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MOLECULAR-PATHOLOGICAL STUDY IN THE EFFECTS' OF VINCRISTINE SULFATE DRUG ON DNA OF SPERM AND SPERMATOGENESIS IN MICE

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ABSTRACT
The weekly treatment of male mice to Vincristine caused marked reduction in testis weight and sperm parameter as well as animals' weight loses.The early reduction in sperm count was observed after 2 wk of treatment with Vincristine and more permeable after 6 and 8 wks of treatments, statistically significant deference were observed in treated groups in comparison to the control group, Although the natural incidence of abnormal spermatozoon was ≈13 % after exposure to Vincristine for 2 wk-4wks, increased the number of morphologically abnormal spermatozoa, approximately three-fold after treatment with Vincristine for 8 wk, the frequency of abnormal spermatozoa was four fold higher than the control spermatozoa was observed, DNA damage of sperms DNA was detected by using FISH molecular test for detection abnormal chromatin structure within DNA of sperm .Early defects were observed after 2 wks of treatment with Vincristine sulfate with and were more prominent after 4, 6, 8 wks of treatments, significantly deference's from the control group Early loss of spermatogenesis appear after 2 wks of treatment but were more prominent after 6 and 8 wks of treatment with Vincristine with more prominent pathological changes represented by severe vacuolation due to hydropic degeneration and empty spaces of somniferous' tubules refers to decreased sperms' number, due to desquamation of sertoli cells, fibrosis' replacement in seminiferous empty space. The result showed that mice treated with Vincristine sulfate showed marked reduction in weight, loss of Spermatogenesis, sperm DNA damage and hydropic degeneration of germinal epithelial linings seminiferous tubules and interstitial testicular edema.

KEY WORDS: Vincristine sulfate, DNA sperms', Spermatogenesis, mice

INTRODUCTION

Vincristine sulfate (VCR)

Vincristine is member of Vinca alkaloids which are large, complex molecules derived from the periwinkle plant used to treatment of cancer they are class of cell- cycle-specific cytotoxic drugs which working by inhibiting the ability of cancer cell to divide. Vinca alkaloids inhibit microtubule polymerization and increase microtubule dissembly.The mitotic spindle apparatus is disrupted, segregation of chromosomes in metaphase is arrested. These effects account for the primary M-phase action of vinca alkaloids, although other antitubulin effects related to cytoskeleton synthetically and used as drug in cancer therapy and as immunity suppressant drugs[1]. These agents do not work to alter DNA structure or function but interfere with mechanics of cell division. During mitosis, the DNA of a cell is replicated and then divided in to two new cells. The process involves spindle fiber, Which are constructed with microtubules and so that these agents called spindle inhibitors[2] Vincristine sulfate (VCR) is admire-indo-alkaloid extracted from the leaves of periwinkle plant (Catharanthusroseus)which is used for the treatment of several forms of malignancy[3,4] however , the clinical use of this drug is limited chiefly because it sensor motor neuropathies [5].

Vincristine sulfate effects on sperm

Vincristine sulfate induce wide spectrum of divisional aberration which result in mitotic arrest, multiple spindlier polyploidy and aneuploidy in a Varity of invitro and in vivo test Systems[6,7,8]. Vincristine treatment of Hodgkin is disease increase the frequencies of the specific types of aneploidy sperm that might elevate the risk of Fathering a child with one of the major clinical aneuploidy syndromes *i.e*. Down (disomy21sperm) Edward (disomy 18 sperm).Turner (nullisomy sex sperm), XYY (disomy Y sperm), triple X (disomy Sperm) or Klinefelter (Xy sperm) and he concluded that VCR chemotherapy for Hodgin's disease transiently induces sperm aneuploidies associated with major clinical aneuploidy syndromes involving Chromosomes X,Y,18 and 21[9]. The gentoxic effects of VCR on germs cells of male mice were investigated and several parameters (the scale and time course of unscheduled and scheduled DNA synthesis in spermatocytes and spermatids and the number of sperm present in caudal epididymides) were analyzed the results show that I/P administration of single dose of VCR resulted in (i) damage to DNA in spermatocytes and spermatids (ii) reduction in the rate of germ-cell development and (iii) killing of the non-proliferating spermatid cells. Damage of DNA in germ cells indicates that VCR may have potential genetic hazards to patients who receive it in antitumor therapy [10] resulting in cell death of resting spermatocytes and cause damage mostly in G1 and S phase of primary spermatocytes. However, at doses causing death of G1 and S-phase spermatocyt, the most sensitive stage of primary spermatocytes development [11] Vincristine crossed the blood. Placenta barrier to produce cellular changes might play an

important role in initiating growth defects[12]. Vincristine reduced fertilization ability of the germ cell stages in male mice results of DNA damage of sperm. Male mice and rats were administered I/P VCR (10µg) for 15 days, VCR caused conspicuous pathological change in the principle and apical cells of the caput and the clear cells of the cauda the study points to toxic effect of VCR on these cell types, suggesting impairment of epididymal function particularly concerning sperm maturation and endocytotic removal of contents of the cytoplasm droplets and dead sperm [13].

MATERIALS & METHODS

Twenty male mice were kept in animal house of Baghdad Medicine College and fed on pellet for lab. Animal and provide with tap water in special tubes and injected with Vincristine sulfate I/P(0.01mg/10gm b.w)for four separated period of time (2-4-6-8)weeks, at four separated subgroups[14] after scarified animals at each end of two weeks, animals submitted to weight as well as testes weight before fixation and sperm specimens collection directly from epididymides of each animals with direct seminal fluid analysis with used CASA[15] (fig.1.Tissue specimens for paraffinized section as well as seminal fluid samples were prepared from each male for done sperms' DNA damage detection by FISH procedure[16] Histopathological processing done [17] and staining methods were performed according [18].

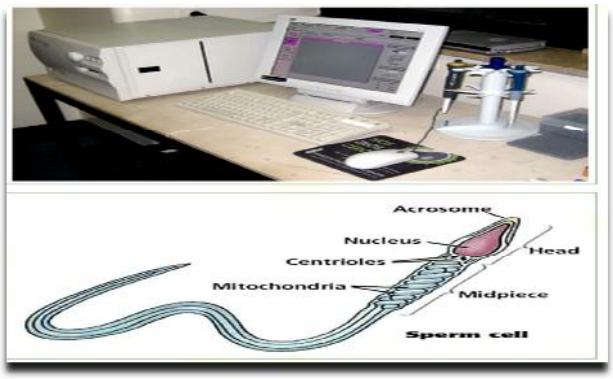

FIGURE 1: Computer Assist Seminal Analysis **(CASA)**

RESULTS & DISCUSSION

1. Results of Vincristine effects on body weight and testes weight after each period of treatment were showed in table (1) as well as sperm count and morphological abnormalities were showed in table (2). our results of animal weighting losses and testes weight lose agreed with [20] who explain that VCR induced weight loss and testicular tissue atrophy and toxic death in mice injected I/P with VCR in dose of 1mg /kg^{-1} VCR due to the effects of starvation, which were comparable to the effects of drug-induced weight loss contributed to its toxic effects to cell-cycle prevents mitosis and reduction of proliferative capacity.

TABLE 1: effects of Vincristine sulfate on body weight and testes weight

Group N=5	Treatment	Initial mean B.W (g)	Terminal mean B.W(g)	Testes mean weight (mg) ± s.d	Testes weight (mg) as a % of control	Testes weight (mg) as a % of B.W
1	VCR(0.1/l0gm b.w) I/P for 2wk	24.5	21.7	181±25.6*	74.5	0.51
2	VCR(0.1/l0gm b.w) for 4wk	25.2	20.1	162±35.6***	53.6	0.40
3	VCR(0.1/l0gm b.w) for 6wk	23.3	17.4	122±38.0***	28.2	0.23
4	0.1/l0gm B.W VCR for 8wk	24.8	14.2	118±33.6***	20.5	0.15
5	control	25.3	35.2	222±25.6	-	0.60

Significantly different from corresponding control as assessed
By analysis of variance: *p< 0.05;** p <0.01;*** p < 0.001.

TABLE 2: effects of Vincristine sulfate on sperm counts and morphology

Group (n=5)	Treatment	Sperm count ×10^6 ± s.d	Abnormal spermatozoa %± s.d
1	VCR (0.1mg /10 gmb.w) for 2wk	2.88±1.21**	31.9±10.1# # #
2	VCR(0.1mg /10 gmb.w) for 4wk	2.30±0.63***	45.7±11.4# # #
3	VCR (0.1mg / 10 gmb.w) for 6wk	2.16±1.43***	52.5±11.7# # #
4	VCR(0.1mg /10gm b.w) for 8wk	1.28±0.63***	77.2±17.3# # #
5	control	6.15±1.22	13.4±1.04

Significantly different from corresponding control as assessed
By the x2 test: ### p < 0.001.

Our results of the reduction of sperm count and abnormal morphology agree with [19, 20] whom improved that VCR (1.0 mg/kg injected I/P to mouse reduced sperm count as well as normal sperm morphology to 79% of controls ($p<0.05$).

2-Results of the effects of Vincristine on DNA of sperm that's detected by FISH procedure Reported in table (3-A & B).

TABLE 3- A: Results of TK (11qE2)/Y DNA mouse probe applied by FISH technique on paraffin embedded section of testicular tissue samples

Group - / Treat n=5	Green signal(a)			Red signal (a)			No Signal (a)
	Score 1	score 2	score 3	Score 1	score 2	score 3	
1-/ 2wk	2	3 A	0	0 A	1	4	0
2- /4wk	5	0 A	0	0 A	1	4	0
3- /6wk	5	0 A	0	2 A	3	0	0
4- /8wk	2	0 A	0	3 A	2	0	3 A
5 -/control	0	3 A	2	0 A	0	5	0

A:significant differences between green and red signals at ($p < 0.01$)
A: significant differences with increase time of treatment at ($p < 0.05$).

TABLE (3-5 B): Results of TK (11qE2)/Y DNA mouse probe applied by FISH technique on Seminal Fluid Samples

Group - / Treat. n=5	Green signal (a)			Red signal (a)			No Signal (a)
	Score 1	score 2	score 3	Score 1	score 2	score 3	
1-/ 2wk	0	2 A	3	0	0 A	5	0
2- /4wk	3	0 A	1	0	1 A	4	1 A
3- /6wk	3	1 A	0	1	4 A	0	1 A
4- /8wk	2 A	0	0	4	1 A	0	3 A
5 -/control	0	2 A	3	0	0 A	5	0

A: significant differences between green and red signals at ($p < 0.01$)
A: significant differences with increase time of treatment at ($p < 0.05$).

The results showed that there were, evidence of DNA damage in germ cells with poor sperm quality and quantity was observed after exposure of all stage of spermatogenesis early and advanced age of mouse and in continuously period from 2-8 wk, and 73-93 % of male mice were found to be infertile after experimental mating with not treated female, this contributed to poor semen quality as results of DNA damage of sperm after treatment with Vincristine and this agreed with[5,13,21,23] in animal and with [22,24,25,26,27] in human.

3-Resuls of microscopic detection of DNA damage which done by Fluorescence's microscopy for genetic examinations methods.fig(2)showed the control picture of XY gene, fig(3)showed the DNA damage signal of XY gene after treatment with Vincristine(green).,fig(4)showed the DNA damage signal of XY gene after Vincristine treatment(red).

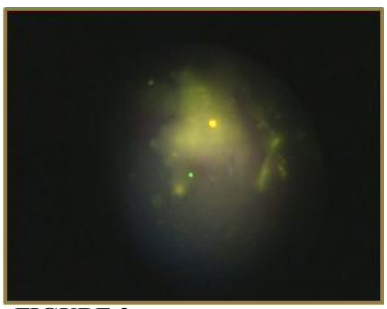

FIGURE 2: signal green and red of contol not treated sperm.

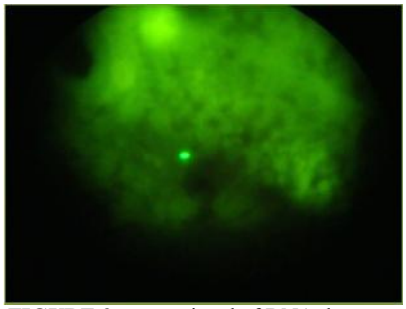

FIGURE 3: green signal of DNA damage of sperm XY Chromosome

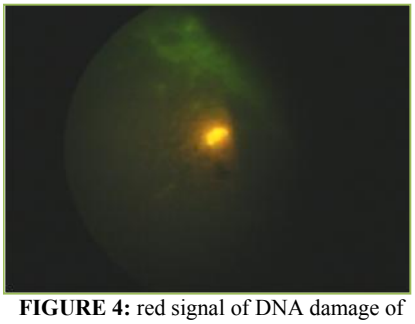

FIGURE 4: red signal of DNA damage of sperm XY chromosome

DNA damage of sperm after treatment with Vincristine agree with what founded by[29, 30] whom improved that the sperm with marked DNA damage examined FISH assay have the strongest effect of decreased in the number of total implantation and the number of live fetuses after sexual mating of exposed male with not treated female mouse, and said that's' sperms DNA defects were resulted from exposure of spermatid and early spermatocytes and Spermatogonia to Vincristine .

4-Results of pathological changes in testes tissue and represented by early loss of spermatogenesis after 2wks of treatment fig.(5)and marked loss of sperm after 4wks of treatment fig. (6) and fibrotic lesion with sever loss of spermatogenesis after 6 and 8 wks of treatments fig (7). with vacuolation and desquamation of sertoli cell and haemohrage foci inter tubular spaces fig. (8) as well as fibrosis replacements in the seminal tubules after 6 and 8 wks of treatment with Vincristine.

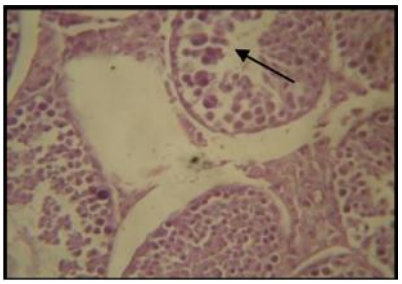

FIGURE 5: Testes: early lose of sperm after 2 wks of treatment

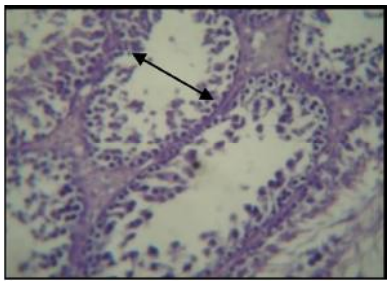

FIGURE 6: Testes: marked lose of sperm after 4 wks of treatment.

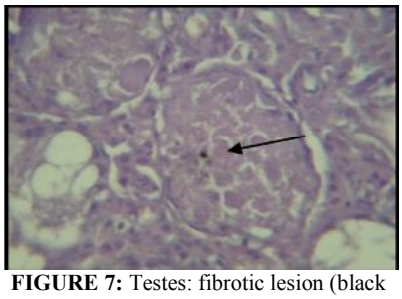

FIGURE 7: Testes: fibrotic lesion (black arrow) due to sperm complete lost.

FIGURE 8: Testes:vaculation (black arrow), sertoli desqumation (red arrow), haemorrage (white arrow)

Cytotoxic effects of Vincristine on spermatogenesis may disrupt sperm for motion by targeting various testicular cell types (Leydig cells, sertoli cells and germ cells) and by activating numerous molecular pathways involved in germ cell life and death decision making genetically modified animal models with deficiencies in specific proapoptotic and prosurvival pathways have become powerful tools in understanding the molecular regulation of spermatogenesis and the response of the semniferous epithelium to toxic injury[10]. Toxic effects of Vincristine are discussed to highlight roles of p53 and fas system as modulators of proapoptotic activity in the testis[28]. Vincristine treatment in mouse induced structural chromosomal aberration on spermatocytes, this attributed to interfering with DNA replication by prevent the cell form entering G1 phase cause an arrest of mitotic and meiotic division to metaphase followed by cell death so result in sperm defeated or no sperm formation , this agree with [5].

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