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Research Article

The Effects of Zn-Deficiency Diet and Zn Suplemantation on the Lipid Peroxidation and Ertyhropoietin Levels in Rats with Experimentally Induced Renal Failure

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ABSTRACT

This study was aimed determineted level of hematological, biochemical, histological value at to may developing toxical effect of kidney nephron and effect oksidatif damage role at possible to may developing tubuler dejeneration this toxic effect with an effect of minority and majority of Zn which known antioxidant after administration of gentamicin. Eight groups were established there are 8 rats in every groups. The acute renal failure groups received 100mg/kg gentamisin ip injection daily for 8 days. Zinc deficient group was given zinc deficient diet and deiyonized water for 4 weeks. Zinc excess group was given combineted with water 227 mg/l $ZnSO_4$ for 2 weeks. Acute renal failure + Zn excess group was given combineted with water 227 mg/l $ZnSO_4$ for 2 weeks and 100 mg/kg gentamicin for 8 days by ip injection. Acute renal failure + Zn deficiency groups was given zinc deficient diet and deiyonized water for 4 weeks and 100 mg/kg gentamicin for 8 days by ip injection. Chronic renal failure group was applied ip 50 mg/kg gentamisin for 15 days. Chronic renal failure + Zn excess group was given combineted with water 227 mg/l $ZnSO_4$ for 2 weeks and 50 mg/kg gentamicin for 15 days by ip injection. The result of analysis after blood and kidney tissue samples were collected, MDA concentration in the blood decreased at Zn excess group, increased in other groups as control groups. GSH concentration in the blood decreased in all groups as control group. MDA level in kidney tissue decreased in acute renal failure + Zn excess group, chronic renal failure + Zn excess group and Zn excess as control group and increased in other groups as control groups. GSH level in kidney tissue increased in acute renal failure + Zn excess group, chronic renal failure + Zn excess group and Zn excess as control group and decreased in other groups as control groups. GSH-Px level in kidney tissue increased Zn excess group but decreased in other groups as control groups. XO level in kidney tissue decreased Zn excess group but increased in other groups as control groups. EPO level in serum increased at acute renal failure + Zn excess, Zn excess groups, This level at chronic renal failure+ Zn excess group same, decreased in other groups as control groups**.** HCT level increased at Zn excess group, decreased at other group as control group. As a result Zn supplementation may beneficial at person with renal failure.

Key words: Antioksidants, Eriythropoietin, Gentamicin, Lipid peroxidation, Zinc

INTRODUCTION

As a trace element that is essential for metabolism, zinc is necessary for the biological functions of more than 300 enzymes. Many studies have stressed the importance of zinc as an antioxidant and therapeutic agent. It is known that zinc takes its antioxidant effect indirectly by maintaining its cell membrane structure, by joining the superoxide dismutase (SOD) enzyme's structure, increasing the tissue concentrations of metallothionein, preventing the oxidation of proteins that contain the thiol group, and also by replacing the redox metals such as copper at critical zones. (Berlett and Stadtman, 1997).

It is known that gentamicin (Genta) gives rise to lysosome, mitochondria, or membrane damage and kidney toxicity by accumulating in proximal renal tubuluses. (Humes and Weinberg, 1986) Zinc is an element that has significant functions in the organism. Its deficiency may lead to many undesired statuses due to the failure of body functions and also give rise to a change in lipid peroxidation products, blood parameters and erythropoietin. Kidney failure will also give rise to changes to these parameters.

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In this study, it is aimed to explain how zinc will have an impact on the erythropoietin level, free radicals, and formation of blood cells.

MATERIALS AND METHODS

Sixtyfour male Wistar Albino rats weighing about 200-250 g were used. The rats were randomly divided into one of eight groups: 1 (control group), 2(Acute Renal failure group), 3 (Zinc deficient group), 4 (Zincexcess group), 5(acute renal failure +Zinc Excess group), 6 (Acute renal failure +Zinc Deficient groups), 7(chronic renal failure), 8 (Chronic renal failure +Zinc excess) each containing 8 animals. Group 1 received normal food and water during experimental. Groups II received 100mg/kg gentamisin ip injection daily for 8 days (Polat *et al.*, 2006). Group III received zinc deficient diet and deiyonized water for 4 weeks. Group IV was given combineted with water 227 mg/l $ZnSO_4$ for 2 weeks (Goel and *et al.*, 2005). Group V was given combineted with water 227 mg/l $ZnSO_4$ for 2 weeks and 100 mg/kg gentamicin for 8 days by ip injection. Groups VI was given zinc deficient diet and deiyonized water for 4 weeks and 100 mg/kg gentamicin for 8 days by ip injection. Group VII was applied ip 50 mg/kg gentamisin for 15 days. Group VIII was given combineted with water 227 mg/l $ZnSO_4$ for 2 weeks and 50 mg/kg gentamicin for 15 days by ip injection. Groups I, III, IV were given normal saline intraperitoneally for 8 days.

A dietary list that lacks zinc was prepared according to the American Instute of Nutrition (AIN- 76, 2007). The dietary list that lacks zinc was prepared by adding sucrose instead of the zinc carbonate of 1.6 grams that is required in normal diet. The rats that are included in the group that lacks zinc were fed on this feed. Deionized water was given to the animals in this group.

Their blood parameters were checked in the blood counting device via QBC vet tubes. Erythropoietin quantity was determined from the acquired serums by using the erythropoietin ELISA kit (Quantikine). Zinc analysis was made through atomic absorption spectrophotometric (AAS) method.

Determination of GSH in blood was made via the method of Rizzi *et al*., while MDA determination in blood was made via the method of Sushil *et al.* (Sushil *et al*., 1989; Rizzi *et al*., 1988).

The tissue extraction and analysis of GSH was performed according to Ball (1966) and Fernandez (1981) method. For the tissue MDA, GSH Px, and XO analyses, the kidney tissue extraction was made via the method of Xia *et al*. (1995) and Marklund (1990) methods.

In kidney tissues, the GSH-Px enzyme activity determination was made by using a commercial kit (Randox – Ransel). Xanthine-oxidase (XO) determination was measured via an Elisa kit. After having been fixed in 10% formalin solution, the kidney tissues were followed daylong in a tissue follow-up device. Thereafter, they were embedded in paraffin blocks in a tissue embedding device. 4-micron thick samples were taken from paraffin blocks by means of a rotary microtome. They were dyed with Hematoxylon-Eosin (HE) colorant. They were analyzed with a binocular microscope via a specially marked ocular (Bancroft and Cook, 1984).

RESULTS

The results of this study, the blood MDA, blood GSH, kidney tissue MDA, GSH, GSH-Px, and XO levels are given in Table 1, serum zinc levels, serum EPO quantity, and some hematologic parameters in Table 2, while histopathologic parameters in Figures 1 and 2. The blood MDA level decreased in Zn-excess group in comparison with the control group, increased in all of the other groups, and remained near the ABY+Zn-excess group. The blood GSH value was found lower in all of the groups in comparison with the control group. The tissue MDA level decreased in ABY+Zn-excess, KBY+Zn-excess, and Zn-excess groups in comparison with the control group, while increased in other groups. The tissue GSH level increased in ABY+Zn-excess, KBY+Zn-excess, and Zn-excess groups in comparison with the control group, while decreased in other groups. The tissue GSH-Px level increased in the Zn-excess group in comparison with the control group, while decreased in all other groups. The tissue XO level decreased in the Zn-excess group in comparison with the control group, while increased in other groups. EPO levels increased in the Zn-excess and ABY+Zn-excess groups in comparison with the control group, the KBY+Zn- excess group remained near the control group, while decreased in other groups. HCT values increased in the Zn- excess group ad decreased in other groups.

DISCUSSION

In the study conducted by Vardı *et al*. (2005), it has been claimed that the MDA level considerably increases in the gentamicin group in case of nephrotoxicity caused by gentamicin. Karahan *et al*. (2005) reports as a result of their study that lycopene has a protective effect in nephrotoxicity that is generated with gentamicin. The opinion that MDA significantly increases in the gentamicin group gains importance. As a result of a study conducted by Nakas-Icindic *et al.* (2005) on rats, it is reported that plasma nitric oxide levels and kidney damage in the group, where acute tubular necrosis is generated with gentamicin, are statistically higher than the control group. In the study conducted by Abdel-Naim *et al.* (1999), it is concluded that gentamicin significantly increases MDA in nephrotoxicity that is induced with gentamicin in rats.

The currently accepted opinion for zinc is that zinc can be used as an antioxidant drug (Powell 2000). A study conducted with older adults has revealed that the zinc application stimulates antioxidant activity and reduces lipid peroxidation (Fortes *et al*., 1997). While MDA production in plasma, liver, and pancreas tissue increases considerably in the rats that are fed on a zinc-poor diet, it is reported that the zinc application significantly represses the increasing MDA levels with the same rats (Shaheen and El Fattah, 1995). A study conducted by Mills *et al*. (1981) has revealed that the blood glutathione level decreases as a result of zinc deficiency. As a result of a study conducted by Karahan *et al.* (2005), it is reported that GSH-Px and CAT activities decrease in the nephrotoxicity induced with gentamicin.

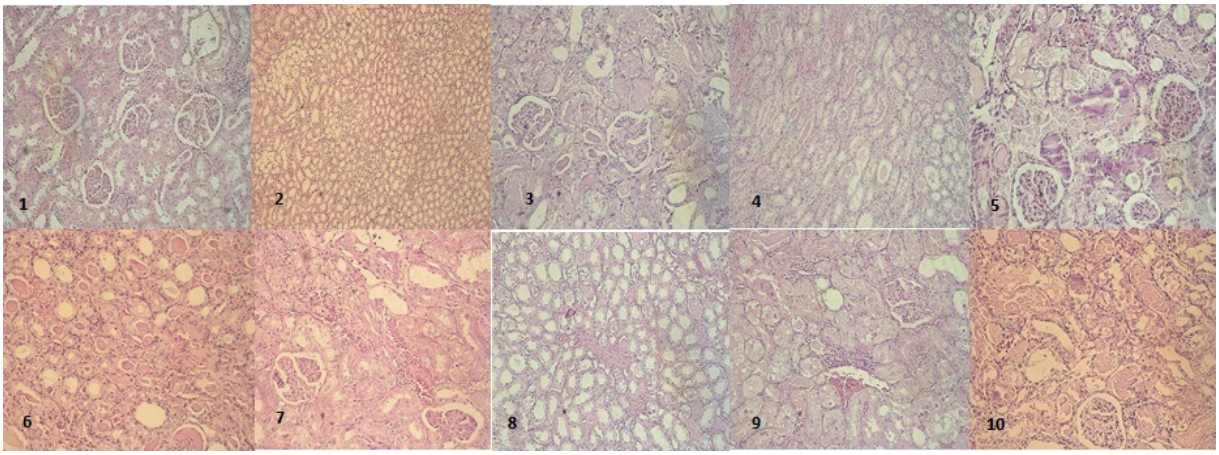

Fig. 1: 1. Control groups: Kidney parenchyma 2.Control groups: Renal medulla 3. Zn excess+ acute renal failure groups: Tubules proksimalis necrosis and hyaline cylinder. 4. Zn excess+ acute renal failure groups: Renal medulla, hyaline cylinder.5. Zn deficiently + acute renal failure groups: Tubules proksimalis necrosis and hyaline cylinder. 6. Zn deficiently + acute renal failure groups: Renal medulla, hyaline cylinder 7. Zn excess + chronic renal failure groups: Tubules proksimalis necrosis and hyaline cylinder 8: Zn excess + chronic renal failure groups: Renal medulla, few hyaline cylinder 9. Chronic renal failure: Tubules proksimalis necrosis and the vacuolization 10. Acute renal failure groups: Tubules proksimalis necrosis and hyaline cylinder.

Table 1: Blood MDA, blood GSH, kidney MDA, GSH, GSH-Px, XO parameters

Parameters	n	Control	Acute renal failure	Chronic renal failure	Acute renal failure+Zn excess	Acute renal failure+Zn Deficiency	Chronic renal failure+Zn excess	Zn excess	Zn Deficiency	P
Blood MDA (nmol/ml)	8	0,67± 0,32^{dc}	0,78± 0,08^{dc}	1,59± 0,30^{a}	0,67± 0,14^{dc}	0,90± 0,19^{cb}	1,10± 0,25^{b}	0,56± 0,37^{ed}	0,87± 0,22^{cb}	***
Blood GSH (mg/dl)	8	45,42± 7,24^{a}	35,85± 15,81^{ba}	36,93± 11,51^{ba}	37,92± 3,17^{ba}	27,87± 7,12^{b}	37,24± 8,21^{ba}	41,26± 4,95^{a}	19,50± 12,14^{c}	***
Kidney MDA (nmol/g tissue)	8	97,74± 5,23^{ab}	110,86± 32,89^{a}	103,06± 68,24^{a}	90,20± 26,65^{ab}	111,20± 25,75^{a}	88,04± 19,37^{ab}	59,88± 25,50^{c}	104,62± 23,37^{a}	*
Kidney GSH (µmol/g tissue)	8	0,45± 0,22^{ab}	0,35± 0,13^{b}	0,30± 0,12^{b}	0,66± 0,21^{a}	0,31± 0,09^{b}	0,53± 0,33^{ab}	0,63± 0,16^{a}	0,41± 0,18^{b}	*
Kidney GSH Px(U/mg tissue)	8	146,49± 28,80^{a}	25,78± 16,69^{c}	75,09± 37,53^{b}	65,00± 24,50^{bc}	24,01± 17,82^{c}	83,87± 49,89^{b}	163,41± 79,43^{a}	56,17± 16,56^{bc}	***
Kidney XO (µU/mg tissue)	8	2242,85± 435,62^{de}	2626,25± 261,51^{cd}	2979,36± 180,43^{bc}	2365,31± 250,63^{de}	3394,74± 583,45^{a}	2566,69± 252,45^{d}	2059,16± 410,41^{e}	3308,56± 346,42^{ab}	***

a, b, c, d, e; The differences among the groups symbolized with different letters in the same line are statistically significant. (*P<0.05, ***P<0.001).

Table 2: Serum Zinc levels, serum EPO levels and hematologic parameters.

Parameters	n	Control	Acute renal failure	Chronic renal failure	Acute renal failure+Zn excess	Acute renal failure+Zn deficiency	Chronic renal failure+Zn excess	Zn excess	Zn deficiency	P
Zinc (mg/L)	8	0.92± 0.22 ^{ab}	0.82± 0.11 ^{bc}	0.59± 0.20 ^{d}	0.73± 0.15 ^{cd}	0.68± 0.12^{cd}	0.93± 0.08^{ab}	1.03± 0.21^{a}	0.68± 0.08^{cd}	***
EPO (pg/mL)	8	135.72± 12.01^{b}	126.51± 14.20^{b}	129.62± 16.15^{b}	140.61± 17.67 ^{b}	102.78± 2.71^{c}	135.82± 13.11^{b}	159.03± 27.59^{a}	126.28± 19.74^{b}	***
HCT (%)	8	52.14± 5.01^{ab}	50.57± 2.93^{abc}	43.71± 10.41^{bc}	50.71± 6.52^{abc}	52.42± 4.35^{ab}	41.42± 14.32^{c}	57.57± 2.07^{a}	48.75± 8.53^{abc}	*
HB (g/dl)	8	17.57± 1.98	17.85± 1.21	16.57± 4.27	17.28± 1.49	16.71± 1.49	17.71± 3.90	16.57± 1.71	16.28± 2.87	
MCHC (g/dl)	8	32± 5.16	34.57± 2.07	32.42± 1.51	34.85± 2.79	31.42± 0.78	33±3.10	33± 1.41	34±2	
WBC (*10^9/L)	8	20.42± 8.2	26.71± 15.19	19.71± 8.84	31.42± 26.3	31.14± 9.65	18.85± 7.6	14±6.5	16.14± 5.87	
GR (*10^9/L)	8	5.85± 2.79	4±0	13.14± 10.79	4.42± 4.5	7.57± 4.5	4.14± 1.46	7.28±1.11	7.14± 2.47	
L/M (*10^9/L)	8	13.85± 5.08^{bc}	20.85± 15.33^{abc}	8.71± 6.12^{c}	28.57± 22.60^{a}	24.14± 5.69^{ab}	8.85± 5.7^{c}	16.71± 15.64^{abc}	9.71± 3.94^{c}	*

a, b, c, d, e; The differences among the groups symbolized with different letters in the same line are statistically significant. (*P<0.05, **P<0.01, ***P<0.001).

At the end of the studies, it is observed that uric acid and xanthine oxidase enzyme activities have a significant role in heart and kidney failure pathophysiology and that this enzyme inhibition may have beneficial clinical outcomes (Doehner *et al*., 2002). In a study conducted on enzyme activities in case of zinc deficiency (George *et al*., 1967), it is claimed that the XO activity in kidney and liver tissues is found higher in the zinc-poor group than the control group.

Holtkamp *et al*. (1993) found the average serum Zn level of 65 hemodialysis patients below the control group. In a study conducted by Bagnis *et al*. (2001), it was observed that the application of EPO in ABY, which is generated with cisplatin-induced nephrotoxicity, for 9 days IP 100 U/kg/day fixed the findings in histological and functional terms. Turi *et al*. (1992) reported that the glutathione levels of hemodialysis patients that receive EPO treatment increase, their MDA levels remain constant, and SOD and GSH-Px enzymes are lower in comparison with the control group. Djordjevic *et al*. (1993) report that erythropoietin does not only fix anemia, but also it increases the SID and catalase (CAT) enzymes, which are the elements of antioxidant defense.

Since majority of zinc in blood is dependent on the carbonic anhydrase enzyme in erythrocytes, it is expected that the zinc deficiency will affect the enzyme activity (Iqbal, 1971). As a result of a study (Dursun, 1988), where zinc deficiency was induced, it is reported that the carbonic anhydrase enzyme decreased during the 30-day feeding, while erythrocyte numbers increased by 39%, haematocrit number by 24% and haemoglobin by 23% before the enzyme activity increases again by the end of the 30-day period. It is reported that haematocrit and haemoglobin values increase in erythrocyte number in case of a zinc deficiency (Huber and Gershoff, 1973). Many studies indicate that zinc increases the numbers of human and animal lymphocytes and; therefore, activates their effects (Baltacı, 1995; Prasad, 1998). The normal functions of lymphocytes predominantly depend on their division, differentiation, and maturing capabilities (McClain *et al*., 1985)

Conclusions

In conclusion, it has been detected that the antioxidant system could weaken for various reasons and the free radicals that increase accordingly could give rise to certain deficiencies in case of an experimentally induced chronic kidney failure. We are of the opinion that the decrease in hematocrit value may relate to the weakening in the antioxidant system. We can say that zinc application prevents oxdative damage in the patients that have kidney failure in a short span of time. Only kidney tissue was used in this study. One should keep in mind that different effects may be observed in different tissues. We have observed that the zinc deficiency has an effect of increasing the oxidative damage and that zinc has an effect of increasing haematocrit value and EPO quantity. The haematological, biochemical, and histopathological findings of the study support the idea that zinc serves as an antioxidant. We can say that zinc will fix the antioxidant system weakening, which is observed in acute or chronic kidney failure, and that it will partly prevent the decrease in haematocrit value. Further, we are of the opinion that administering zinc supplement to individuals with kidney failure could be beneficial.

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