incubate the tubes for 10 minutes at room temperature (16-25°C) or for 5 minutes at 37°C.
6. Read the absorbance (A) of the Standard and the Sample at 600 nm against the Blank. The colour is stable for at least 2 hours.

CALCULATIONS

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

$$\frac{A_{\text{Sample}}}{A_{\text{Standard}}} \times C_{\text{Standard}} \times \text{Sample dilution factor} = C_{\text{Sample}}$$

If the Urea Standard provided has been used to calibrate (Note 3):

	Serum and plasma	Urine
$\frac{A_{\text{Sample}}}{A_{\text{Standard}}}$	x 50 = mg/dL urea x 23.3 = mg/dL BUN x 8.3 = mmol/L urea	x 2500 = mg/dL urea x 1165 = mg/dL BUN x 415 = mmol/L urea

REFERENCE VALUES

Serum and plasma[4]: 15-39 mg/dL urea = 7-18 mg/dL BUN = 2.5-6.5 mmol/L urea. Concentrations in the neonatal period are lower, and in adults over 60 years of age are higher than in adults. Concentrations also tend to be slightly higher in males than in females.

Urine[4]: 26-43 g/24-h urea = 12-20 g/24 h BUN = 428-714 mmol/24-h urea

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the 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.

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

METROLOGICAL CHARACTERISTICS

- Detection limit 1.3 mg/dL urea = 0.60 mg/dL BUN = 0.21 mmol/L urea
- Linearity limit: 300 mg/dL = 140 mg/dL BUN = 50 mmol/L urea. For higher values dilute sample 1/5 with distilled water and repeat measurement.
- Repeatibility (within run):

Mean urea concentration	CV	n
26 mg/dL = 4.3 mmol/L	1.6 %	20
86 mg/dL = 14.2 mmol/L	0.8 %	20

- Reproducibility (run to run):

Mean urea concentration	CV	n
26 mg/dL = 4.3 mmol/L	2.4 %	25
86 mg/dL = 14.2 mmol/L	1.3 %	25

Sensitivity: 8.6 mA·dL/mg = 0.143 mA·L/mmol

- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 3). Details of the comparison experiments are available on request.
- Interferences: Lipemia (triglycerides 10 g/L) and bilirubin (20 mg/dL) do not interfere. Hemolysis (hemoglobin 2 g/L) and elevated ammonia interfere. Other drugs and substances may interfere[5].

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

DIAGNOSTIC CHARACTERISTICS

Urea is synthesized in the liver as a by-product of the deamination of amino acids. Its elimination in the urine represents the major route for nitrogen excretion.

Elevated urea concentration in plasma is found as a result of a high protein diet, increased protein catabolism, after a gastrointestinal hemorrhage, mild dehydration, shock and heart failure or treatment with glucocorticoids (pre-renal uremia)[4,6].

Post-renal uremia is caused by conditions that obstruct urine outflow: nephrolithiasis, tumor or prostatic hypertrophy. The usefulness of urea as an indicator of renal function is limited by the variability of its plasma concentration as a result of nonrenal factors[4,6].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. It is advisable to wash the Reagent A2 vial with a small volume of the prepared mixture in order to completely rinse the vial and avoid any losses.
2. The stability of Reagent A may be drastically reduced when it is not stored at 2-8°C.
3. Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analyzers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator cod. 18011 and 18044).

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BioSystems S.A. Costa Brava 30, Barcelona (Spain)
Quality System certified according to
EN ISO 13485 and EN ISO 9001 standards

COD 11802	COD 11502	COD 11542
2 x 50 mL	4 x 50 mL	1 x 1 L
STORE AT 15-30ºC		
Reagents for measurement of creatinine concentration Only for *in vitro* use in the clinical laboratory		

CREATININE

BioSystems
REAGENTS & INSTRUMENTS

CE

CREATININE
ALKALINE PICRATE

PRINCIPLE OF THE METHOD

Creatinine in the sample reacts with picrate in alkaline medium forming a coloured complex. The complex formation rate is measured in a short period to avoid interferences[1,2].

CONTENTS

	COD 11802	COD 11502	COD 11542
A. Reagent	1 x 50 mL	2 x 50 mL	1 x 500 mL
B. Reagent	1 x 50 mL	2 x 50 mL	1 x 500 mL
S. Standard	1 x 5 mL	1 x 5 mL	1 x 5 mL

COMPOSITION

A. Reagent. Sodium hydroxide 0.4 mol/L, detergent.

Irritant (Xi): R36/38: Irritating to eyes and skin. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S37/39: Wear suitable gloves and eye/face protection.

B. Reagent. Picric acid 25 mmol/L.

S. Glucose/Urea/Creatinine Standard. Glucose 100 mg/dL, urea 50 mg/dL, creatinine 2 mg/dL (177 µmol/L). Aqueous primary standard.

STORAGE

Store at 15-30ºC.

Reagents and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

- Reagents: Presence of particulate material, turbidity, absorbance of the blank over 0.350 at 500 nm (1 cm cuvette).
- Standard: Presence of particulate material, turbidity.

REAGENT PREPARATION

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 2-8ºC.

ADDITIONAL EQUIPMENT

- Thermostatic water bath at 37ºC
- Analyzer, spectrophotometer or photometer able to read at 500 ± 20 nm.

SAMPLES

Serum, plasma or urine collected by standard procedures. Dilute fresh urine 1/50 with distilled water before measurement. Heparin, EDTA, oxalate and fluoride may be used as anticoagulants.

Creatinine in samples is stable for 24 hours at 2-8ºC.

PROCEDURE

1. Bring the Working Reagent and the photometer to 37ºC.
2. Pipette into a cuvette: (Note 1)

Working Reagent	1.0 mL
Standard (S) or Sample	0.1 mL

3. Mix and insert cuvette into the photometer. Start stopwatch.
4. Record the absorbance at 500 nm after 30 seconds (A_1) and after 90 seconds (A_2).

CALCULATIONS

The creatinine concentration in the sample is calculated using the following general formula (Note 2):

$$\frac{(A_2 - A_1)_{Sample}}{(A_2 - A_1)_{Standard}} \times C_{Standard} \times \text{Sample dilution factor} - \text{Corrective Factor}^{4,5} = C_{Sample}$$

If the Creatinine Standard provided has been used to calibrate (Note 3):

	Serum and plasma	Urine
$\frac{(A_2 - A_1)_{Sample}}{(A_2 - A_1)_{Standard}}$	x 2] -0.37 = mg/dL creatinine	x 100 = mg/dL creatinine
	x 177] -33 = µmol/L creatinine	x 8840 = µmol/L creatinine

REFERENCE VALUES

Serum and plasma[3]:

Men: 0.9-1.3 mg/dL = 80-115 µmol/L
Women: 0.6-1.1 mg/dL = 53-97 µmol/L

Urine[3]:

Men: 14-26 mg/kg/24-h = 124-230 µmol/kg/24-h
Women: 11-20 mg/kg/24-h = 97-177 µmol/kg/24-h

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) and the Biochemistry Control Urine (cod. 18054) to verify the performance of the measurement procedure.

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

METROLOGICAL CHARACTERISTICS

- Detection limit: 0.03 mg/dL creatinine = 2.65 µmol/L creatinine
- Linearity limit: 20 mg/dL = 1768 µmol/L creatinine. For higher values dilute sample 1/2 with distilled water and repeat measurement.
- Repeatibility (within run):

Mean concentration	CV	n
1.7 mg/dL = 150 µmol/L	2.9 %	20
5.3 mg/dL = 468 µmol/L	1.3 %	20

- Reproducibility (run to run):

Mean concentration	CV	n
1.7 mg/dL = 150 µmol/L	3.9 %	25
5.3 mg/dL = 468 µmol/L	2.9 %	25

- Sensitivity: 31 mA·dL/mg = 0.351 mA·L/µmol
- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 2). Details of the comparison experiments are available on request.
- Interferences: Hemoglobin (10 g/L), bilirubin (10 mg/dL), protein and ketonic bodies do not interfere. Lipemia (triglycerides > 2 g/L) may interfere. High concentration of reducing compounds may interfere. Other drugs and substances may interfere[6].

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

DIAGNOSTIC CHARACTERISTICS

Creatinine is a catabolic end product of creatine (or phosphocreatine). The amount produced each day is related to the muscle mass. Creatinine is freely filtered by the glomerulus (small amounts are reabsorbed and are also secreted by the renal tubules).

Creatinine measurement is used almost exclusively in the assessment of kidney function (impaired renal perfusion, loss of functioning nephrons) and in the monitoring renal dialysis[3,7].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. These reagents may be used in several automatic analysers. Instructions for many of them are available on request.
2. For measurement in serum or plasma, introduce the corrective value for the reaction of nonspecific proteins as a constant factor subtracted from the concentration value obtained[4,5]
3. Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analyzers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011 and 18044).

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