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# INVESTIGATION OF ZINC DEFICIENCY ON CHROMOSOMAL ABNORMALITIES IN MICE

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***Key Words**: Zinc Deficiency, Chromosomal Abnormalities*

## SUMMARY

The present study investigated the effect of zinc-deficiency on chromosomal abnormalities and mitotic index, and the interaction of zinc deficiency and methotrexate. Therefore, two zinc-deficient and two zinc-adequate diet received mice groups were constituted. Methotrexate was injected to one of zinc-deficient and one of zinc-adequate diet groups. There were no structural and numerical abnormalities in all groups. However, mitotic index was significantly lower than other groups in zinc-deficient and methotrexate administered mice.

## ÖZET

**Farelerde Çinko Eksikliğinin Kromozomlar Üzerine Etkisinin İncelenmesi**

Bu çalışmada çinko eksikliğinin; kromozom anomalisi, mitotik indeks ve metotreksat ile etkileşimi araştırıldı. Bu amaçla iki çinko eksik, iki çinko normal dört grup oluşturuldu. Metotreksat çinko eksik grubun birisi ile çinko normal grubun birisine tek doz olarak verildi. Bütün gruplarda yapısal ve sayısal anomali tesbit edilemedi, fakat çinko eksik-methoteraksat grubunda diğer gruplara göre mitotik indeks düşük bulundu.

## INTRODUCTION

Zinc is a common element in the human environment and constitutes an important trace element intervening in many biological processes(1). Zinc plays an important role in the biosynthesis of nucleic acids, mainly by affecting thymidine kinase and DNA polymerases. Toxicity of zinc is low, zinc deficiency represents, however, a hazard for human health. It is known that, zinc deficiency causes a reduction in the specific activity of many zinc-requiring enzymes(2). The most important role is attributed to thymidine kinase(3) which phosphorylates thymidine prior to its incorporation into DNA during replication and the lack of which can reduce the rate of DNA replication and cell proliferation(4). Additionally, it is known that methotrexate(MTX) is an analogue of folic acid with higher affinity for dihydrofolate reductase than folic acid, so that the synthesis of folinic acid potently inhibited. Folinic acid is required for the production of thymidine which in turn is required for DNA synthesis. In this condition, it would be

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expected an interaction between zinc deficiency and MTX administration. Studies on mutagenicity of zinc suggest strongly that zinc does not represent a mutagenic risk(5) and does not induce misrepairing in vitro between nucleic acid bases(6). Whereas, no information appears to be available on possible mutagenic effect of zinc deficiency(1) and MTX administration in zinc deficiency. The purpose of this study has amied to explain these questions partially.

## MATERIALS AND METHODS

Swiss albino mice (n=20) of both sexes, weighing from 22-26 g and bred in our laboratory were subjected to the present study. Animals were maintained according to "Guide to the care and use of experimental animals" by the Canadian Council on Animal care (7). All mice were divided equally into four experimental groups. Two groups of mice were fed with a zinc-deficient diet. The others were fed with a zinc-adequate diet. Diets were prepared as previously described (8,9). The zinc-deficient diet contained 2.8 $mg.kg^{-1}$ zinc. Food and water were available continuously ad libitum (deionized distilled water was given to the zinc-deficient groups). After eight weeks of this dietary treatment, 0.5 $mg.kg^{-1}$ MTX was injected to one of zinc-deficient and one of zinc-adequate diet groups intraperitoneally as a single dose (10). MTX injection was not applied to the other groups. Twenty four hours later MTX injection, 4 $\mu g.g^{-1}$ colchicine was injected to all mice intraperitoneally to block the cells in metaphase. Two hours after the colchicine injection, mice were killed by decapitation and blood was collected and then, plasma was separated by centrifugation. The plasma levels of zinc were determined by atomic absorbtion spectrophotometry (Hetachi Z-8000 Model) using an air-acetylene flame after a 1:3 dilution of plasma with deionized-distilled $H_20$. The femur was dislocated from each mouse and bone marrow preparations were performed as described by Schmid (11), with some modification. Bone marrow cells were incubated in 0.07M KCI for 20 min at $37^0$ C and centrifuged at 200g for 5 min. then the pellets were resuspended in 6ml of 3:1 methanol:acetic acid fixative 3 times. They were prepared and stained with 5 % Giemsa in Gurr's buffer for 6 min. at room temperature.

Fifty metaphase cells were examined for each mouse. Numerical and structural abnormalities were evaluated. Hypodiploid cells were not evaluated because of some known reasons (Chromosome loss or preparational artifact). Proportion of aneuploid cells were calculated as twice the proportion of hyperdiploid cells. Additionally, one thousand cells were counted for each mouse to determine the mitotic index and metaphase cells were included only.

Data were analyzed using the z-approximation to compare independent proportions (12). The level of significance was set at 0.05.

## RESULTS

There were no structural and numerical abnormalities in the two zinc deficient groups (one of these received no MTX) and the zinc-adequate-MTX groups when they were compared to the zinc-adequate group(control) ($p>0.05$). These results are presented in Table 1. The mitotic index was significantly lower in zinc deficient-MTX group than the others ($p<0.05$). The same parameter was also lower in the zinc-deficient without MTX

group and the zinc-adequate-MTX group, but these differences were not statistically significant ($p>0.05$). These findings are presented in Table 2. Plasma zinc levels of mice are given Table 3. Plasma zinc levels-deficient mice were significantly lower than control.

Table 1:Distribution of diploid and metaphase chromosomes in mice bone marrow:
MTX:Methotrexate

| Group | Total Meta-phase cells | Structural Abnormalities | | | | Numerical Abnormalities | | |
|---|---|---|---|---|---|---|---|---|
| | | Break | Deletion | Gap | Ring | Hypo-diploid ≤39 | Normal 40 | Hyper-diploid ≥ 41 |
| Zinc-Adequate | 264 | 1 | - | 2 | - | 5 | 257 | 3 |
| Zinc-adequate-MTX | 259 | - | - | 3 | - | 9 | 254 | 2 |
| Zinc-deficient | 280 | 2 | 1 | 4 | - | 11 | 247 | 5 |
| Zinc-deficient-MTX | 292 | 1 | - | 4 | - | 12 | 252 | 4 |

Table 2:Mitotic index proportions
MTX:Methotrexate
* :$p<0.05$

| Group | Proportion % |
|---|---|
| Zinc-adequate | 3.9 |
| Zinc-adequate-MTX | 2.92 |
| Zinc-deficient | 2.86 |
| Zinc-deficient-MTX | 1.56* |

Table 3:Plasma zinc levels of mice

| Groups | Plasma Zinc Level(μg/ml) mean ± s.e.m. |
|---|---|
| Zinc-deficient | 1.14 ± 0.23* |
| Control | 2.42 ± 0.51 |

*$p<0.05$

## DISCUSSION

We found no information on possibility of mutagenicity of zinc deficiency in the literature. Our results suggest that zinc deficiency did not cause structural and numerical abnormalities chromosomes of mice and it has no significantly efect on the mitotic

index. However, zinc-deficient and MTX treated animals had lower mitotic indxes than the other groups ($p<0.05$). In view of these findings, it was suggested that zinc deficiency may cause a sensitization to the effect of cytotoxic agents (perhaps mutagenic agents too). This condition is expected because the role of zinc on DNA replication and cell proliferation via thymidine kinase has been known (3). On the other hand, MTX is a folic acid analogue and impairs the production of thymidine under these condition, a decrease of the mitotic index may be expected. In this study, MTX only one dose has been assayed, besides mitotic index suppressing effect of MTX is known clearly. The higher MTX dose than 0.5 $mg.kg^{-1}$ suppresses mitotic index strongly. Therefore, the effect of zinc deficiency on mitotic index might be covered when it was administered higher than our MTX dose. Although, according to our results, MTX alone appeared no to have a forceful effect on mitotic index; in our opinion this condition may be related to MTX dose as discussed above. Besides, decreasing of mitotic index is clear in the zinc-deficient and MTX administered mice and it is not surprising. However, it needs new detailed investigations on this area.

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