ORIGINAL ARTICLE

ROLE OF ASCORBIC ACID ON ALTERED LEVELS OF TESTOSTERONE DURING CHEMOTHERAPY

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Background: Improvement in quality of life of cancer survivors has become a significant healthcare dilemma. Despite having important part in the support of spermatogenesis, few studies are conducted on Leydig cells secretion, i.e., testosterone which has an important role in fertility The objective of this study was to demonstrate altered serum testosterone levels by anthracycline on mice and to study changes observed in serum level in DOX affected mice with co-administration of antioxidant ascorbic acid. **Methodology:** Male mice aged five weeks were used. The animals were divided into 3 groups of 10 mice each. Animals in Group A were given normal saline intraperitoneally (IP). Group B mice were given DOX IP alone. In Group C, DOX and ascorbic acid were given orally. Blood samples were taken through cardiac puncture. Sera from centrifuged blood were tested for testosterone using ELISA kit. **Results:** Serum testosterone levels were 1.61±1.77, 6.22±4.78, and 1.98±0.45 ng /ml in Group A, B and C respectively. The administration of DOX induced significant increase (p<0.001) in the serum testosterone level. However co-administration of Ascorbic acid with DOX significantly reduced (p<0.001) the serum testosterone level. **Conclusion:** Ascorbic acid has a role against DOX induced altered hormonal levels.

Keywords: Doxorubicin, Ascorbic acid, Mice, Antioxidant, Testosterone

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INTRODUCTION

Usage of chemotherapeutic agents is significantly growing owing to increased number of malignancies globally.[1] Despite their side effects on healthy proliferating cells, their use has become a cornerstone in the process of treatment and has proven to be mitigating effect on cancer complications.[2] Doxorubicin is a widely used chemotherapeutic drug that belongs to anthracycline family. From the very beginning after its induction in cancer guideline therapy, it has been approved and used as a first-line treatment of various cancers both in adults or children.[3] Its mechanism of action is chiefly by inhibiting enzymes crucial for DNA replication.[4] In addition, it produces free radicals that overcame the antioxidant capacity of cells resulting in cytotoxicity.[5]

Experiments have supplied enough evidences regarding doxorubicin's adverse effect on testicular tissue through inducing oxidative stress and apoptosis.[6] Its intercalation into strands of DNA results in double-strand breaks and causes cell death[7,8] in dividing testicular germ cells[9]. The very high vulnerability of sperms and testes to doxorubicin induced damage might be due to a weak anti-oxidant defence mechanism in testicular tissue.[10] Earlier studies have demonstrated pivotal role of antioxidants in improving the quality of the different testicular parameters in infertile males.[11] Keeping this in mind, various antioxidants have been administered to save the testes from doxorubicin injury.[12,13]

Ascorbic acid is from the free radical scavenging group of antioxidants.[14,15] Ascorbic acid has been observed to decline the chances of development of breast cancer.[16] As one of the strong antioxidant, ascorbic acid seems to produce hurdles in DOX producing free radical formation.[17] Marked deficiency in the levels of antioxidants including ascorbic acid was seen in the therapy of DOX in breast cancer. It increases the tendency of healthy tissues to radical damage, proving the call to use antioxidants along with anticancer therapy.[18] The objective of this study was to see the levels of testosterone in DOX affected animals with co-administration of ascorbic acid.

MATERIAL AND METHODS

This study was conducted at the Institute of Basic Medical Sciences, Dow University of Health Sciences (DUHS) Karachi with the approval from the Local Research Committee, Institutional Review Board (IRB), Funding Committee of Dow University and Board of Advance Studies Review (BASR) DUHS Karachi.

Only health thirty male mice of NMRI Strain which were 5 weeks old and caged in animal house of, under normal circadian rhythm. The animals were divided into 3 groups of 10 each. Group A (Control Group) with 10 male mice received 1 ml of normal saline IP on 6th, 8th and 10th day of the study. In Group B

(DOX group) , animals had DOX in dosage of 0.003 mg/g or 0.003 mg in 0.03 ml/gm body weight IP[6] up to 3 doses on 6th, 8th and 10th day of study (total cumulated dose 0.009 mg/gm). Group C (DOX+ascorbic acid group) animals received DOX in a dose of 0.003 mg/gm or 0.003 mg in 0.03 ml/gm body weight IP[6] and ascorbic acid in dose of 0.5 mg/gm or 0.5 mg in 0.01 ml/gm body weight per oral.[19] DOX on 6th, 8th and 10th day of experiment, and ascorbic acid daily.

After exposure, blood samples were collected via cardiac puncture from each mouse. They were run in serum testosterone rat/mouse ELISA kit by Demeditec DEV9911 Germany in Dow Diagnostic Researcj and Reference Laboratory, DUHS.

One-way Analysis Of Variance (ANOVA) was used and then Tukey-Multiple Comparisons Post Hoc test was applied to check the pair-wise comparison at 5% level of significance (95% confidence interval CI).

RESULTS

The Mean±SD serum testosterone in the control group A was 1.61±1.77 ng/ml and in DOX group B it was 6.22±4.78 ng/ml. When the means of control and DOX groups were compared, it showed the *p*-value 0.005 at 95% Confidence interval. A significant increase was observed in the serum testosterone level in DOX group.

Comparison between B and C, Mean±SD observed for serum testosterone group B was 6.22±4.78 ng/ml whereas the Mean±SD observed in DOX+ ascorbic acid group was 1.98±0.45 ng/ml. The *p*-value for this comparison was 0.009 at 95% CI using Tukey-Multiple Comparisons Post Hoc test. A significant decrease was observed in the serum testosterone level in DOX+ascorbic acid group.

Comparison between the A and C, the Mean±SD observed for serum testosterone in control group was 1.61±1.77 ng/ml whereas in DOX+ascorbic acid treated group the Mean±SD was found to be 1.98±0.45 ng/ml. An insignificant increase was observed in the serum testosterone level in DOX+ ascorbic acid group. The *p*-value observed for this comparison was 0.958 at 95% CI (Table-1).

Table-1: Comparison of mean serum testosterone levels in groups (ng/ml)

Groups	Mean±SD	95% Confidence Interval		Tukey HSD Post Hoc multiple comparisons test	
		Lower Bound	Upper Bound		
Control (A)	1.61±1.77	-7.88	-1.33	A *vs* B	*p*=0.005*
DOX (B)	6.22±4.78	0.9582	7.5218	B *vs* C	*p*=0.009*
DOX+ascorbic acid (C)	1.98±0.45	-2.9118	3.6518	C *vs* A	*p*=0.958

*Significant

DISCUSSION

The protective effects of ascorbic acid over other organs of the body have already been established. It seemed to have nullifying effect against the adverse actions of various substances including gamma irradiation, cadmium, mercury, other anthracyclines and organ phosphorous.[20] Role of ascorbic acid in diseases is evident from various studies. Its beneficial part to health may be attributed to its immunomodulator activity along with antioxidant property.[21] The potential of the healthy nutritional environment demonstrated on male reproductive tract in animals.[22] Animals with deficiency of vitamin C show disturbed spermatogenesis process leading to hormonal imbalance.[10] The significant change was observed between DOX treated and DOX+ ascorbic acid treated groups and insignificant change between control and DOX+ascorbic acid groups was noticed.

Alteration in Leydig cells could lead to imbalance in hormones. Increased serum testosterone level in our study was in contrast with the findings by Ward *et al*[23], who reported serum testosterone levels were not significantly affected by this chemotherapeutic treatment. Another study showed that doxorubicin caused a decrease in free testosterone and FSH levels, the later significantly increased as a result of pre-treatment with rutin and/or hesperidin[24] which was in contrast to both our and another study[23]. In another study vitamin C had significant effect on testosterone level and quality of sperm in gentamicin-induced Wistar rats[25]. The germinal cell, because of active division of mitosis and meiosis seems to be more susceptible to chemotherapy. It can be assumed that secretion of testosterone by Leydig cells remains active after treatment with chemotherapeutic agent[26], but need to be further evaluated.

CONCLUSION

Change in level of serum testosterone is indicating increase number of Leydig cells by chemotherapeutic agent. At the same time alteration in levels after receiving ascorbic acid suggest that its effect on chemotherapeutic agent.

RECOMMENDATION

Clinical studies must be conducted to determine both the short-term and long-term impact of antioxidants, singly and in combination, on the efficacy of cancer chemotherapy and on the development of chemotherapy induced side effects especially their effect on hormonal levels and the mechanism of action through which they impact on the levels of hormone.

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AAH: Concept and study design
MK: Drafting, Critical review
DSR: Data analysis and interpretation
RR: Data acquisition and analysis
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DS: Final revision and approval

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