Vol 4 Issue 3 Dec 2014

ISSN No : 2249-894X

Monthly Multidisciplinary Research Journal

Review Of Research Journal

Chief Editors

Welcome to Review Of Research

RNI MAHMUL/2011/38595 **ISSN No.2249-894X**

Review Of Research Journal is a multidisciplinary research journal, published monthly in English, Hindi & Marathi Language. All research papers submitted to the journal will be double - blind peer reviewed referred by members of the editorial Board readers will include investigator in universities, research institutes government and industry with research interest in the general subjects.

Advisory Board

Address:-Ashok Yakkaldevi 258/34, Raviwar Peth, Solapur - 413 005 Maharashtra, India
Cell : 9595 359 435, Ph No: 02172372010 Email: ayisrj@yahoo.in Website: www.ror.isrj.org

Review Of Research
Vol. 4 | Issue. 3 | Dec. 2014
Impact Factor : 2.1002 (UIF)
ISSN:-2249-894X

Available online at www.ror.isrj.org

ORIGINAL ARTICLE

TUMOR NECROSIS FACTOR (TNF-ά), INTERLEUKIN-6 AND NITRIC OXIDE SERUM LEVELS AMONG EGYPTIAN CHILDREN WITH CHRONIC RENAL FAILURE

[1]Essam Shawky A.E.H. Khattab, [2]Khadega Kamel El Gohary and [2]Hala Hassen El Deeb

[1]Chemistry Department (Biochemistry Division), Faculty of Science, Al-Azhar University, Cairo, Egypt.
[2]El Sahel Teaching Hospital, Cairo, Egypt.

Abstract:

Renal failure is characterized by the accumulation of nitrogenous waste products (urea and creatinine), and the inability to regulate fluid and electrolyte homeostasis. The glomerular filtration rate (GFR) must usually decline by 50% or more, before clinical significant increase in the serum creatinine and urea concentration. The present study were conducted through 60 Egyptian children, ages from (2-14) years, divided into two groups; 40 patients were diagnosed as renal failure and 20 healthy control group.The objective of our present study was to estimate the serum level of TNF-α and IL-6 and to evaluate the possible role of nitric oxide in these patients.The results showed highly significant increase in TNF-a, and IL-6. In contrast, nitric oxide showed highly significant decrease in chronic renal failure group compared to healthy control group. Correlation coefficient between TNF-a & IL-6 and urea, creatinine, phosphorous & potassium, showed highly positive significant correlation. Correlation coefficient between nitric oxide, and urea, creatinine, phosphorous & potassium, showed highly negative significant correlation.

KEYWORDS:

Chronicrenal failure, nitric oxide and cytokines, Tumor necrosis, Interleukin-6.

INTRODUCTION

Chronic renal failure (CRF) is defined as the stage at which the irreversibly damaged kidneys are unable to maintain the homeostasis of the body. Patients with established CRF do not recover, but instead experience a continuous loss of function even when the original disease that damaged the kidneys is no longer active (Papadopoulou, 1989). In addition, CRF is a gradual deterioration of renal glomerular function manifested by progressive proteinuria, azotemia and impaired metabolism of water and various electrolytes (Potter et al., 1997).The etiology of CRF in childhood correlates closely with the age of the patient, when the renal failure is first detected. CRF in children younger than 5 years is commonly a result of anatomic abnormalities (hypoplasia, dysplasia, obstruction, malformation), whereas after 5 years of age acquired glomerular diseases or hereditary disorders (Alport syndrome, cystic disease) predominate (Behrman et al., 2000). When nephrons are progressively damaged, the first sign of functional deterioration is diminution of renal reserve and a decrease of GFR which is strong predictor for the onset and complications of CKD. Before this becomes clinically evident and during the initial phase of loss of renal function, the body uses various physiological mechanisms to maintain homeostasis so that there is a

clinically normal interval and the time of onset therefore is determined by chemical rather than clinical criteria (Ledger, 2006).

The approach to the child during this early phase is to limit any further injury to the kidney. During later phase the aim is to prevent the metabolic consequences that follow from progressive loss of renal function (Chantler and Holliday, 1988).The causes of CRF appear to vary according to the country. Differences among specific countries may represent different prevalence of disease or simply different diagnostic terminology (Chantler and Holliday, 1988). The two main groups of diseases leading to CRF in Egypt are: Parenchymal renal diseases (67.5%) and Obstructive (Post renal) diseases (32.5%) (Bahaa El-Din el al.,1991).

Renal failure is characterized by accumulation of nitrogenous waste products (urea and creatinine) and the inability to regulate fluid and electrolyte homeostasis. The glomerular filtration rate (GFR) must usually decline by 50% or more before clinically significant increase in the serum creatinine and blood urea concentrations occur (Arieffet al., 1995). With progressive deterioration of renal function to less than 30 or 25% of normal, the adaptive mechanisms eventually become limited and overt clinical signs develop. Nitrogen retention and the appearance of uremia start to affect ion transport and cellular metabolism. In addition, metabolic abnormalities, biochemical changes (including impaired energy utilization and abnormal metabolism of carbohydrate, fat and protein), advancing anemia due to bone marrow failure and endocrine disturbances become evident (Papadopoulou, 1989).

CRF was defined as a serum creatinine (s.Cr) concentration two or more times higher than normal for age and gender or as a GFR below 30 ml/min /1.73 m² for at least 3 months. ESRD was defined as a SCr concentration four or more times higher than normal for age and gender, or a GFR below 15 ml/min/1.73m² for at least 3 months (Lagomarsimoet al., 1999). El-Nahas and Tamimi, (1999) suggested that proteinuria may be nephrotoxic, thus contributing to the progression of renal disease, the rate of decline of renal function was proportional to the severity of proteinuria, and patients with heavy proteinuria (>3 gm/24h) having the worst prognosis.

Cytokines are soluble mediators which controlled in many critical interactions among cells of the immune system. These cytokines are a diverse group of intercellular signaling peptides and glycoproteins with molecular weights (MW) between 6.000 and 60.000, and most of them are genetically and structurally unrelated to one another (Ryszet al., 2006). They regulate not only the immune and the inflammatory responses but also wound healing, hematopoiesis, angiogenesis and many others biologic processes. These cytokines are derived primarily from activated monocytes and macrophages, T and B lymphocytes and other cells. Cytokines produced by lymphocytes are called lymphokine, whereas those produced by monocytes or macrophages are called monokines (Oppenheim et al., 2001).

Human Interleukin - 6 (IL-6) is a 184 A.A. polypeptide with potential O and N-glycosylation sites, and a significant homology with G-CSF. It is produced by various cells including "T-cells & B-cells", monocytes, fibroblasts, keratinocytes, endothelial cells, mesangial cells, astrocytes, bone marrow stroma cells and several tumor cells. It regulates the growth and differentiation of various cell types with major activities on the immune system, hematopoiesis, and inflammation. These multiple actions are integrated within a complex cytokine network, where several cytokines induce (IL-1, TNF, PDGF, IFNs,) or are induced IL-6 and the final effects result from either synergistic or antagonistic activities between IL-6 and the other cytokines (IL-1, IL-2, IL-4, IL-5, IFNγ, IL-3, GM-CSF, GM-CSF, CSF). IL-6 induces final maturation of B-cells into antibody producing cells and is a potent growth factor for myeloma/plasmacytoma cells. It co-stimulates T-cell growth and cytotoxic T-cell differentiation. It promotes megakaryocyte development and synergizes with other cytokines to stimulate multipotent hematopoietic progenitors. It can also induce differentiation and growth inhibition of some leukemia -or non-hematopoietictumoral cell lines. IL-6 is also a major inducer of the acute phase reactions in response to inflammation or tissue injury. The elevation of IL-6 precedes that of acute phase proteins, e.g. in postoperative phenomenon, and may thus be a sensitive early parameter to investigate inflammatory conditions.Serum IL-6 has already been described in association with surgical or traumatic tissue injuries, infectious diseases, auto-immune diseases including arthritis, graft rejection, alcoholic liver cirrhosis, malignancies, etc.

Tumor Necrosis Factor)TNF-a(is a pleiotropic protein, which has a wide range of biological activities. It is a product of macrophages and is the principal host mediator of septic shock and cachexia of chronic diseases. A related molecule, TNF-β or lymphotoxin, is produced by activated T-lymphocytes in response to antigen or mitogen (Goh, 1990). TNF-a is a potent paracrine and endocrine mediator of inflammatory and immune functions. TNF-a is selectively cytotoxic for many transformed cells, especially in combination with IFN-γ (Manogue, 1991). TNF works alongside other cytokines to stimulate and coordinate the immune and inflammatory responses to antigenic challenges. It also initiates the energy substrate mobilization required to meet the heightened fuel demands associated with immunological,

inflammatory and wound healing activities. In chronic infection, and malignancy, this may lead to severe depletion of host tissues with its own attendant morbidity and mortality (McColl and Parry, l990).

In response to antigenic stimulation, T-cell and macrophages secrete a set of glycoproteins termed as lymphokines and monokines such as IL-1,IL-2, IL-3, IL-4, IL-5, IL-6, granulocyte monocyte-colony stimulating factor (GM-CSF), TNF-a and lymphotoxin (TNF-β). These glycoproteins mediate immune and inflammatory responses by regulating proliferation, differentiation and maturation of lymphocytes, hematopoietic cells and endothelial cells. Most cytokines are pleiotropic and have multiple biological activities (Arai et al., 1990b). TNF is synthesized by various activated phagocytic and non-phagocytic cells including macrophages,monocytes, lymphocytes, natural killer cells, astrocytes,microglial cells of the brain, kupffer cells of the liver (Tracey et al., 1989) and also produced by human placental cytotrophoblastic cells (Yang et al., 1993).

Studies have demonstrated that high blood concentrations of soluble tumor necrosis factor receptors (STNFRs) is associated with malnutrition in a variety of diseases and that the blood (STNFRs) concentration is elevated in hemodialysis patients (HD) (Mari et al., 2002).Cytokine concentrations often have been found to be increased in both dialyzed and un-dialyzed chronically uremic patients. TNF-a clearance is reduced in uremia because this cytokine is catabolized and excreted mainly by the kidneys. In addition, we found that gene expression for TNF-a in circulating blood cells is enhanced in chronically uremic patients, suggesting that an activation of the systemic inflammatory response may contribute to the metabolic, vascular, and immune complications of this disease (Guarnieriet al., 2003).Graziaet al. (2002) suggested that changes in immune response to infections agents in patients on hemodialysis might be due to impaired monocyte function. Uremia and hemodialysed patients over produce proinflammatory cytokines, such as IL-1β, TNF-a and IL-6.Hemodialysis session markedly decreases IL-8 concentration, which is significantly affected by pre-dialysis concentrations, indicating that removal of IL-8 is a concentration gradient-dependent action, but does not change the serum level of IL-1 β, SIL-2R, IL-6, and TNF-alpha, underlining importance of the structure characteristics of the molecules (Tarakciogluet al., 2003).Nitric oxide is a gaseous free radical that serves cell signaling, cellular energetics, host defense, and inflammatory function in virtually all cells (Kone, 2004).In the kidney and vasculature, NO plays fundamental roles in the control of systemic and intrarenal hemodynamics, the tubuloglomerularfeedback response, pressure natriuresis, release of sympathetic neurotransmitters and renin, and tubular solute and water transport.

The objective of the present study is to investigate the changes occurred in the TNF-a & IL-6 levels and to evaluate the possible role of nitric oxide in chronic renal failure Egyptian children.

SUBJECT AND METHODS

The present study was conducted through 60 Egyptian children, their ages ranged from 2 to 14 years old. These cases were divided into the following groups: Group-I:40 children were choosing from the chronic renal failure outpatient clinic of the Internal medicine department of the Kasr El- Aini Hospital and Group-II:20 healthy control group

Blood sampling and Serum Separation:

About (10-12) ml venous blood sample from each patient of these mentioned groups were taken for the present investigation. The blood was incubated at 37 ºc for 20 minutes for clot formation, then centrifuged for (10-12) minutes at 1200 rpm the separated serum was used to estimate the following parameters: kidney function (urea, creatinine, calcium and phosphorous), nitric oxide were measured by using colorimetric method assays, Na & K were determined by using automated analyzer (Easylyte), TNF-a was measured by using Enzyme Linked Immuno Sorbent Assay (ELSA) and IL-6was measured by using Enzyme Amplified Sensitivity Immunoassay (EASIA).

Determination of serum urea:

Determination of urea was done by Berthelot's reaction. (Patton and Crouch, 1977),(Randox kit).

Calculation:

$$\text{Serum ure (mg/dl)} = \frac{\text{A specimen}}{\text{A standard}} \text{ X } 50$$

Determination of serum creatinine:

Determination of serum creatinine were occurred using the kinetic method of Jaffe (Henry, 1974),(Diamond Kit).

Calculation:

A2 - A1 = A standard or A sample

$$\text{Serum creatinine (mg/dl)} = \frac{\text{A specimen}}{\text{A standard}} \text{ X } 2$$

Determination of calcium:

Using colorimetric method by Bamettet al. (1973), (Randox Kit).

Calculation:

$$\text{Serum calcium (mg/dl)} = \frac{\text{A specimen}}{\text{A standard}} \text{ X10}$$

Determination of serum phosphorus:

Using the method of (Young, 1991), (spectrum diagnostics kit).

Calculation:

$$\text{Serum phosphorus (mg/dl)} = \frac{\text{A specimen}}{\text{A standard}} \text{ X } 5$$

Determination of serum protein:

Using the method of Gornallet al.(1968), (Diamond diagnostics kit).

Calculation:

$$\text{Serum protein (mg/dl)} = \frac{\text{A specimen}}{\text{A standard}} \text{ X } 6$$

Determination of serum albumin:

According to the method of Drupt(1974),(Diamond diagnostics kits).

Calculation:

$$\text{Serum albumin (mg/dl)} = \frac{\text{A specimen}}{\text{A standard}} \text{ X } 4$$

Determination of serum sodium and potassium:

It was determined using automated sodium and potassium analyzer (Easylyte, 13127NK). The Easylyte is an automated, microprocessor- controlled analyzer for measurement of sodium, potassium and chloride in serum, plasma, whole blood, and urine. 100µl of serum was injected for measure sodium and potassium, the analysis takes 55 seconds. This is through, ion selective electrodes. Calibration is automatic, but can be performed on demand. A unique solutions pack contains standard solutions and a wash solution.

Determination of Tumor necrosis factor-alpha (TNF-α):

TNF-a was measured by using Enzyme Linked Imnunosorbent Assay (ELISA) of (Theresa, 1994).

Preparation of TNF-a standard:

a.Six test tubes were labeled (2-6) and "0 dose" Then 600 µl of diluent(1)was added to each of the tubes.
b.The lyophilized TNF-a standard was reconstituted with 1000µl of diluent (1), diluent (2) and vortex.
c.Standards (2-6) were then prepared by performing 1: 4 dilution of preceding standard

Assay procedure:

1.100 µl of standards (0-6) was dispensed into theirdesignated wells.
2.100 µl of serum was added to 200 µl of diluent (1) + 100 µl of diluent (2) in atest tube and vortex. To each of designated wells 100 µl of each diluted sample preparation was dispensed.
3.25 µl of reconstituted human TNF-a antibody was dispensed into each well, then the plate was covered with the plate sealer and incubated 3 hours at room temperature (R.T.)
4.25 µl of reconstituted human TNF-a conjugate was added into each well and then incubated at room temperature for 30 minutes.
5.Wash step:By using a multi-channel pipette 250 µl of diluted wash buffer was added to each well then the fluid was removed from the wells.This procedure was repeated a total of 4 times, then 250 µl of diluted wash buffer was dispensed a fifth time and plate soaked for 10 minutes. At the end of 10 minutes,excess fluid was removed from each well.
6.50 µl of the diluted streptavidin-alkaline phosphatase was dispensed into each well, then the plate was sealed and incubated at R.T, for 30 minutes.
7.After removal of plate sealer, the plate was washed 5 times using the wash method described above.
8.200 µl of prepared color reagent solution was dispensed into each well, and then the plate was sealed and incubated at R.T. for 20 minutes.
9.The plate was read at 492 nm during the 20 minutes incubation to monitor the speed at which color is generated.
10.50 µl of stop solution was dispensed into each well in the same order that the color reagent solution was added.

CALCULATION OF RESULTS:

The standard curve was plot on semi-log paper where known concentrations or TNF-a were plotted on the log scale (X-axis) and the corresponding optical density (OD) on the linear scale (Y-axis).The concentration of TNF-a in unknown samples were then determined by plotting the sample OD on the Y-axis, then drawing a horizontal line to intersect with the standard curve. A vertical line dropped from this point Intersects the X-axis at the concentration of TNF-a in the unknown Sample.There is an inverse relationship between OD and concentration, the higher the OD the less TNF-a in the Sample.

DETERMINATION OF NITRIC OXIDE:

a.Determination of nitrite level

Nitrite level in the serum sample was measured by direct reaction of the sample with Greiss reagent according to (Granger et al., 1990).

b.Determination of nitrate level

Nitrate level in the serum sample was measured by reduction of nitrate to nitrite by the enzyme nitrate reductase and the total nitrite was measured by reaction with Greiss reagent and then read on the spectrophotometer.According to the method by (Schmidt et al., 1992) nitrate reductase enzyme was used with NADPH and FAD as cofactors to reduce nitrate in the serum to nitrite.

1.After centrifugation of the serum sample, 450 µl of thesupernatant was added to 30 µl of the enzyme.
2.Mixture and incubation for 15 minutes at 37 ºC to reduce the entire nitrate in the sample to nitrite.
3.400 µl of the incubated mixture was added to 800 µl of 1% sulphanilamide and 800 µl of 0.5% naphthylenediaminedihydrochloride.
4.Absorbance of the product was detected directly by spectrophotometer at 543 nm.
5.Concentrations were determined on linear standard curve from (10-200) mmol sodium nitrite.

Determination of Interleukin-6 (IL-6):

IL-6 was measured by using Enzyme amplified sensitivity immunoassay (EASIA) (Kita et al., 1994), (Gamma Trade Kit).

The biosource of IL-6 EASIA is a solid phase Enzyme Amplified Sensitivity Immunoassay (EASIA) performed on microtiter plate. The assay is based on an oligoclonal system in which a blend of monoclonal antibodies (MAbs) directed against distinct epitopes of IL-6 are used. Antibody-producing cells are immortilized using the myeloma cell fusion method of Kohler and Milstein. A hybridoma cell is produced which secretes specific homogeneous antibodies. The use of a number of distinct MAbs avoids hyperspecificity and allows high sensitive assays with extended standard range and short incubation time. Standard or samples containing IL-6 react with capture monoclonal antibodies (MAbs 1) coated on the microtiter well. After incubation, the occasional excess of antigen is removed by washing. Mab 2, the horseradish peroxidase (HRP)-labelled-antibody t is then added. After an incubation period allowing the formation of a sandwich: coated MAbs 1 - IL-6 - MAb 2 - HRP, the microtiter plate is washed to remove unbound enzyme labelled antibodies. Bound enzyme - labelled antibodies are measured through a chromogenic reaction. Chromogenic solution (TMB + H2O2) is added and incubated. The reaction is stopped with the addition of stop solution (H2SO4) and the microtiter plate is then read at the appropriate wavelength. The amount of substrate turnover is determined colourimetrically by measuring the absorbance which is proportional to the IL-6 concentration.

A standard curve is plotted and IL-6 concentrations in a sample is determined by interpolation from the standard curve. The use of the EASIA Reader (linearity up to 3 OD units) and a sophisticated data reduction method (polychromatic date reduction) result in high sensitivity in the low range and in an extended standard range.

RESULTS

The present study was conducted through 60 Egyptian children, their ages ranged from 2 to 14 years old. These cases were divided into two groups; Group-I:forty patients were chosen from the chronic renal failure outpatient clinic of the Internal medicine department of the Kasr El- Aini and El Sahel Teaching Hospitals; Group-II: 20 healthy control group the following laboratory investigations were carried out: Kidney function (urea, Creatinine, Na, K, P, Ca), Interleukin-6 (IL-6) and Tumor necrosis factor-alpha (TNF-a)

Kidney function test in Egyptian children with chronic renal failure:

The obtained results indicated that, the concentration of urea, creatinine, phosphorous and calcium in healthy Egyptian children were 30.05±5.64, 0.94±0.23, 4.27±0.55 and 11.60±0.60 mg/dl respectively; but these results in the chronic renal failure children were 106.48±15.98, 5.34±0.97, 7.46±1.01 and 8.34±0.81 mg/dl respectively. Besides, the concentration of sodium and potassium in healthy Egyptian children were 134.55 ±1.96 and 5.98±0.33 mEq/dl respectively; but these results in the chronic renal failure children were 124.75±4.06 and 8.80±1.60 mEq/dl respectively. The obtained results revealed that these results of urea, creatinine, potassium and phosphorous showed a high significant increased concentrations (P value <0.005) in a comparison with healthy Egyptian children control group. In contrast, our results of sodium and calcium showed significant decreased concentrations (P value <0.05) (Table 1).

IL-6 and TNF-a in children with chronic renal failure:

The results within hands appeared that, the concentration of IL-6 and TNF- a of healthy Egyptian children were 5.99±0.74 and 19.30±4.09 Pg/ml respectively; but these results in the chronic renal failure Egyptian children were 23.67±5.65 and 37.88±6.65 Pg/ml respectively. Our results of IL-6 and TNF- a showed a high significant increased concentrations (P value <0.005) (Table 2).

Table 1: Kidney Function Tests in Egyptian Children with Chronic Renal Failure.

Parameter \ Group		Mean Values ± SD	
		Control	Chronic Renal Failure
Urea	(mg/dl)	30.05 ± 5.64	106.48** ±15.98
Creatinine	(mg/dl)	0.94 ± 0.23	5.34** ± 0.97
Na	(mEq/dl)	134.55 ±1.96	124.75* ± 4.06
K	(mEq/dl)	5.98 ± 0.33	8.80** ± 1.60
P	(mg/dl)	4.27 ± 0.55	7.46** ± 1.01
Ca	(mg/dl)	11.60 ± 0.60	8.34* ± 0.81

* Significant change for a comparison between the chronic renal Egyptian children group with the healthy control group ($P<0.05$); ** Significant change for a comparison between the chronic renal Egyptian children group with the healthy control group ($P<0.005$).

Table 2: TNF-α, IL-6 and NO Serum Levels in Egyptian Children with Chronic Renal Failure.

Parameter \ Group		Mean Values ± SD	
		Control	Chronic Renal Failure
TNF- α	(pg/ml)	19.30 ± 4.09	37.88** ±6.65
IL-6	(pg/ml)	5.99 ± 0.74	23.67** ± 5.65
NO	(µmol/L)	38.00 ±7.75	25.35** ± 4.42

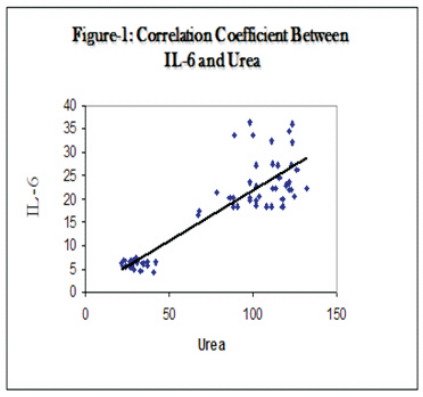
*Significant change for a comparison between the chronic renal Egyptian children group with the healthy control group ($P<0.05$); **Significant change for a comparison between the chronic renal Egyptian children group with the healthy control group ($P<0.005$).

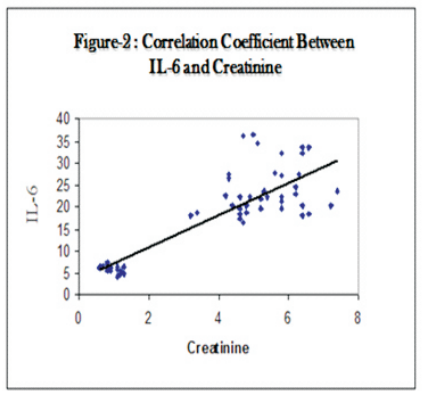
Correlation coefficient Urea, Creatinine and IL-6 & TNF-α within control and pathogenic groups:

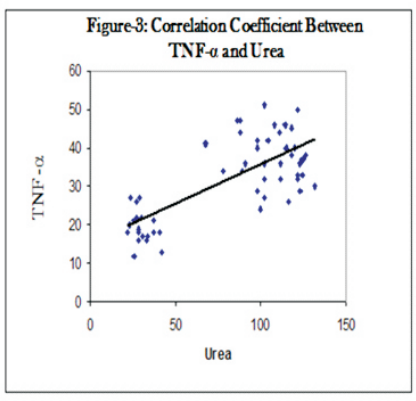
The obtained results revealed that, the correlation coefficient between urea, creatinine and IL-6 & TNF-a showed a high positive significant correlation (P value < 0.005) between Egyptian chronic renal failure children and healthy control group (Figs.1-4).

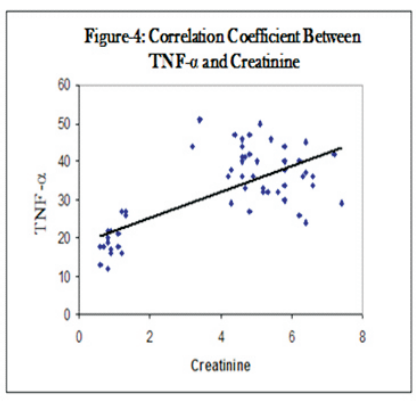
Correlation coefficient Sodium, Potassium and IL-6 & TNF-a within control and Chronic Renal Failure groups:

The results indicated that, the correlation coefficient between sodium and IL-6 & TNF-a showed a high negative significant correlation, P value < 0.005 (Figs. 5&7). While the correlation coefficient between potassium and IL-6 &TNF-a showed a high positive significant correlation, P value < 0.005 (Figs. 6 &8).

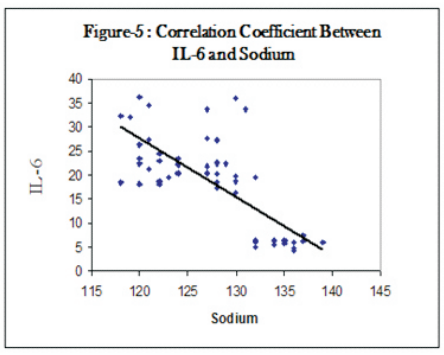


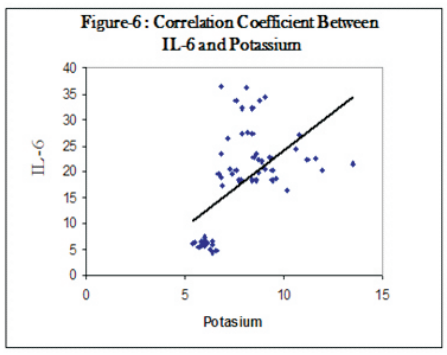


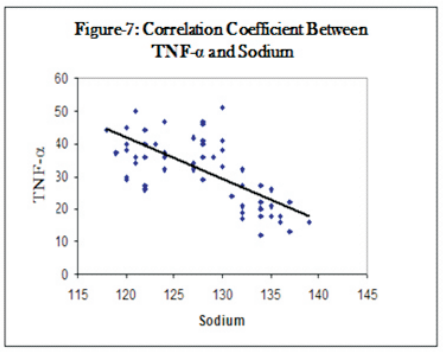


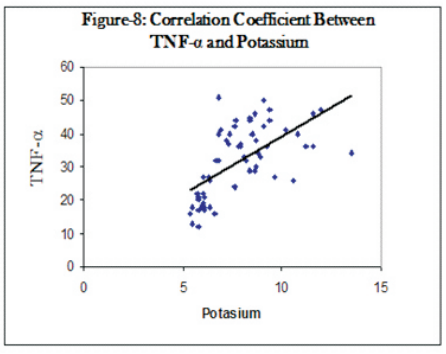


Figs. 1-4: Correlation coefficients between IL-6 &TNF-α and each of urea and creatinine.









Figs. 5-8: Correlation coefficients between IL-6 &TNF-α and each of sodium and potassium.

Correlation coefficient Phosphorous, Calcium and IL-6 & TNF-a within Healthy control Egyptian children and Chronic Renal failure Egyptian Children:

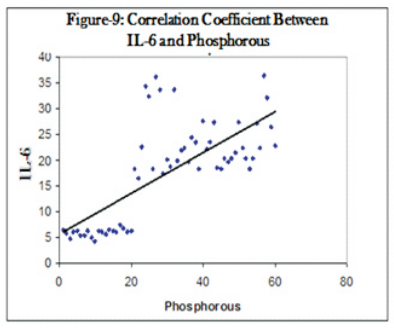
The obtained results explained that, the correlation coefficient between phosphorous and IL-6 & TNF-a showed a high positive significant correlation, P value <0.005 (Figs. 9&11). While the correlation coefficient between calcium and IL-6 & TNF-a showed a high negative significant correlation, P value < 0.005 (Figs.10&12).

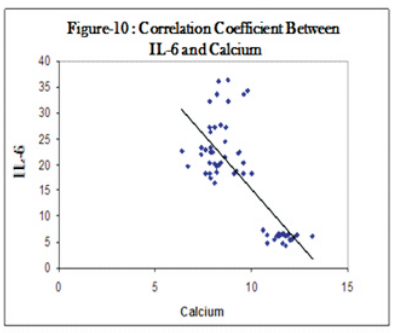
Serum Nitric Oxide Levels in an Egyptian children with chronic renal failure:

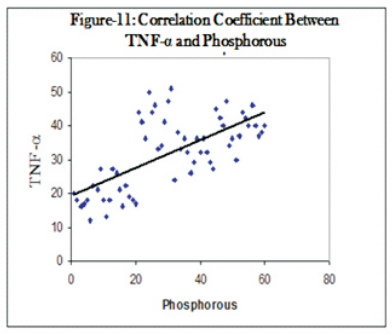
The obtained results showed that, the concentration of nitric oxide for healthy Egyptian children was 38.0±7.75 µmol/L; but these results in the chronic renal failure children were 25.35±4.42 µmol/L.Our results indicated that nitric oxide showed high significant decreased concentrations (P value <0.005) (Table 2).

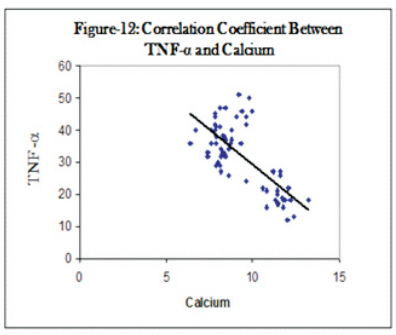
Correlation coefficient of urea, creatinine and Nitric Oxide within healthy control and An Egyptian children with chronic renal failure groups:

The results revealed that the correlation coefficient between urea, creatinine& nitric oxide showed high negative significant correlation, P value < 0.005 for the chronic renal failure Egyptian children group as a comparison with healthy control group (Figs. 13 & 14).

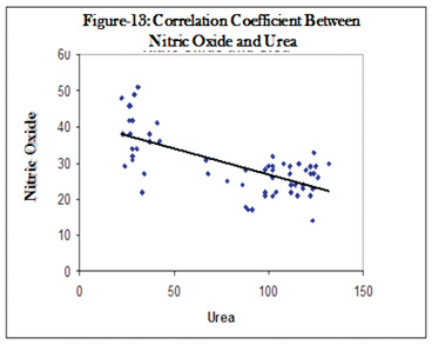


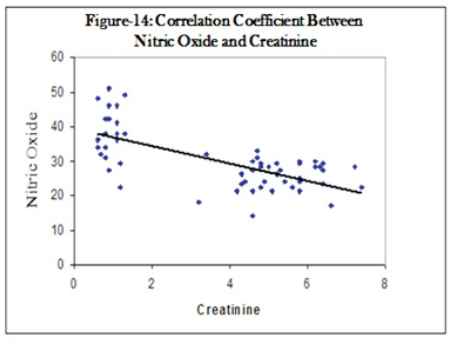






Figs. 9-12: Correlation coefficients between IL-6 &TNF-α and each of phosphorus andcalcium.

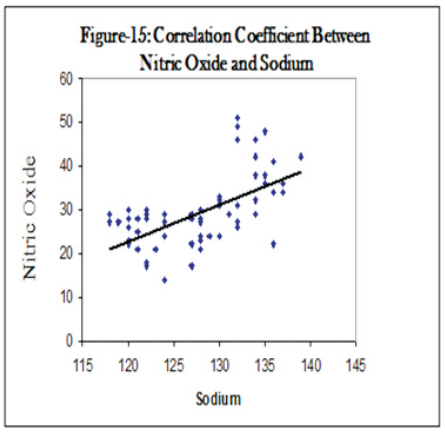


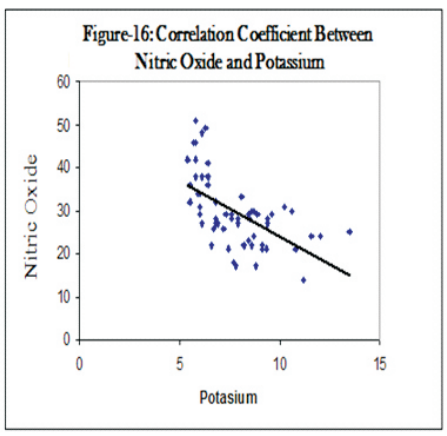


Figs. 13&14: Correlation coefficients between nitric oxide and each of urea and creatinine.

Correlation coefficient Sodium, Potassium and Nitric Oxide within healthy Egyptian children control and chronic renal failure Egyptian children groups:

The results indicated that, the correlation coefficient between sodium and nitric oxide showed high positive significant correlation, P value < 0.005. However, the correlation coefficient between potassium and nitric oxide showed a high negative significant correlation, P value < 0.005 (Figs. 15& 16).

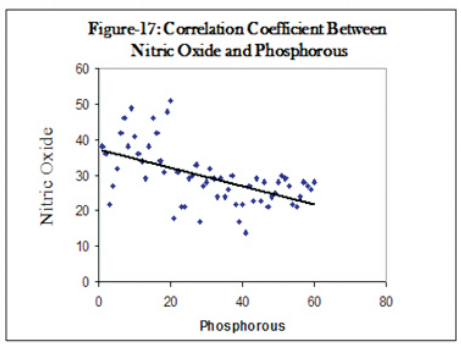


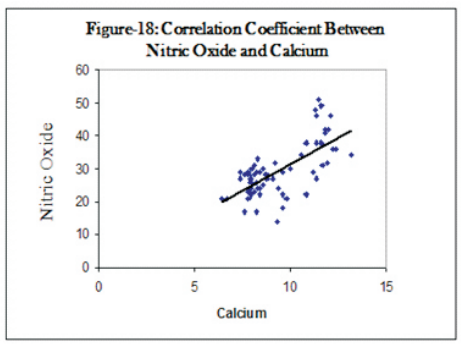


Figs. 15 & 16: Correlation coefficients between oxide acid and each of sodium and potassium.

Correlation coefficient Phosphorous, Calcium and Nitric Oxide within healthy Egyptian children control and chronic renal failure Egyptian children groups:

The results explained that, the correlation coefficient between phosphorous and nitric oxide showed highly negative significant correlation, P value < 0.005. Whereas, the correlation coefficient between calcium and nitric oxide showed a highly positive significant correlation, P value < 0.005 (Figs. 17& 18).





Figs. 17 & 18: Correlation coefficients betweennitric oxide and each of phosphorus and calcium.

DISCUSSION

The objective of our present study is to investigate the concentrations of TNF-α and IL-6 and to evaluate the possible role of nitric oxide in chronic renal failure Egyptian children.Reduced renal function is associated with a variety of biochemical abnormalities. However, the extent of these changes and their magnitude in relation to renal function is not well defined, especially among individuals with mild to moderate chronic renal insufficiency (Chi-Yuan and Glenn, 2002).

Kidney function in chronic renal failure Egyptian children:

The present studies showed highly significant increased concentration of urea, creatinine, potassium and phosphorous in CRF Egyptian children, in a comparison withhealthy control Egyptian children group. In contrastour results revealed that, calcium and sodium showed a significant decreased

concentration (Table 1). It is important to be noted that elevation of serum creatinine and blood urea is late sign of renal dysfunction. Creatinine is formed in the breakdown of muscle creatinine and proportional to the muscle mass. It should be stable from day-to-day.Any changes in the serum creatinine level would usually be a result of change in the glomerular filtration rate. Abrupt caessation of glomerular filtration causes the serum creatinine to rise by 1-3 mg/dl daily. Examination of serum electrolytes, calcium, phosphorus and magnesium are also to be considered (Portaleet al., 1984). Portal(1984) showed that serum phosphate concentration generally begins to rise when the GFR falls under 30 ml/min /1.73m². In the setting, even in the presence of an elevated PTH level, there is no further increase in the urinary excretion of phosphate. Also, Hsu 2002 showed that, significant elevation in serum potassium in patients with mild to moderate CRI not be related to the use of drugs known to cause hyperkalaemia in susceptible subjects, and the associations between reduced renal function and increased level of potassium and phosphorous are physiologically plausible, and so on despite the fact that various homeostatic, compensatory mechanisms may be in play throughout the course of CRI that serve to minimize changes in serum chemistries, of great importance among these mechanisms are hormonal changes (e.g. aldosterone promoting urinary potassium excretion , or parathyroid hormone promoting urinary phosphorus excretion and calcium mobilization from bone. Their results suggest that these adaptations are unable to compensate completely for the decrease in GFR. However, Mahdavi(2003) compliance with dietary phosphate restriction in children is poor as most of their favorite foods are rich in phosphate. Thus, phosphate binders may become necessary to prevent phosphate absorption from the gastro-intestinal tract.

Monkawaet al.(2000) showed that hypercalcemia is usually caused by an autonomous production of 1,25-dihydroxyvitamin D (calcitriol) by macrophages within the granuloma. These macrophages are able to convert 25-hydroxyvitamin D, produced by the liver, into calcitriol by possessing the 1a-hydroxylase enzyme. Calcitriol then travels to the intestinal cells and promotes luminal absorption of calcium and phosphate into the circulation. Hypercalcemia may also cause renal failure by inducing renal vasoconstriction, thereby reducing the glomerular filtration rate with consequent renal insufficiency. Liach(1995) suggests that an elevation serum phosphorous level is unlikely to play an important role in the pathogenesis of secondary hyperparathyroidism in mild to moderate CRI. instead, the initiation and maintenance of hyperparathyroidism was ascribed to a deficiency in 1,25(OH)2 vitamin D and this differs from several previous studies (Wilson et al., 1985) found in 12 CRI subjects (CrCl range 38-78 ml/min) and (Liachand Massry, 1985) found in 13 CRI subjects CrCl range 34-93 ml/min/1.73m² that serum phosphorus was lower than in control.

TNF-a and IL-6levels in chronic renal failure Egyptian children:

The present study, TNF- a and IL-6 showed highly significant increase concentration in chronic renal failure as compared to control group (Table 2).

Correlation coefficient between urea &creatinine and IL-6 & TNF- a showed highly positive significant correlation (Figs. 1-4).

Correlation coefficient between sodium and IL-6 & TNF- a showed highly negative significant correlation (Figs.5&7), but correlation coefficient between potassium and IL-6 & TNF- a showed highly positive significant correlation (Fig.6& 8).

Correlation coefficient between phosphorous and IL-6 &TNF- a showed highly positive significant correlation (Figs.9& 11), but correlation coefficient between calcium and IL-6 & TNF- a showed highly negative significant correlation (Fig.10& 12). Ertenet al. (2005) and Ghobrialet al. (2013) showed that the mean predialysis serum level of IL-6 and TNF-a were significantly higher in HD patients compared to healthy subjects. Tumor necrosis factor alpha (TNF-a) is a potent cytokine which is secreted by macrophages in response to lipopolysaccharide stimuli which mimic host invasion and mediates an array of immunological and metabolic responses (Charles et al., 1992).TNF-a has important effects on whole body lipid and glucose metabolism (Gokhanet al.,1997). It increases triglyceride and very low density lipoprotein levels as well as increasing hepatic lipogensis and lipolysis (Feingold and Grunfeld, 1992). In adipocytes, it decreases most of the lipogenic enzymes as lipoprotein lipase (Gokhan*et al.*, 1994). It also decreases glucose uptake in fat and muscle and increases the counter regulatory hormones as cortical and epinephrine (Lang *et al.,* 1992). Elevated serum levels of TNF-a in the nephropathic group may be to increased accumulation of advanced glycation end products (AGES) on glomerular basement membrane (Goji*et al.*, 1991).Plasma cytokine levels are related to a range of other aspects of vascular adhesion and coagulation. Since both TNF-a and IL-6 are involved in the regulation of acute-phase proteins (Gauldie*et al.*, 1990). it is plausible that a cause-and-effect relationship exists between elevated circulating cytokine levels and acute-phase reactants in CRF.

Irish *et al*., (1998) observed increased fibrinogen, reduced HDL cholesterol, and increased IL-6 in

patients with CRF, and suggested that this could predispose to vascular disease. Although many factors influence the inflammatory response and immune function once of cytokines could also contribute to increased production of acute-phase proteins. Furthermore, macrophage IL-6 secretion may be increased in CRF (Libetta*et al*., 1996). The inflammatory response is orchestrated by cytokines, especially tumor necrosis factor-a (TNF-a) and interleukin-6 (IL-6). Both regulate the production of acute-phase proteins, have potent effects on lipid and carbohydrate metabolism, and are linked with an increased risk of IHD in subjects with normal renal function (Mendall*et al*., 1997 &Jovinge*et al*., 1998).Pro-inflammatory cytokines also regulate vascular adhesion. TNF-a in particular promotes the expression of CAMs by endothelial cells (Bevilacquaet al., 1993). Further-more, increased plasma levels of soluble CAMs have been reported in CRF patients (Bonomini*et al*., 1998). Thus it is possible that cytokines could drive the increased levels of acute-phase proteins and CAMs observed in patients at risk of IHD.

A higher blood concentration of TNF-a in HD patient may be the result of monocyte activation by circulating endotoxin. However, decreased renal clearance of this cytokines, in correction with renal function damage, could be responsible for the rise in blood concentration. Hypoalbuminemia was associated with high serum concentration of soluble receptor P80 for TNF-a and IL-6 in HD outpatients with no concomitant diseases, malignancy, and liver disease or collagen disease (Mari *et al.*, 2002).Grazia*et al.* (2002) suggest that IL-1B, TNF- a and IL-6 acting synergistically, contribute to a monocyte activation that is also inversely correlated to dialytic age. They confirmed that the enhanced basal secretion of cytokines and the impaired ability of monocytes from HD patients to respond to stimulation. The progressive impairment of monocytes increased with dialytic age, supporting the fact that prolonged haemodialytic treatment with cuprophane might be responsible for the poor immune response and may contribute to the deterioration of the immunodeficient status of HD patients.Tarakcioglu*et al*. (2003) demonstrate that a hemodialysis session markedly decreases IL-8, which is significantly affected by predialysis concentrations, indicating that removal of IL-8 is a concentration gradient dependent action, but does not change the serum levels of IL-1 beta, SIL-2R, IL-6, and TNF- a, underlining importance of the structural characteristics of the molecules.

Trying to compare patient group with compromised growth to those with fair growth, it was found that those with fair growth showed lower creatinine, higher GFR, higher serum Ca level, lower ALP, lower serum cholesterol and higher HB level. This comes in agreement with many studies (Kari *et al*., 2000 & tom *et al*., 1999) who found that the combination of increased dialysis and adequate nutrition promote normal growth with CRF. deGrooteet al.(1989) stated that many septic patients don't have elevated or even detectable TNF- a levels. Bone, (1991) documented also that too many patient with many disorders, have elevated TNF- a levels without circulatory collapse, and too many patient with sepsis have no or only low TNF- a are not a prerequisite for the development of sepsis.Another explanation was given by (Barrier and Lowry, 1995) who indicated that TNF-a detection in a given population is inconsistent and depends on the sensitivity of the assay used timing of the sampling in relation to the insult. Also, they found that local release of TNF- a by activated cells may not be reflected in circulating levels and that the variability of TNF- a assays may well account for much of the disparity in results. Westendrop*et al*., (1997) stated that family studies show that up to 60% of the variability in TNF- a production between individuals may be genetically determined. Molving*et al.*, (1988) Stated that there is stable inter- individual variation in levels of production of TNF- a suggesting inherited individual difference, this can be explained by the location of TNF- a within the MHC region on chromosome (6) that is a highly polymorphic region and the TNF- a itself contains a large number of polymorphism as described by (Hajeer and Hutchinson, 2002).Solariet al. (2004) suggest that patient with vericoureteral reflux and the cytokine TNF-a AA genotype may have increased susceptibility to reflux nephropathy.

NITRIC OXIDE INCHRONIC RENAL FAILURE:

In the present study, nitric oxide showed highly significant decrease concentration in chronic renal failure Egyptian children as compared to healthy control group (Table 2). Correlation coefficient between urea &creatinine and nitric oxide showed highly negative significant correlation (Fig. 13&14). Correlation coefficient between sodium and nitric oxide showed highly positive significant correlation (Fig. 15), but correlation coefficient between potassium and nitric oxide showed highly negative significant correlation (Fig.16). Correlation coefficient between phosphorous and nitric oxide showed highly negative significant correlation (Fig.17), but correlation coefficient between calcium and nitric oxide showed highly positive significant correlation (Fig.18). Goonasekera*et al*. (1997) and Ghobrial*et al*. (2013) proof that higher nitric oxide level was associated with a lower glomerular filtration rate (GFR) in normal children and in children with hypertension due to renal failure. The tendency of NO concentration in the plasma to decrease with age in normal children, may be a reflection of age related increase in (GFR). Goonasekera*et al*., and Higashi *et*

al., (1997) did not find any significant correlation between NO level and the age of the child. Shannon et al. (2000) showed that urinary nitrate levels are significantly decreased with a serum creatinine ≥1 0.Nitrate and nitrite are excreted by the kidney, and thus, renal function and clearance should be considered if they are intended to be used as marker of NO production.

Mean plasma nitrate is approximately three fold to four fold higher than normal in patients with renal disease prior to dialysis as described by (Ellis *et al*., 1998).Endogenous inhibitors of NOS, such as symmetrical and asymmetrical dimethylarginine, are increased in patients with low glomerular filtration rates, and thus NOS products such as nitrate may be decreased in the patient's urine (Ellis *et al*., 1998).Most workers have found elevated serum nitrate levels with transplant rejection or graft versus host disease, with infection, nitrate has been reported to be elevated by (Ioannidis *et al*., 1995) or unchanged by (Weiss *et al*., 1995).Boger, (2004) stated that, both asymmetric dimethylarginine (ADMA) and its regioisomer, symmetric dimethylarginine, are eliminated from the body by renal excretion. Plasma ADMA levels are increased in humans with hypercholesterolemia, atherosclerosis, hypertension, chronic renal failure, and chronic heart failure. Increased ADMA levels are associated with reduced NO synthesis as assessed by impaired endothelium – dependent vasodilatation. Boger andCherla*et al.* (2004) proved that administration of L-arginine has been shown to improve endothelium – dependent vascular function in subjects with high ADMA levels, and L-arginine has a protective role in ischemic acute renal failure.

Accumulation of ADMA (a competitive inhibitor of NOS) in patients with chronic renal failure may be responsible for up regulation of low density lipoprotein receptor-1 (Lox-1) and increased oxidized LDL uptake, thus contributing to lipidosis and foam cell formation. The date illustrate and additional nonendothelial mode of antiatherogenic action of NO prevention of Lox-1 induction and lipid accumulation macrophages (Smirnova*et al*., 2004).Elevated ADMA levels, i.e. in patients with renal failure, may be responsible for endothelial accumulation of oxidized LDL (ox LDL) via up regulated Lox-1receptor, thus contributing to endothelial lipidosis and dysfunction (Smirnova*et al*., 2004).

REFERENCES

1.Arai, K. Nishida, J. and Yokota, T. 1990b. Co-ordinate regulation of immune and infammatory responses by cytokines. Rinsho. Byri, Abs., 38: 347.
2.Arieff, A. and Defronzo, R. 1995. Fluid, Electrolyte, and Acid-base Disorders, 2nded. New York: Churchill livingstone; 643.
3.Bahaa EI-Din, HK. El-Naggar, M. Abd El-Salam, A. Anwar, O. and Maher R. 1991. Chronic renal failure in pediatric ICU over a period of four years: Etiology, clinical features, and outcome. (Medical Journal of Cairo University Vol. 5A, No.2: 479-489.
4.Bamett, RN. et al.1973. the quantitative determination of calcium in serum, plasma and urine. Amer. J. Clin.Path. 69:836
5.Barriere, S.L. and Lowry, S.F. 1995. An overview of mortality Risk prediction in sepsis. Crit. Care Med. 23: 376- 93.
6.Behrman Richard, E. Kliegman Robert, M. and Jenson Hal, B. 2000. (Nelson Text Book Of Pediatrics, 16 TH Edition 1609-1612) WB Saunders Company.
7.Bevilacqua, M.P. 1993. Endothelial-leukocyteadhesionmolecules. Annu. Rev. Immunol. 11: 767-804.
8.Boger, R.H. 2004. Asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, explains the L-"arginine paradox"and acts as a novel cardiovascular risk factor. J Nutr. Oct; 134(10 Suppl.): 2842S-2847S; discussion 2853S.
9.Bone, R.C. 1991. Pathogenesis of sepsis. Ann. of Internal Medicine, 115: 457- 69.
10.Bononmini, M., Reale, M. Santarelli, P. and Stuard, S. 1998. Serum levels of soluble adhesion molecules inchronic renal failure and dialysis patients. Nephron 79: 399-407.
11.Chantler, C. and Holliday, M. 1988. (Progressive loss of renal function in pediatric nephrology., 773-804 Williams & Wilkins.
12.Charles, H.L. Cormel, D. and Gregory, J.B. 1992. Tumor necrosis factor impairs insulin action on peripheral glucose disposal and hepatic glucose output, Endocrinology, 130 (1): 43 – 52.
13.Cherla, G. and Jaimes, E.A. 2004. role of L-arginine in pathogenesis and treatment of renal disease.J. Nutr. Oct; 134 (10 Suppl.): 2801S-2806S; discussion 2818S.
14.Chi-Yuan Hsu, Glenn M Chertow, Gary C Curhan. Methodological issues in studying the epidemiology of mild to moderate chronic renal insufficiency. Kidney International, 2002; 61(5) 1567–1576 ICID: 588428.
15.Chi-Yuan, Cummings, Steven R., Mcculloch, Charles E., Chertow, Glenn M. Bone mineral density is not diminished by mild to moderate chronic renal insufficiency.Kidney International, 61: 1814-1820.

16.deGroote, M.A. Martin, M.A .Densen, P. Pfaller, M.A. and Wenzel, R.P. 1989. Plasma tumor necrosis factor levels in patients with presumed sepsis. Results in these treated with anti-lipid antibody vs. placebo. JAMA. 262(2): 249-51.
17.Drupt, F. 1974. Determination of albumin spectrophoto- metric bromocresol green pharm .Boil. 9, P: 777.
18.Ellis, G. Adatla, I. Yazdanpanah, M. and Marela, S. 1998. Nitric and nitrate analyses: A clinical biochemistry perspective. Clin. Biochem 31: 195-220.
19.El-Nahas, A. M. and Tamimi, N. 1999. The progression of renal failure: a harmful quartet. Q.J Med 92: 421-424.
20.Feingold, K.R. and Grunfeld , C. 1992. Role of cytokines in inducing hyperlipidemia,Diabetes, 41 (Suppl. 2): 97-101.
21.Gauldie, J. Northeman, W. Fey, GH. et al. 1990. IL-6 function as an exocrine hormone in inflammation. J. Immunol. 144: 3804-3808.
22.Ghobrial EE, Mahfouz N.N., Fathy G.A., Elwakkad A.A., Sebaii H.M.. 2013. Oxidative stress in Egyptian hemodialysis children. Iran J Kidney Dis. 2013. 7(6): 485-91.
23.Goh, C.R. 1990. Tumor necrosis factor in clinical practice. Am. Acad. Med. Singapore. Vol. 19: 235-39.
24.Goji, H. Koj, I.N. Manabu, S. Manabu, Kazuko, U. Yoji, S. Kazuharu, I. and Motyoharu, K. 1991. Possible role of tumor necrosis factor and interleukin- 1 in the development of diabetic nephropathy. Kidney International, 40: 1007-1012.
25.Gokhan, S.H Adriane, B., David, M.and Bruce M.S 1994. Reduced tyrosine kinase activity of the insulin receptor in obesity diabetes: central role of tumor necrosis factor αJ. Clim. Invest., 94: 1543 – 1549.
26.Gokhan, S.H., Peter, A., Richard, L.A. and Bruce M.S. 1997. Differential regulation of the P80 tumor necrosis factor receptor in human obesity and insulin resistance diabetes, 46(3): 451-455.
27.Goonasekera, C. D. A. Shah, V. Rees, D. D. and Dillon, M. J. 1997. NO activity in childhood hypertension. Archives of diseases in Childhood 77: 11 – 16.
28.Gornall, A. et al. 1968. Colorimetric determination of total protein. J. Boil. Chem. 177: 751.
29.Granger, D.L. Hibbs, J.B. Perfed, J.R. and Durack, D.T. 1990. Metabolic fate of L-arginine in relation to microbiostatic capability of murine macrophages. J. Clin. Invest., 85:264-273.
30.Grazia, M. Valentina, B. Pasquale F. and Francesco, R. et al. 2002. IL – 1B, TNF- αand IL-6 release from monocytes in haemodialysis patients in relation to dialytic age.
31.Guarnieri, G. Antonione, R. and Biolo, G. 2003. Mechanisms of malnutrition in uremia. J. Ren. Nutr. 13(2): 153-7.
32.Hajeer, A.H. Hutchinson, I.V. 2000. TNF- alpha gene polymorphism: clinical and biological implications. Microsc. Res. Tech., 50(3): 216- 28.
33.Henry,R.J.(1974):Clinical chemistry, principle and technics, 2and edition, Harper and Row, p.525.
34.Higashi, Y. Oshima, T. Ozono, R. Matsuura, H. and Kajiyama, G. 1997. Aging and severity of hypertension attenuate endothelium- Dependent renal vascular relaxation in humans. Hypertension. 30(2 pt 1): 252-8.
35.Hsu,Cy and Chertow, GM. 2002. Elevations of serum phorus and patassium in mild to moderate chronic renal insufficinecy. Nephrol Dial Tramsplant 17(8): 1419-25.
36.Irish, A. 1998. Cardiovascular disease, fibrinogen and acute phase response association with lipids and blood pressure in patients with chronic renal disease. Atherosclerosis, 137: 133-139.
37.Jovinge, S. Hamsten, A. and Tornvall, p. et al. 1998. Evidence for a role of tumor necrosis factor alpha in disturbances of triglyceride and glucose metabolism predisposing to coronary heart disease Metabolism, 47: 113 118.
38.Kari, J.A. Gonzalez, C Ledermann, S.E. Shaw and Ress, L. 2000. Outcome and growth of infants with severe chronic renal failure. Kidney Int., 57(4): 1681-7.
39.Kita, Y. et al. 1994. Evaluation of sequential serum interlukine-6 level in liver allograft recipients. Transplantation, 57: 1037-1041.
40.Kone, B.C. 2004. Nitric oxide synthesis in the kidney: isoforms, biosynthesis, and functions in health.
41.Lagomrarsimo, E. Valenzuela, A. Cavagnaro, F.andSolar, E. 1999. Chronic renal failure in pediatrics.Chilean survey. Pediatric Nephrology 13(4): 288-291.
42.Lang, C. H. Dobrescu, C. and Bagby, G.J. 1992. Tumor necrosis factor improves insulin action on peripheral glucose disposal and hepatic glucose output. Endocrinology, 130: 43-52.
43.Ledger, S. 2006. Laboratory assessment of kidney function. Cannt J. Apr-Jun; 16(2): 34-7; quiz 38-9.
44.Liach, F. 1995. Secondary hyperparathyroidism in renal failure: the trade off hypothesis revisited Am J Kidney Dis 25: 663- 679.
45.Liach, F. and Massry, S.G. 1985. On the mechanism of secondary hyperparathyroidism in moderate

renal insufficiency, J ClinEndocrinolMetab 61: 601- 606.
46.Libetta, C., De Nicola, L., Rampino, T. et al. Inflammatory effects of peritoneal dialysis: evidence of systemic monocyte activation. Kidney Int. 1996; 49: 506–511
47.Mahdavi, H. Kuizon, BD. Gales, B. et al. 2003. Sevelamer hydrochloride; an effective phosphate binder in dialyzed children, pediatrNephrol., 18: 1260.
48.Manogue, K.R. 1991. The molecular structure of TNF. From the Cytokine Handbook, Thomson A.W., ed., Academic Press. London, pp 241-256.
49.Mari, O. Akihiko, K. Takako, T. and Mitsuyoshi, F. et al. 2002. Role of soluble receptors for tumor necrosis factor alpha in the development of hypoalbuminemia in hemodialysis patients. Am. J. Nephrol., 22: 73-80.
50.McColl, J.L. and Parry, BR. 1990. Tumor necrosis factor n surgical illness. Aust. N.Z.J. Surg., (60): 503.
51.Mendall, M., Patel, P., Ballam, L., Strachan, D., & Northfield, T. (1996). C-reactive protein and its relation to cardiovascular risk factors: A population based cross sectional study. British Medical Journal, 312: 1049-1050.
52.Monkawa, T. Yoshida, T. Hayashi, M. et al. 2000. Identification of 25- Hydroxyvitamin D3 1a-hydroxylase gene expression in macrophages. Kidney Int., 58: 559- 568.
53.Oppenheim, J.J. Ruscetti, F.W. and Faltynek, C. 2001. Cytokines. In: Basic and Clinical Immunology, 7th Ed. By Stites, D.P. Terr. A.I. Appletar and large. Norwalk, Connecticut/California p. 78-99.
54.Papadopoulou, Z. L. 1989. Chronic renal failure. Renal Disease In Children: Clinical Evaluation And Diagnosis, 286-305.
55.Patton, C.J. and Crouch, S. R. 1977. Spectrophotometric and kinetic investigation of the Berthelot reaction for the determination of ammonia. Analytical Chemistry 49:464–469.
56.Portal, AA. Booth, BE. Halloran, BP. and Marris, RC. JR. 1984. Effect of dietary phosphorus on circulating concentration of 1.25 dihydroxyvitamin D and immumoreactive parathyroid hormone in children with moderate renal insufficiency. J Clin invest; 74: 1580- 1589.
57.Portale, AA. and Morris, RC. JR. 1991. Pathogenesis of secondary hyperparathyroidism in chronic renal insufficiency. Miner Electrolyte Metab., 17: 211-220.
58.Potter Gregg S., Johnson Ron J., and Fink Gregory D. 1997. Role of endothelin in hypertension of experimental chronic renal failure. Hypertension, 30: 1578-1584.
59.Rysz,J., Banach M.et al. 2006.Blood serum levels of Il-2,IL-6, IL-8, TNF-alpha and IL-1beta in patients of maintenance hemodialysis. Journal of Cellular and Molecular Immunology,, 3:151-4
60.Schmidt, D.M. et al. 1992.The C-terminus of the succinate dehydrogenase IP peptide of Saccharomyces cerevisiae is significant for assembly of complex II. Biochemistry 31(36):8442-8
61.Shannon, D.S. Marcia, A.W. Marc, I.L. and Robert, M.W. 2000. Temporal change of cytokines and nitric oxide products in urine from renal transplant patients Kid. Int., 58:829-837.
62.Smirnova, I. V., Kajstura, M., Sawamura, T. and Goligorsky, M. S. 2004. Asymmetric dimethylarginineupregulates LOX-1 in activated macrophages: role in foam cell formation. Am. J. Physiol. Heart Circ. Physiol.287, H782-H790
63.Solari, V. Enmis, S. Cascio, S. and purip. 2004. Tumor mecrosis Factor – alpha gene polymorphi reflux mephropathy. J urol. 172 (4pt2): 1604 - 6.
64.Tarakcioglu, M. Erbagic, AB. Usalan, C. and Deveci, R. et al. 2003. Acute effect of hemodialysis on serum levels of the proinflammatory cytokines- Mediatiorsinflamm, Fed., 12(1): 15-9.
65.Theresa, L. 1994. Cytokine measurements and interpretations of cytokine assay in human disease. J.Clin.Immunol., 14: 327-339.
66.Tom, A. McCauley, L. Bell, L. Rodd, C. and Espinosa, P. 1999. Growth during maintenance hemodialysis: impact of enhanced nutrition and clearance. J. Pepiatr. 134(4): 464-471.
67.Tracey, K.J. Vlassara, H. and Germai, A. 1989. Cachectin/tumor necrosis factor. Lancet. 1: 1122-26.
68.Weiss, G. Schwaighofer, H. Herold, M. Nachbaur, D. Wachter, H. Niederwifser, D. Werner, ER. 1995. Nitric oxide formations as predictive parameter for acute graft- versus-host disease after human allogenic bone marrow transplantation, transplantation 60: 239 – 1244.
69.Wilson, L. Felsenfeld, A. Drezner, MK. and Liach, F. 1985. Altered divalention metabolism in early renal failure: role of 1.25 (OH)2 D. Kidney Int., 27: 564 – 573.
70.Yang, Y. Yelavarthi, K.K. Chen, H.L. Pace, J. L. Terranova, P. F. and Hunt, J.S. 1993. Molecular, biochemical and functional characteristics of TNF- αproduced by human placental cytotrophoblastic cells. J. Immunol. 15; 150 (12): 5614-24.
71.Young, DS. 1991. Effect of drugs on clinical laboratory tests. 3ed ed., AACC press, Wacshington DC.