Serum Neutrophil Gelatinase-Associated Lipocalin (NGAL) in Acute Kidney Injury and Its Relation to Troponin T in cardiac and renal patients.

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**Background:** Neutrophil gelatinase-associated lipocalin (NGAL) might be investigated as a potential early and sensitive marker of kidney impairment/injury in order to select the appropriate strategy for reducing the risk in the setting of cardiorenal syndrome (CRS). NGAL is a 25 kDa protein covalently bound to matrix metalloproteinase-9 (MMP-9) from neutrophils, which has been shown to be highly increased in patients with acute and chronic renal injury in different clinical stages. **Aim of the work:** study the relation between NGAL, Troponin and AKI in cardiac non renal patients and renal non cardiac patients. **Subject and method:** 50 subjects classified into five groups: Group 1: 10 healthy control subjects. Group II:10 patients with Acute Decompensated Heart failure (ADHF). Group III:10 patient with acute myocardial infarction (ACS). GroupIV:10 patients with hypertensive encephalopathy (HTN). GroupV:10 patients with acute kidney injury due to toxic exposure (AKI). **Methods:** Routine investigations: Complete blood picture, Fasting and post prandial plasma glucose level, liver function tests, Lipid profile, serum urea and creatinine and urine analysis. Specific investigations: Measurement of NGAL (ELISA). Measurement of Troponin by ELFA (Enzyme-Linked-Fluorescent-Assay). **Results:** in AKI (acute kidney injury) high statistical significant difference betweenall studied parameter(NGAL1,NGAL2,Troponin1,Troponin2) among the different groups. NGAL1 was significantly higher in AKI group versus all other groups, also NGAL1 was significantly higher in ADHF than in control group. NGAL2 was significant higher in AKI and ADHF groups. Troponin1 and 2 was significantly higher in ACS versus all other groups. no significant correlation between NGAL1 and all other parameters in Control group and AKI group. Also there was non significant correlation between NGAL and Troponin i.e no relation between cardiac troponin and renal troponin, no statistical significant difference between NGAL1 and the different parameters in ACS group, HTN encephalopathy group, ADHF group, and control group. in AKI group NGAL1 positive correlate with NGAL2, and this prove high sensitivity of serum NGAL due to kidney affection although non significant correlation between NGAL and creat.1,2 and –ve correlation with GFR. no statistically significant difference between NGAL2 and the different parameters In ADHF group, in ACS group and in HTN encephalopathy group, except between NGAL2 and NGAL1 where there is positive correlation in ADHF patients. in AKI group no significant correlation was found between NGAL 2 and other parameters except that NGAL2 positive correlate with NGAL1. That explain NGAL is the early marker of (AKI) and not Troponin. **Conclusion:** NGAL 1&2 are good indicators of early renal affection in ADHF and AKI patients, but neither in ACS nor in HTN encephalopathy.
patients. NGAL had no relation to Troponin serum values in renal non cardiac patients and also in cardiac non renal patients.

Introduction:
Acute kidney injury (AKI) is diagnosed in 5% of all hospitalized patients and in up to 50% of all Intensive Care Unit (ICU) patients. In recent years a dramatic rise in the prevalence of AKI has been observed with virtually no change in mortality, reaching up to 50–80% in all dialyzed ICU patients. AKI may progress to end-stage renal disease, and even sub clinical episodes of AKI, which are common, may also progress to end-stage renal disease.

Renal dysfunction, as measured by decreased glomerular filtration rate (GFR), is common in chronic systolic heart failure (CHF) and is associated with severely increased mortality and morbidity, which is apparent even in the early stages of border line renal dysfunction.

In primary renal disease, renal impairment is not only associated with decreased GFR and increased urinary albumin excretion (UAE), but also with the presence of structural tubular damage, as measured by increased urinary concentrations of specific tubular marker proteins.

One of these markers is neutrophil gelatinase associated lipocalin (NGAL), which has been shown to be highly increased in patients with acute and chronic renal injury in different clinical stages.

NGAL is a member of the lipocalin family, NGAL is a 25-kDa monomer (polypeptide chain of 178 amino acids) associated with neutrophil gelatinase.

NGAL expression has been shown to be induced rapidly in renal tubules in response to acute injury. NGAL seems to be one of the earliest kidney markers of ischemic or nephrotoxic injury in animal models and is detected in the blood and urine of humans soon after acute kidney injury (AKI).

Zografos et al., (2009) reported that serum NGAL levels were significantly higher in the presence of Coronary artery disease (CAD) and correlated with its severity.

Also, in Heart Failure (HF), increased myocardial expression of NGAL might be one of the mechanisms for its prognostic value, independent of coexisting renal injury.

Troponin T, I and C are components of the contractile apparatus of striated muscle. Specific forms of troponin T and I are present in the heart muscle, namely cTnT and cTnI. After myocardial cell damage, cTnT are released from the myocytes and their levels are detectable 3–12 h after the injury, with the concentrations in direct proportion to the extent of myocardial injury. Mean time to peak cTnT level is approximately 12–48 h. The concentration returns to the normal range after 5–14 days. It has been established that elevated cardiac troponin T (cTnT) is commonly elevated in patients with end-stage renal dysfunction, regardless of myocardial necrosis.

This phenomenon represents a confounding factor for the interpretation of cTnT in the setting of acute coronary syndromes (ACS) in individuals with renal dysfunction. Moderate renal impairment is present in 40% of individuals with ACS.


Such finding warrants us to study relation between cardiac troponin T and renal troponin (NGAL) and their serum changes during cardiac insults which may affect the kidney.
Aim of the work:-
Study of NGAL in certain states supposed to develop AKI, also to study the relation between NGAL, Troponin and AKI in cardiac non renal patients and renal non cardiac patients.

Subject and methods:
This work carried out Internal Medicine ICU and clinical pathology departments, Faculty of Medicine, Zagazig University, during the period from Sept. 2013 to Dec.2015.

* Subjects:
50 subjects were included in this study and were classified into five groups:

**Group 1:** (GpI) Including 10 healthy volunteer control subjects.

**Group 2:** (GpII) Including 10 patients with Acute Decompensated Heart failure with history of chronic valvular heart disease. **Group 3:** (GpIII) including 10 patient suffering from acute myocardial infarction. **Group 4:** (GpIV) including 10 patient presented with hypertensive encephalopathy. They all showed history of complicated hypertension of primary type. **Group 5:** (GpV) including 10 patient presented with acute kidney injury due to toxic exposure.

* Methods: All subjects were subjected to Full history and thorough clinical examination.

**Collection of blood samples:**
6 ml of peripheral venous blood were taken from each subject under complete aseptic conditions the samples were left for spontaneous clotting then centrifuged at 3000 rpm for 5 minutes. Samples were separated and divided into 4 tubes for measurement of basal values of routine laboratory, serum creatinine, plasma NGAL. This process was repeated after 48 hours of the procedure.

**A) Routine investigations:** Complete blood picture, Fasting and post prandial plasma glucose level, Liver function tests, Lipid profile, serum urea and creatinine. Urine and stool analysis

**Calculation of glomerular filtration rate using MDRD equation**
\[
GFR \text{ (mL/min/1.73 m2) } = 175 \times (S.cr)^{-1.154} \times \text{Age}^{-0.203} \times (0.742 \text{ if female})^{16.}
\]

**B) Specific investigations:**

1-Measurement of NGAL by Enzyme Immunoassay (ELISA).

2-Measurement of Troponin by ELFA (Enzyme-Linked-Fluorescent-Assay).

**Statistical analysis:**
Data were analyzed with SPSS version 15.0 (statistical package for the Social Science, Chicago, IL). Quantitative data were expressed as mean±standard deviation (SD); data were analyzed by one way analysis of variance (ANOVA). While qualitative data were expressed as number and percentage and were analyzed by Chi square (X2) test. Correlation was done using Pearson correlation test-value was considered significant if <0.05, highly significant if <0.001 and non significant if >0.05.

**Results:**

<table>
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</tr>
<tr>
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</tr>
<tr>
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<td>10</td>
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<tr>
<td>P</td>
<td>0.97(NS)</td>
<td>0.63(NS)</td>
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</table>

Table (1) Demographic Data and classification of the study population using analysis of variance (A NOVA test) .
high statistical significant difference in between the means of all studied parameter (NGAL1, NGAL2, Troponin1, Troponin2) among the different groups of the study with p. value (<0.001) for all by applying (KRUSKAL WALLIS-LT) . NGAL1 was significantly higher in gpV versus all other groups, also NGAL1 was significantly higher in gpII than in control group.

NGAL2 was significant higher in gpV versus all other groups, also NGAL2 was significantly higher in gpII than control group with no significant elevation among gpIII and gpIV. It may be worthily mentioning here that NGAL2 was elevated similarly to NGAL1 in gpV and II but with higher values.

Troponin1 was significantly higher in gpIII versus all other groups and it did not differ in the other gps (II,IV and V) from the control value.

Troponin2 was significantly higher in gpIII versus all other groups and it did not differ in the other gps (II,IV and V) from the control value.
No significant correlation between NGAL1 and all other parameters in Control group.
in ADHF group no significant difference between NGAL1 and the different parameters except between NGAL1 and NGAL2 where there is positive correlation meaning that an early renal affection occurred in ADHF patient. Also NGAL and Troponin there was non significant correlation that in agreement with early kidney affection (AKI) not affect cardiac Troponin. i.e no relation between cardiac troponin and renal troponin.

In ACS group no statistical significant difference between NGAL1 and the different parameters except troponin2 where there is positive significant difference as clear Troponin normally increase in ACS.

In HTN encephalopathy group no statistical significant difference between NGAL1 and the different parameters. Positive non significant correlation between NGAL and serum creat.1,2 are expected also negative non significant correlation with GFR.

In AKI group no significant correlation was found between NGAL 1 and other parameters in (AKI) group except that NGAL1 positive correlate with NGAL2, and this prove high sensitivity of serum NGAL due to kidney affection although non significant correlation between NGAL and creat.1,2 and –ve correlation with GFR.

Also no significant correlation between NGAL and Troponin. i.e Troponin not sensitive to early kidney affection so Troponin cannot be a marker of Acute Kidney Injury (AKI)

In ADHF group no statistically significant difference between NGAL2 and the different parameters except between NGAL2 and NGAL1 where there is positive correlation meaning that an early renal affection occurred in ADHF patient.

In ACS group no statistical significant difference between NGAL2 and the different parameters except troponin2 where there is positive significant difference as clear Troponin normally increase in ACS.

In HTN encephalopathy group no statistical significant difference between NGAL2 and the different parameters. Positive non significant correlation between NGAL2 and serum creat.1,2 are expected also negative non significant correlation with GFR. in AKI group no significant correlation was found between NGAL 2 and other parameters in (AKI) group except that NGAL2 positive correlate with NGAL1. That explain NGAL is the early marker of (AKI) and not Troponin.

Discussion:-
NGAL seems to be one of the earliest kidney markers of ischemic or nephrotoxic injury in animal and is detected in the blood and urine of humans soon after acute kidney injury (AKI) (Haase et al., 2009) i.e. early detector and biomarker of acute kidney injury and is considered as one of renal Troponins. It has been reported that elevated cardiac troponin T (cTnT) is commonly found in patients with end-stage renal dysfunction, regardless of myocardial necrosis (Donnino et al., 2004). A highly significant increase in NGAL in acute kidney injury group (154ng/ml (98-168) at the base and 202 ng/ml (191-225) after 48h was found. These values came in consistence with the findings of Waikar and Bonventre (2006).

In the same group, serum NGAL1 level was also significantly higher than normal parallel to the level of serum creat1, creat2, blood urea and decreased GFR. Our results are supported by the results of Mori et al., (2005) and Haase et al.,( 2009) Who mentioned early elevation of serum NGAL in acute kidney injury but Nickolas et al. (2008), did not find superiority of NGAL level over serum creatinine level predicting subsequent AKI in ICU patients.

In group II of Acute Decompensated Heart Failure patients, although serum creat1, 2, urea and GFR did not show any renal affection, NGAL was found to be higher than normal indicating early renal function affection or acute kidney injury, but not failure. Our findings are consistent with the finding of Kevin et al., in 2008 and Mullens et al., (2009) who studied NGAL serum level in such patients and they referred this finding to the occurrence of low cardiac output which may lead to renal ischemia and renal tissue damage proved by increased serum NGAL.
Serum Troponin1, 2 were not found elevated in ADHF patients in our study however no data has been reported confirming serum Troponin elevation in ADHF. It may be worth mentioning that elevated serum NGAL in those patients did not correlate to serum Troponin values in the same patients denoting no relation between renal affection and cardiac Troponin levels in ADHF patients.

Serum NGAL values in ACS patients was not significantly different from the control subjects i.e. ACS does not cause Acute Kidney Injury (AKI). This is supported by the findings of Mikko H. et al., (2011)20.

In ACS patients serum NGAL1,2 although did not increase in values, it correlated positively to Troponin 2 but not Troponin 1, this may point to a delayed 48 h renal injury in such patients which was not found at admission. This supported by Devarajan (2008)21, But In paradox to Luis et al., (2010)22. We did not find significant changes in either NGAL1&2 or in Troponin 1&2 values among the group of hypertensive encephalopathy patients that may point to no acute kidney or cardiac injury in such patients, in contrary to Jolanta et al., in 200823, who Concluded that Hypertension is associated with kidney injury as reflected by elevated serum NGAL and cystatin C. we found a negative; non-significant correlation between GFR and both NGAL1&2 in the groups of ADHF,ACSs, HTN, and AKI. This points to early deterioration in renal functions with mild decrease of GFR, but before the frank appearance of renal disease by the gold standard serum creatinine and blood urea.

Conclusion:
Our study clearly showed that NGAL 1&2 are good indicators of early renal affection in Acute Decompensated Heart Failure and Acute kidney Injury patients, but neither in ACS nor in HTN encephalopathy patients Troponin 1& 2 are only of values in diagnosis of ACS patients and did not show useful values in ADHF,HTN and AKI patients diagnosis. NGAL serum values in our study had no relation to Troponin serum values in renal non cardiac patients and also in cardiac non renal patients.

References:-


