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Neutrophil Gelatinase-Associated Lipocalin And Omentin As Biomarkers Of Chronic Kidney Disease.

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ABSTRACT

The prevalence of End Stage Renal Disease (ESRD) has been increasing worldwide. It is an important health problem which causes high mortality and morbidity. The main pathophysiologic mechanism underlying the high mortality of ESRD is premature atherosclerosis and chronic inflammation. A large number of biomolecules have been shown to predict the atherosclerotic and inflammatory process in ESRD. To investigate the level of Omentin and Neutrophil gelatinase - associated lipocalin (NGAL) in patients with ESRD receiving hemodialysis. The present study included 60 patients with end stage renal disease(ESRD)in addition 30normal healthy individuals were enrolled in this study as control group. This study revealed that there was high significant with the mean level of Omentin 1 concentration in ESRD group (488,38 ± 371,08 $p< 0.001$) in compared to healthy group, also there was significant elevation in the mean level of NGAL (902,90 ± 698,35 $p< 0.001$). Omentin and NGAL were consider better markers of End Stage Renal Disease.

Keywords: End Stage Renal Disease (ESRD), NGAL, Omentin .

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INTRODUCTION

Chronic kidney disease (CKD) is a devastating illness with an incidence and prevalence rapidly approaching epidemic proportions worldwide (Daijun *et al*., 2014).

CKD is progressive loss in kidney function over a period of months or years. The symptoms of kidney function are not specific, and might include feeling generally unwell and experiencing a reduced appetite. Often, chronic kidney disease is diagnosed as a result of screening of people known to be at risk of kidney problems, such as those with high blood pressure or diabetes and those with a blood relative with CKD. It is differentiated from acute kidney disease (acute kidney injury) in that the reduction in kidney function must be present for over 3 months. CKD is an internationally recognized public health problem affecting 5–10% of the world population. (Martínez *et al.,* 2014).

Traditional blood markers and urinary markers of kidney injury have been used for decades in clinical studies for diagnostic and prognostic purposes. For prevalent nephropathies such as diabetic kidney disease, they remain as useful clinical markers of renal injury and even of CKD progression. However, considering the variety of causes that lead to kidney damage, these markers seem to be neither sensitive nor specific, mainly in the early disease stages (Waikar *et al*., 2009).

Additionally, these markers do not directly reflect injury to renal cell signaling pathways and internal organization. Unlike cardiac troponin, most of these markers represent functional and late consequences of injury, and do not explain the type and intensity of aggression that is taking place in the renal parenchyma. Thus, new biomarkers of renal injury and function are required in clinical practice to permit accurate diagnosis, guide treatment, and predict disease progression (Wasung *et al*., 2015). However, a single biomarker may not be adequate to capture the spectrum of CKD, given the inherent heterogeneity of renal structure and the diverse settings under which kidney injury occurs (Schiffi and Long 2012).

Creatinine is a breakdown product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body depending on muscle mass Creatinine is a commonly used as measure of kidney function (Yuegang *et al.,* 2008) ..

The creatinine clearance test is used to monitor the progression of renal disease. The diagnosis of renal failure is usually suspected when serum creatinine is greater than the upper limit of the “normal” interval. In chronic renal failure and uremia, an eventual reduction occurs in the excretion of creatinine by both the glomeruli and the tubules (Edmund *et al*., 2006).

Cholesterol is required to build and maintain membranes; it modulates membrane fluidity over the range of physiological temperatures. The hydroxyl group on cholesterol interacts with the polar head groups of the membrane phospholipids and sphingolipids, while the bulky steroid and the hydrocarbon chain are embedded in the membrane, alongside the nonpolar fatty acid chain of the other lipids. Through the interaction with the phospholipid fatty acid chains, cholesterol increases membrane packing, which reduces membrane fluidity (Sadava *et al.,* 2011).

Serum albumin is the most abundant blood plasma protein and is produced in the liver and forms a large proportion of all plasma protein. It normally constitutes about 60% of human plasma protein, being synthesized primarily by hepatic parenchymal cells except in early fetal life, when it is synthesized largely (Fried man *et al*., 2010).

The serum enzymes of patients with ESRD are commonly abnormal. This is due in part to the absence of renal excretion and to the frequent presence of multiple comorbid conditions. The serum enzymes most commonly used to assess the diagnosis of hepatobiliary disorders include the alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). ALT and AST are routinely measured to assess liver functions in patients with and without renal failure (Lomashvili *et al*., 2008).

Omentin (34 kDa) is a recently identified fat deposition specific adipokine codified by two genes (1 and 2). It is considered to be highly and selectively expressed in visceral omental adipose tissue (AT). Omentin is predominantly expressed in the AT stromal vascular cells, however, it is also expressed in the heart (epicardial

fat), lungs, ovary and placenta, but in these organs, (Tan *et al*., 2010). Omentin 1 is the major circulating form of omentin and its biological role is still not well known (Zhong *et al*., 2011). The reason of increased level of omentin related to impaired renal clearance , and defective degradation and excretion, and this agreed with few studies which found higher levels of omentin in end stage renal disease patients. This may due to the size of omentin which is relatively large protein, which during hemodialysis may not be cleared from plasma most adipokines are elevated in patients with chronic kidney disease (Al Celik *et al*., 2012) .

NGAL (also known as human neutrophil lipocalin, lipocalin-2, siderocalin, 24p3, or LCN) is a small molecule of 25 kDa having 178 amino acids that belongs to the superfamily of lipocalins (Yim *et al*.,2014) which are proteins specialized in binding and transporting small hydrophobic molecules. NGAL was originally isolated from the supernatant of activated neutrophils (Jain *et al*., 2016) and identified as apolypeptide covalently bound to gelatinase -. NGAL is also normally expressed at very low levels in several human tissues, including the kidneys, lungs, stomach, and colon, (Bianca *et al.,* 2016).

Injured kidney tubular cells produce and secrete several biological substances associated with innate and acquired inflammatory immune responses, including NGAL (sanjeevani *et al*.,2014).NGAL was one of the top upregulated genes in damaged kidneys and fulfilled the criteria of apromising biomarker of tubular damage following the start of acute kidney injury . NGAL is systemically synthesized in response to kidney damage Hence, NGAL appears to deliver a 'real-time' assessment of tubular injury. Depending on finding in mice and patients, a model of NGAL trafficking across the nephron was developed (schemidt-Ott *et al.,* 2011). The circulating NGAL is freely filtered, under physiological conditions, through the renal glomeruli (as a protein with low-molecular-weight and positive charge) and reabsorbed in the proximal tubule. Thus low levels of plasma NGAL is predictable in the absence of kidney disease (Watanabe *et al.,*2014).

SUBJECT, MATERIALS & METHODS

This study was conducted on 90 Egyptian individuals classified into two different groups as the following: **groups 1** consist of 60 patients with End stage renal disease from Urology center, Mansoura University, and **groups 2** consist of 30 healthy individuals with no kidney disease.

Blood samples were collected from all patients and healthy control group Common biochemical parameters, including Creatinine , Ca , Hb , FBS , Na , K , Albumin , ALP , AST and Cholesterol were measured at baseline in all patients, according to standard methods in the routine clinical laboratory.

Creatinine was determined by the method of (Henry *et al.,*1974) by using a commercially available kit (Spinreact, Co., Spain).

Serum sodium and potassium were estimated by flame photometer. Serum calcium was estimated by titration method using ethylene diamine tetracetic acid. Serum phosphate was estimated by colorimeter using Fiske and Subbarow method.

Hemoglobin concentration (Hb %) was determined by the method of (Doglas *et al.,*1984) by using a commercially available kit.

Cholesterol was determined by the method of (Meiattini *et al*.,1978) by using a commercially available kit (Spinreact, Co., Spain).

Albumin was determined by the method of (Doumas *et al.,*1971) by using a commercially available kit (Spinreact, Co., Spain).

Alkaline Phosphatase (ALP) activity was determined by colorimetric method of Belfield and Goldberg,(1971) using assay colorimetric kit (Spectrum , Egypt).

Serum aspartate aminotransferase (AST) activity was determined by colorimetric method of Reitman and Frankel,(1957) .using assay colorimetric kit (In vitro, Egypt).

NGAL was measured in the blood using a commercial available ELISA kit (Antibody Shop, Gentofte, Denmark), according to the manufacturer's instructions. All specimens were diluted often to obtain concentration for the optimal density according to the ELISA kit instruction.

Serum omentin was determined using the immunoenzymatic method with the application of the Human omentin kit (BioVendor, Czech Republic).

Statistical Analysis

Data were analysed using SPSS version 16.0 for Windows (Statistical Package for Social Science, Inc., Chicago, IL, USA), (Levesque, 2007) was used in the analysis. Frequency and percentage are presented for qualitative variables. Significance level (p) value was expressed as follows: p > 0.05 = Insignificant, p < 0.05 = Significant and p < 0.001 = highly significance. One-way ANOVA test was used for comparing between different groups .

RESULTS

The mean value of Creatinine in negative control was found to be 0.58±0.13 and this was increased to 9.84±3.34 (p<0,001) in ESRD patients. the mean value of K in negative control was found to be 4.14±0.43 and this was increased to 5.10+_0.69 (p<0,001) . The mean value of PO_4 in negative control was found to be 3.43±o.64 and this was increased to 4.80±1.69 (p<0,001)inESRD patients. The mean value of ALP in negative control was found to be 80.83±23.45 and this was increased to 195.91±132.19 (p<0,001). The mean value of albumin in negative control was found to be3.95±0.24 and this was decreased to 3.59|±0.31(P~0.001) in ESRD patients. The mean value of Hb% in negative control was found to be14.48±1.28 and this was decreased to11.0±1.32 (P<0.001) The mean value of Ca in negative control was found to be 9.85±0.52 and this was decreased to 8.01±0.82 as shown in Table (1).

Table 1: Clinical and hematological characteristics of all studied groups

Test	Groups		P value
	Healthy (I) (N=30) mean±SD	ESRD (II) (N=60) mean±SD	
Creat (mg/dl)	0.58± 0.13	9.84± 3.34**	0.000
FBS (mg/dl)	98.80± 26.32	111.46± 86.40	0.436
Na (mEq/L)	137.43± 2.0	136.05± 4.51	0.114
K^+ (mg/dl)	4.14± 0.43	5.10± 0.69**	0.000
Ca (mg/dl)	9.85± 0.52	8.01± 0.82**	0.000
PO (mg/dl)	3.43± 0.64	4.80± 1.69**	0.000
Hb% (g/dl)	14.48± 1.28	11.0± 1.32**	0.000
ALP (U/L)	80.83± 23.45	195.91± 132.19**	0.000
ALB (g/dl)	3.95± 0.24	3.59± 0.31**	0.000
ALT (U/L)	15.83± 6.43	13.36± 6.52	0.093
AST (U/L)	21.1± 5.98	18.43± 5.08*	0.030
Chol. (mg/dl)	187.56± 38.27	184.43± 42.37	0.734

P-value < 0.05 is considered significant, where P*<0.05 and **<0.001 compared to control group.

Omentin was increased in ESRD patients compared with healthy control group Mean ± SD .488.38±371.08 and78.71±121.56 (P<0,001), NGAL was increased in ESRD patients compared with healthy control group Mean±SD .902.90±698.35 and147.99±128.24 (P<0,001) as shown in Table 2 .

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Table 2: Omentin-1 and NGAL in all studied groups

Test	Groups		P value
	Healthy (I) (N=30) mean±SD	ESRD (II) (N=60) mean±SD	
Omentin-1 (ng/ml)	78.71± 121.56	488.38± 371.08***	0.000
NGAL (ng/ml)	147.99± 128.24	902.90± 698.35***	0.000

P-value < 0.05 is considered significant, where P*<0.05, **<0.01 and *<0.001 compared to control group.**

In this study, Receiver Operating Characteristic (ROC) analysis in ESRD patients revealed that serum NGAL continued to be the best predictor of CKD with a sensitivity of %100 and specificity of % 80 with Auc of %0.957 and Omentin showed a sensitivity of %95 and specificity of %60 with Auc **%**0.913 as shown in fig (1).

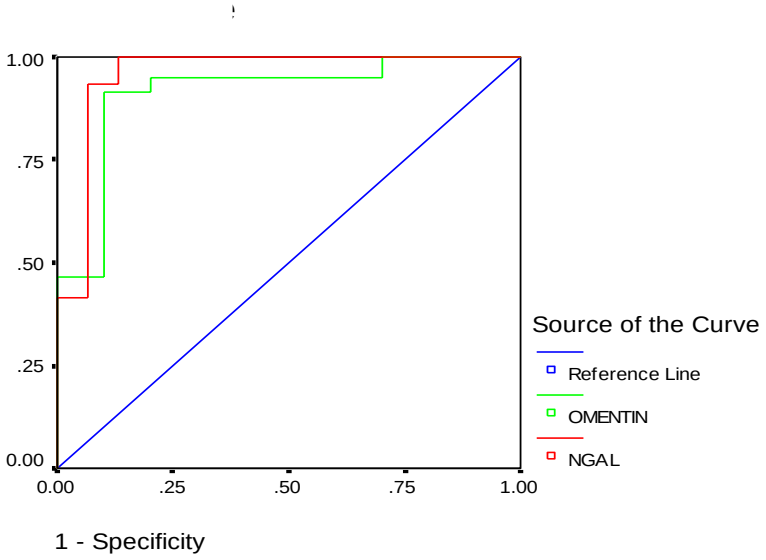

Fig 1: ROC curve for serum NGAL levels (ng/ml) and serum omentin levels in ESRD patients

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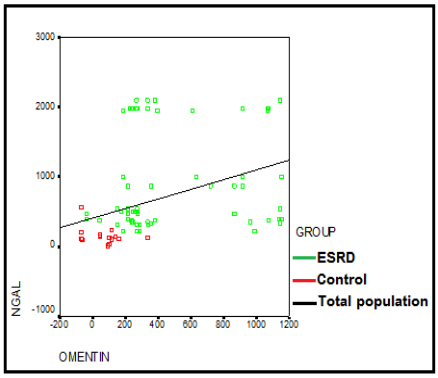
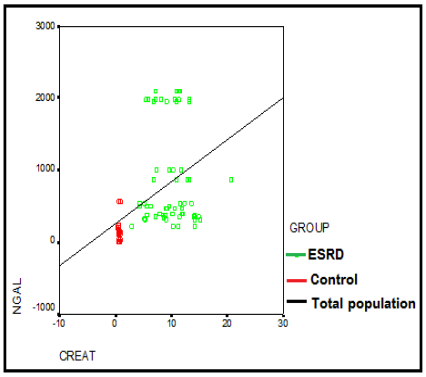
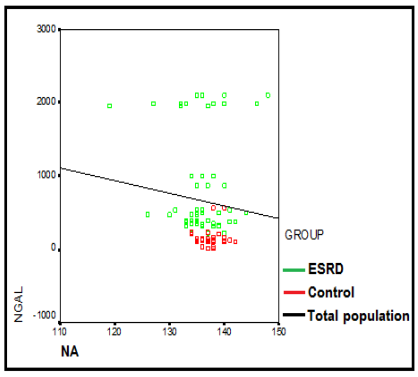


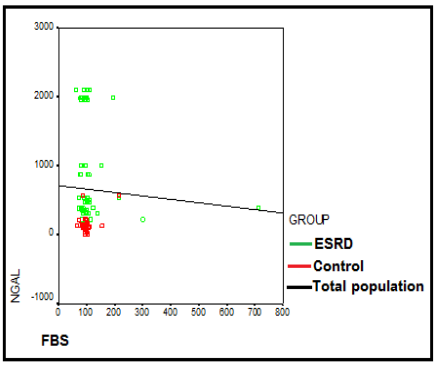
correlation between NGAL&OMENTIN



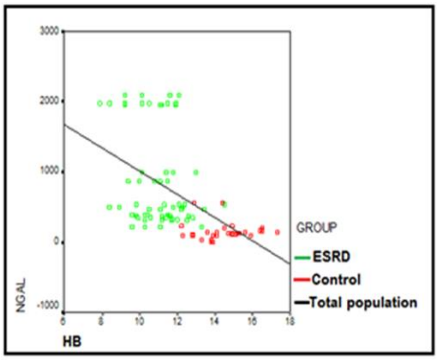
correlation between GAL&CREAT



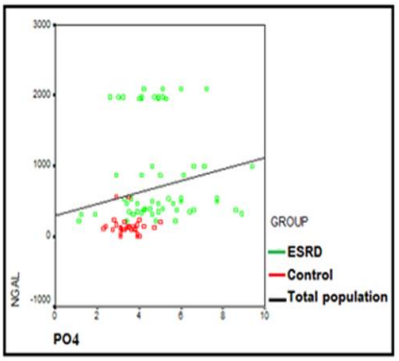
correlation between GAL&NA



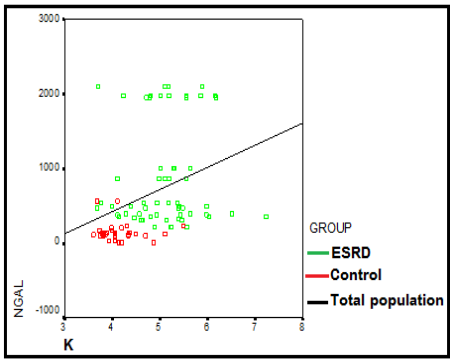
correlation between GAL&FBS



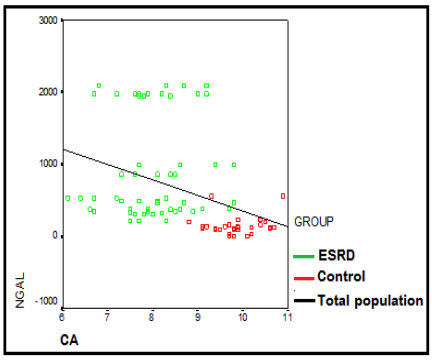
correlation between NGAL &HB



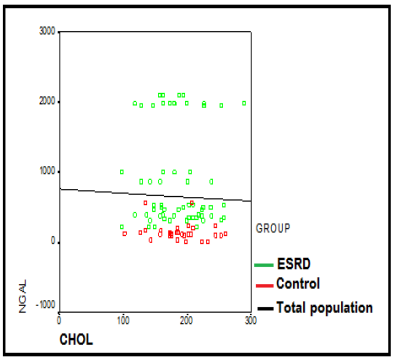
correlation between GAL&PO4



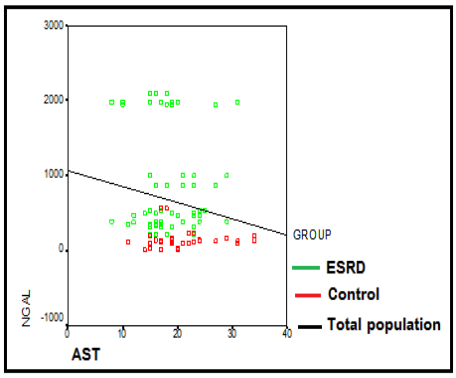
correlation between NGAL&K



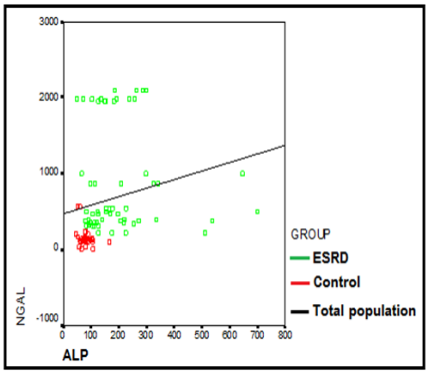
correlation between NGAL&CA



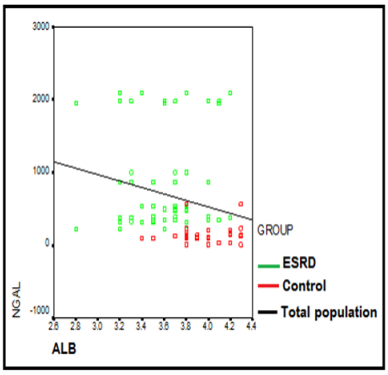
correlation between NGAL&CHOL



correlation between NGAL&AST



correlation between NGAL&ALP



correlation between NGAL&ALB

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DISCUSSION

There is concern in the nephrology community about strategies for early identification of kidney damage in patients at risk of developing CKD. CKD affects more than 10% of the general population in the U.S. alone and is associated with high rates of all-cause and cardiovascular mortality, imposing a high cost to the public health services. Serum and urinary biomarkers that have been employed for decades to detect renal dysfunction and damage, such as creatinine and proteinuria respectively, cannot capture accurately the extension of the injury within the glomerular, tubular, or interstitial compartments, nor can they predict disease progression, renal survival, and mortality. In this scenario, novel biomarkers of CKD that could reflect early kidney damage and thus provide a window for early clinical intervention have been sought intensively (Saran *et al*.,2016).

In this present study Creatinine was increased in end stage renal disease patients significantly compared with healthy control group Mean ± SD were 9.84±3.34 and 0.58±0.13 respectively (p<0,001) also K increased in end stage renal patients significantly compared with healthy control group Mean ± SD were 5.10+_0.69 and 4.14±0.43respectively (p<0.001) .Po4 was increased in end stage renal disease significantly compared with healthy control group Mean ± SD were 4.80±1.69 and 3.43±o.64 (P<0.001) ALP was increased in end stage renal disease patients compared with healthy control group Mean ± SD were 195.91±132.19 and 80.83±23.45 (P<0.001). In our study albumin was decreased in end stage renal disease patients compared with healthy control group Mean± SD were3.59|±0.31 and 3.95±0.24 (P~0.001) Hb% was decreased in end stage renal disease patients compared with healthy group Mean ± SD were 11.0±1.32 and 14.48±1.28 (P<0.001) .Ca was decreased in end stage renal disease patient compared with with healthy control group Mean ± SD were 8.01±0.82 and 9.85±0.52.

Decrease in serum Na+ concentrations between the CRF patients and control groups without statistically significant decrease result to reduce Na+ intake and humoral natriuretic factor in CKD which helps to increase sodium excretion and maintain normal Na+ balance. K+ concentrations a statistically significant increased in CRF patients the hyperkalemia is thought to result from the failure to follow dietary potassium restrictions and ingestion of medications that contain potassium, or from an endogenous release of potassium, as in case of trauma or infection. In other hands, our data shown significant decrease in serum Ca+ concentration in CRF patients and this interpreter the reduction of renal production of 1,25. Dihydroxycholecalciferol (active metabolites of vitamin D) and hence reduced the intestinal absorption of calcium and lead to hypocalcaemia as well as abnormalities of Ca, phosphate, parathyroid hormone (PTH), and renal osteodystrophy and decreased renal production of calcitriol contributes to hypocalcemia (Nicki *et al*., 2010).

Serum liver enzymes of patients with end-stage renal disease (ESRD) are commonly abnormal. This is due in part to the absence of renal excretion and to the frequent presence of multiple conditions. serum enzymes most commonly used to assess the diagnosis of hepatobiliary disorders include the alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

ALT and AST levels may be lower in ESR patients because of a deficiency in vitamin B6, which is a coenzyme of ALT and AST (Lopes *et al.,* 2009). Total cholesterol was assays in serum of patients with ESRD and the results indicated that there was a significant decreased ($P < 0.05$) in patients compared with control group which was (184.43 ± 42.37 mg/dl vs. 187.56 ± 38.27 mg/dl).

These results were similar to that found by (Korcagora *et al.,* 2003). The decrease of cholesterol concentration could be due to increased hepatic syndrum defective triglyceride removal(Shah *et al*., 1994).

Omentin is a novel hydrophilic adipokine of 313 amino acids (35 kDa), which contains a secretory signal sequence and a fibrinogen-related domain, and appears as a glycolized trimer of 120 kDa molecular weight in its negative form (Fain *et al*.,2008) . In this study Omentin was increased in ESRD patients compared with healthy control group Mean ± SD .488.38±371.08 and78.71±121.56 (P<0,001) this was in agreement with Al Celik who show significant elevation of serum omentin in End Stage Renal Disease .

Our results were in contrast to the study was done by Moreno-Navarrete *et al.,* (2011) who stated that Omentin levels were lower in patients with ESRD .

NGAL Was small 25-kD protein, belonging to the "lipocalins" superfamily, is massively released in blood from injured tubular cells after various conditions potentially detrimental to the kidney in experimental and human clinical models. No less important, NGAL release from renal tubule occurs soon after damage, notably preceding the rise in serum creatinine and thus allowing the initiation of preventive therapeutic measures in a timely manner. The NGAL molecule is freely filtered by the glomerulus and reabsorbed in the proximal tubules; its secretion in urine occurs after direct renal insults. In chronically damaged tubular cells, high quantities of NGAL are produced due to intracellular stress and protein overload, perhaps as a compensatory response to counteract intracellular complement-induced oxidative stress (Bolignano *et al.*,2008). In this study NGAL was increased in ESRD patients compared with healthy control group Mean±SD .902.90±698.35 and147.99±128.24 (P<0,001) this was in agreement with(Sin ger *et al.*,2013). who show significant elevation of serum NGAL in End Stage Renal Disease because of increasing distal renal tubular expression and synthesis of NGAL and NGAL secretion increasing . Our results were contrast to the study was done by Nickolas *et al.,* who stated that NGAL levels were lower in patients with ESRD .

CONCLUSION

We found NGALand omentin levels were elevated in patients with ESRD.NGAL and Omentin were useful markers for detection cronic kidney disease .

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