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RESEARCH ARTICLE

IMPACT OF ZINC SUPPLEMENTATION ON INFLAMMATION AND FATIGUE IN HEMODIALYSIS PATIENTS.

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Abstract

Background: Inflammation and fatigue are now considered as predictors of cardiovascular and all cause mortality and up till now have no valid treatment strategies. Zinc deficiency occurs frequently in HD patients and is assumed to be related to inflammation and fatigue in HD patients. This study was designed to determine the effects of Zn supplementation on inflammation and fatigue in zinc deficient hemodialysis patients.

Methods: a prospective randomized placebo-controlled single blinded study was conducted on long-term HD patients with serum Zn concentration < 80 µg/dL. Fifty two patients were randomized to 2 groups; group 1 received 25 mg/day oral Zinc supplement for twelve weeks and group 2 received placebo for twelve weeks. Serum high sensitive-CRP (hs-CRP), interleukin-6 (IL-6) and Fatigue Severity Scale (FSS) were assessed at baseline and at the end of the study.

Results: At the end of the study, there were a significant decrease in FSS in group 1 ($p = 0.001$) with a significant difference between the two groups in percent change ($p < 0.001$). There were no significant differences either in serum hs-CRP or in IL-6 levels within or between the two groups.

Conclusions: Zinc supplementation in HD patients at dose of 25 mg/day for 12 weeks improved fatigue but did not affect inflammatory markers.

Introduction:-

Although dialysis has improved the life expectancy of End Stage Renal Disease (ESRD) patients, the mortality rate still remains disappointingly high (Qureshi et al., 2002). Inflammation and its complications such as cardiovascular diseases, protein-energy wasting and erythropoietin-resistant anemia are prevalent in hemodialysis (HD) patients and increase mortality in these patients(Akchurin and Kaskel, 2015) .

Prevalence of inflammation in HD patients is 30–50 %(Khalatbari-Soltani and Tabibi, 2015). A variety of factors contribute to chronic inflammatory status in CKD, including increased production and decreased clearance of pro-inflammatory cytokines, oxidative stress, acidosis, chronic and recurrent infections, altered metabolism of the adipose tissue, and intestinal dysbiosis (Akchurin and Kaskel, 2015).

Extracorporeal factors, such as repeated contact of blood mononuclear cells with dialysis tubes and dialyzer membranes, impurities in the dialysis water and/or dialysis solution and bio-incompatible factors in the dialysis circuit play an additional role(Bergstrom et al., 2000; Kalantar-Zadeh et al., 2003; Akchurin and Kaskel, 2015).

Zinc deficiency is prevalent (40–78%) in renal failure patients on dialysis (Lee et al., 2000; Vanholder et al., 2002; Roozbeh et al., 2009). Low serum Zn concentration is attributed to Zn removal during HD, decreased serum albumin

levels as well as inadequate dietary intake and reduced gastrointestinal absorption of Zn. Serum Zn levels can also be reduced by increased expression of intracellular metallothioneins following oxidative stress (Kobayashi et al., 2015).

The relationship between Zn deficiency and inflammation is bidirectional. Increasing evidence indicates that zinc has anti-inflammatory properties, and zinc deficiency promotes systemic inflammation. However to date the precise mechanisms by which zinc deficiency contributes to enhanced inflammation is not well understood, it is hypothesize that zinc deficiency can promote chronic inflammation in part by triggering immune cell activation, as well as by altering DNA methylation status of genes involved in the induction of a proinflammatory response (Wong et al., 2015).

Moreover, *in vivo* and *in vitro* experiments demonstrate that IL-6 up-regulates zinc transporter Zip14 expression in liver which most likely plays a major role in the mechanism responsible for hypozincemia that accompanies the acute-phase response to inflammation in general(Rink and Haase, 2007). It has been found that Zn deficiency contributes also to fatigue in patients with Chronic Fatigue Syndrome (CFS)(Werbach, 2000).

Fatigue is one of the most common symptoms experienced by dialysis patients, with its prevalence ranging from 60% to 97%(Horigan, 2012). Some evidence indicates that immunological regulation is disturbed in CFS and therapies that modulate the immune system can result in a clinical improvement of fatigue(Bansal et al., 2012). Zinc as a modulator of both innate and adaptive immunity (Bonaventura et al., 2015) is therefore supposed to improve fatigue in HD patients.

Numerous physiological, sociodemographic, psychological, and dialysis-related factors have been suggested as possible factors implicated in the onset of fatigue in chronic HD patients such as age, sex, race, educational status, anemia, malnutrition, uremia, dialysis inadequacy, hyperparathyroidism, and symptoms of depression or depression (Bossola et al., 2009). Moreover, inflammation appears to be associated with fatigue in HD patients (Bossola et al., 2015).The following study was conducted to determine the effects of Zn supplementation on inflammation and fatigue in zinc deficient hemodialysis patients.

Patients and Methods:-

Study design and setting:-

Prospective randomized placebo- controlled single blinded study was conducted at National Institute of Urology and Nephrology. All subjects signed an informed consent statement prior to inclusion in the study. The study protocol was approved by research ethics committee of Faculty of Pharmacy, Ain shams University.

Patients:-

Eligibility Criteria:-

Adult outpatients receiving four-hour HD sessions three times per week (age 18-65) with low plasma Zn concentrations (< 80 μg/dL) were included. Patients were excluded if they were on HD duration less than 6 months or they had gastrointestinal disorders, liver diseases, cancers, mental retardation or dementia, psychiatric illness, and chronic inflammation as hepatitis, received blood transfusions three months before the study, or were receiving immune suppressant drugs and supplementat with natural herbs, antioxidants, vitamins/minerals, and fish oils..

Material and Methods:-

Patients were randomized into two groups, group 1 received oral supplementation with 25 mg elemental Zn per day and group 2 who received placebo for twelve weeks. Zn was supplied as 110 mg Zn Sulphate capsule hard gelatin capsules under trade name of Octozinc manufactured by OCTOBER PHARMA S.A.E. , Egypt. Zinc was administered after dinner.

At baseline the patients were subjected to physical examination and thorough history taking.
Blood samples were collected from patients before HD session after 8-12 hours fasting at baseline and after 12 weeks to determine serum levels of Zn, high sensitive c reactive protein (hs-CRP), and interleukin-6 (IL-6). Evaluation of Zn was done by colorimetric method using commercial kit supplied by Biodiagnostic company, Giza, Egypt ,while hs-CRP and IL-6 were determined using Enzyme Linked Immunosorbent Assay using commercial kits

supplied by DRG International Inc., USA and Ani Biotech Oy Orgenium Laboratories Business Unit, Vantaa, Finland respectively.
Quality of life of patients in both groups was assessed by Fatigue Severity Scale (FSS) at baseline and 12 weeks after (at the end of the study).

Statistical methods:-

Data management and analysis were performed using Statistical Package for Social Sciences (SPSS) vs. 21.
Numerical data were summarized using means and standard deviations or medians and ranges, as appropriate. Categorical data were summarized as numbers and percentages. For normally distributed data, Student's t-test was done while for non normally distributed data by Mann-Whitney test and Wilcoxon Rank Signed test were done. Chi-square was used to compare between the groups with respect to categorical data. All p-values are two-sided. P-values < 0.05 were considered significant.
Spearman's rho correlation coefficient was used to analyze the correlation between inflammation markers and Fatigue Severity Scale.

Results:-

A total of 226 patients were assessed for eligibility and 64 fulfilled the inclusion criteria and included in the study as shown in figure1.

Twelve patients did not complete the study for the following reasons:
In group 1; three patients were withdrawn due gastrointestinal side effects (nausea, vomiting, diarrhea and abdominal cramps) and one patient was excluded due to hospitalization for vascular access complication.

In group 2; two patients died, one patient moved to another center, two patients were excluded due to hospitalization for surgical removal of polycystic kidney and stroke and three patients dropped out due to missed post-study data.

Twenty eight patients of group 1 and twenty four patients of group 2 completed the study.

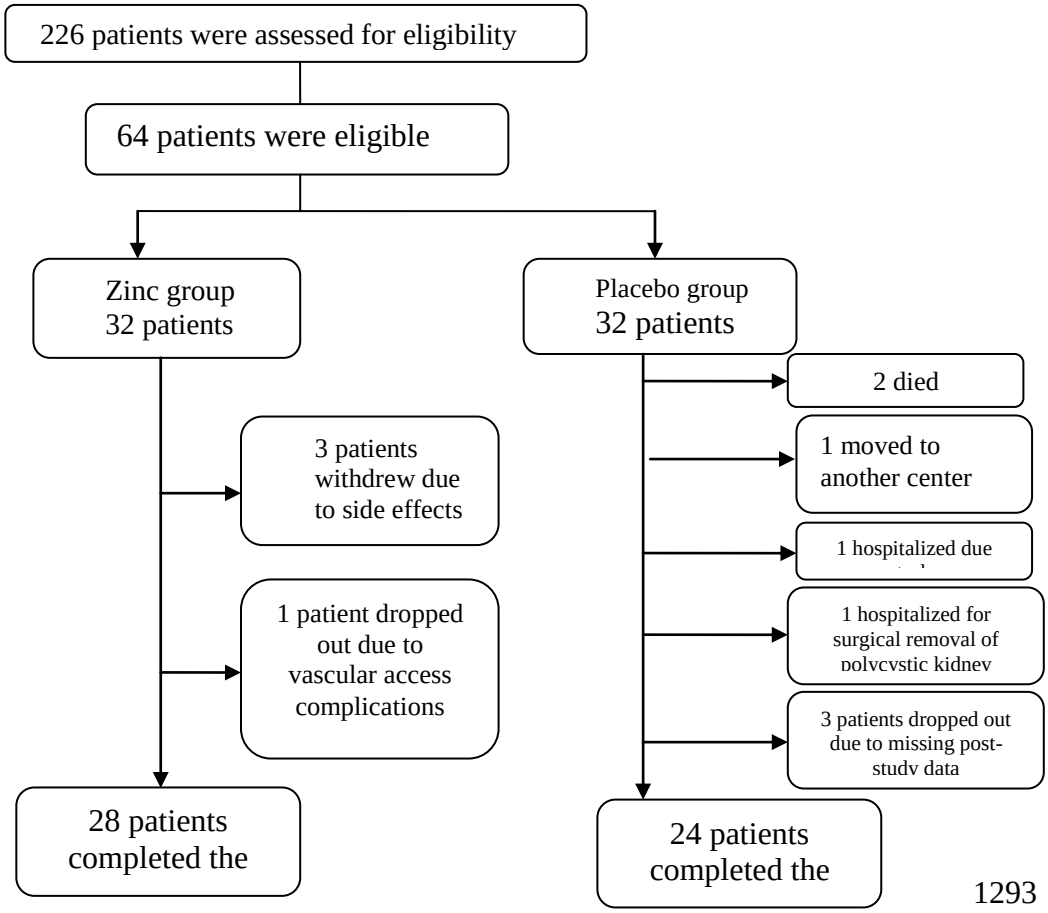

Figure 1: Flow chart of patient's enrollment and drop out.

Fifty-two patients (28 males and 24 females) with a mean age of 48.21 ± 12.07 years completed the study. Sixty six percent of the patients were Zn deficient (serum Zn less than 80µg/dL). Median length of time on dialysis in group 1 and group 2 is 4.5 and 3.5 years respectively. The two groups were comparable at baseline except for gender there were a statistically significant difference between the two groups as shown in table 1.

Table (1): Baseline characteristics of the study group subjects:-

Parameter	Group 1	Group 2	P-value
Age (years) (mean ± SD)	50.1 ± 10.4	46.0 ± 13.7	0.225[a]
Gender (Number (%))	Female 9 (32.1%) Male 19 (67.9%)	Female 15 (62.5%) Male 9 (37.5%)	0.029 [b]*
Weight (Kg) (mean ± SD)	81.1 ± 20.1	76.5 ± 21.9	0.431[a]
Height (Cm) (mean ± SD)	169 ± 10.7	164.9 ± 9.7	0.157[a]
Body Mass Index (Kg/m²) (mean ± SD)	28.1 ± 5.2	27.8 ± 6.5	0.846[a]
Years of RHDx (median (IQR))	4.5 (2.38-7)	3.5 (2-5.25)	0.573[c]
Serum Zn level (µg/dl) (median (IQR))	66.2 (55.9-71.6)	63.0 (53.6-67.0)	0.300[c]
Serum hs-CRP (mg/L) (median (IQR))	9.0 (7.5-10)	9.25 (7.38-12)	0.632[c]
Serum IL-6 (pg/ml) (median (IQR))	17.85 (13.84-31.18)	20.35 (15.14-30.63)	0.666[c]
FSS (median (IQR))	4.95 (4.44-5.69)	5 (4.53-5.53)	0.927 [c]

SD:- standard deviation, RHDx: regular hemodialysis, IQR:Interquartile range, a: Student's t test, b: Chi square test, c: Mann-Whitney test, *(P<0.05): significant,
Group 1 received 25 mg elemental Zn daily for 12 weeks and group 2 received placebo daily for 12 weeks.

Effect on inflammatory parameters:-

a. Serum high sensitivity C-reactive protein:

Although there were no statistically significant differences between the 2 groups at the end of the study, five patients (18 %) of group 1 show normalization of hs-CRP level (< 3mg/L) while only one patient (4%) normalized in group 2 (Table 2).

b. Serum interleukin-6:-

At the end of the study, serum interleukin-6 did not change in group 1 and slightly decreased in group2 but this was not statistically significant and may be due to chance.

Table (2): Comparison between the study groups regarding inflammatory parameters before and after the study:-

Inflammatory parameters	Timing	Group 1 Median (IQR)	Group 2 Median (IQR)	P-value
Serum hs-CRP (mg/L)	Before	9.0 (7.5-10)	9.25 (7.38-12)	0.632 [a]
	After	9.75 (7.35-12)	9.55 (5.5-11.25)	0.652 [a]
	P-value	0.724 [b]	0.475 [b]	
Serum IL-6 (pg/ml)	Before	17.85 (13.84-31.18)	20.35 (15.14-30.63)	0.666 [a]
	After	17.95 (13.37-27.58)	19.31 (13.16-26.81)	0.811 [a]
	P-value	0.665 [b]	0.563 [b]	

IQR: interquartile range, a: Mann-Whitney test, b: Wilcoxon signed rank test,
Group 1 received 25 mg elemental Zn daily for 12 weeks and group 2 received placebo daily for 12 weeks.

Effect on fatigue:-

At the end of the study, there was a significant decrease ($p = 0.001$) in FSS in group 1 while the FSS non-significantly increased in group 2 patients. Moreover, there were a significant difference ($p < 0.001$) between the two groups in percent change.

FSS	Group 1 Median (IQR)	Group 2 Median (IQR)	P-value
Before	4.95 (4.44-5.69)	5.0 (4.53-5.53)	0.927 [a]
After	4.84 (4.28-5.44)	5.11 (4.41-5.56)	0.286 [a]
P-value	0.001 [b] *	0.285 [b]	
Change%	-5.66 (-6.89 – 2)	2.03 (-2.36 -3.19)	<0.001 [a] **

IQR: interquartile range, a: Mann-Whitney test, b: Wilcoxon signed rank test,
, ($P \geq 0.05$): non-significant, *($P<0.05$): significant, **($p<0.001$): highly significant,
Group 1 received 25 mg elemental Zn daily for 12 weeks and group 2 received placebo daily for 12 weeks.

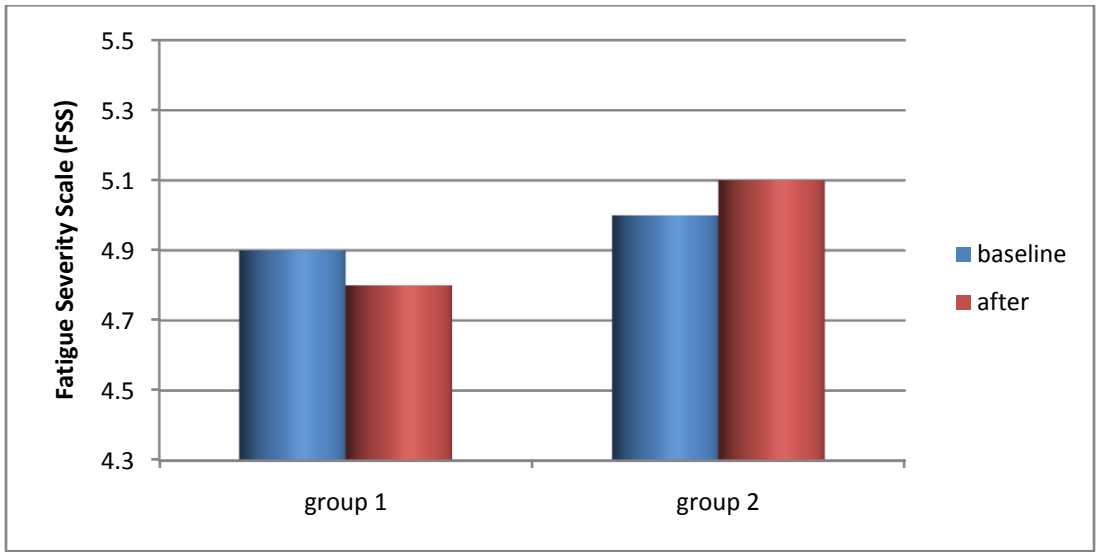

Figure (): Median Fatigue Severity Scale in study groups before and after the study

At the end of the study, there were no correlation between FSS and inflammatory markers ($r < 0.5$ and $p > 0.05$).

Discussion:-

Inflammation in HD patients results from an imbalance between the effects of pro-inflammatory and anti-inflammatory cytokines synthesized and secreted by circulating monocytes, tissue macrophages, Kupffer cells, and endothelial cells. Interleukin-6 seems to be the major mediator of acute-phase reactants synthesis. Plasma concentrations of acute-phase proteins such as C-reactive protein, fibrinogen and serum amyloid A, are clinical indicators of systemic inflammation (Guarnieri et al., 2005).

Numerous studies have been performed to find treatment strategies for reducing inflammation in HD patients which falls in three broad categories: lifestyle modifications, pharmacological interventions, and optimization of dialysis but up till now none of them are valid(Akchurin and Kaskel, 2015).

In the study presented here, Zn supplementation in Zn deficient HD patients at dose of 25 mg/day for 12 weeks does not ameliorate inflammation in HD patients as evidenced by unchanged hs-CRP and IL-6 levels at the end of the study.

Four studies have investigated the effect of Zn supplement on CRP in HD patients. Two of them reported a ignificant reduction in CRP levels upon Zn supplementation while the others did not show a significant difference in CRP levels at the end of the study.

In agreement with our results, *Matson et al* and ***Rashidi et al*** reported non-significant difference in CRP levels after 6 weeks of 50 mg/day Zn supplementation(Matson et al., 2003; Rashidi et al., 2009).

On the other hand, a significant decrease in CRP level were reported by Guo and Wang who used Zn supplement at dose of 11 mg/day for eight weeks and *Roozbeh et al* upon using a dose of 57 mg/day for six weeks(Roozbeh et al., 2011; Guo and Wang, 2013).

The effect of Zn supplementation on serum IL-6 was not studied before in HD patients. Interleukin-6 was chosen as inflammatory marker in this study due to the growing evidence of its importance as predictor of all cause mortality (ACM) and cardiovascular mortality (CVM)in HD patients in addition to CRP or even a stronger predictor (Panichi et al., 2004; Zhang et al., 2013).

A growing wave of research examining fatigue in HD patients revealed its role as predictor of cardiovascular events and mortality in addition to its negative impact on quality of life in this population(Jhamb et al., 2009; Davison and Jhangri, 2010; Koyama et al., 2010).

L-carnitine, growth hormone, nandrolone decanoate and vitamin C were tested for treatment of fatigue in HD patients but none of them can be recommended(Bossola et al., 2011).

Fatigue and depression coexist, but importantly fatigue can be present where there is no depression. Therefore, the study of fatigue needs to discriminate between these two states. The Fatigue Severity Scale (FSS) is a valid and reliable instrument used to determine physical fatigue which is able to discriminate between depression and fatigue (Bonner et al., 2008).

There was a significant improvement of fatigue upon 12 weeks of 25mg/day Zn supplementation in Zn deficient HD patients.

Conclusion:-

Although fatigue has been found to be associated with IL-6 level, Zinc supplementation at dose of 25mg/day for 12 weeks improved fatigue without affecting IL-6 and hs-CRP levels indicating that Zn might improve fatigue by other mechanism rather than modulating inflammation which need further investigation .In conclusion, Zinc supplementation may be beneficial to ameliorate fatigue in Zinc deficient HD patients.

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