Histological Grading of Ethambutol Induced Optic Toxicity in Rabbits

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ABSTRACT

BACKGROUND: Ethambutol is one of the first line anti tuberculous drug. Its toxicity is low with no evidence of teratogenecity. Optic toxicity is one of its major side effects which is dose and duration dependant. Etambutol causes both bulbar and retrobulbar neuritis, latter being more common ,which manifests as painless, subacute, symmetrical, progressive visual loss, reduced visual acuity, scotomas and dyschromatopsia.

OBJECTIVES: To study and grade the dose and time related ethambutol induced toxic histological changes in optic nerves of rabbits.

MATERIAL & METHODS: This experimental study was conducted on 24 rabbits of either sex weighing 1.5- 2.5 kg. Rabbits were randomly divided in to four groups comprising six animals in each group. Group A served as control (no drug given),while Group B,C and D were given ethambutol in doses of 35, 50 and 100 mg/kg/day. Three animals from each group were sacrificed at 6 and 12 weeks and their both optic nerves were taken out and subjected to histopathological examination. The histological changes were recorded and graded according to rating scale and analysed with SPSS version 17 to see the dose and time related ethambutol induced optic neuritis. Statistical analysis was done using SPSS version 17. Paired "t" test was applied to compute the differences in histological changes between various groups. The differences were statistically significant (P <0.05).

RESULTS: The histological findings showed that optic nerves of group A were quite normal.At 6 weeks the histological changes at Ethambutol doses of 35, 50,100mg/kg/day were 3.17± 753, 4.00±.894 and 5.33±.816 respectively.At twelve weeks the histological changes at Ethambutol doses of 35, 50,100 mg/kg/day were 4.00±.894, 5.00±.00 and 7.00±00 respectively. Ethambutol toxicity did not increase significantly at 6 weeks versus 12 weeks with 35mg/kg/day (P value 0.185). Ethambutol toxicity increased with 50mg/kg/day at 6 weeks versus 12 weeks (P value 0.041). While Ethambutol toxicity also significantly increased with 100mg/kg/day at 6 weeks versus 12 weeks (P value 0.004).

CONCLUSION: Our study confirmed that ethambutol induced optic toxicity is both dose and duration dependent in rabbits, which were graded on the basis of histological changes.

keywords: *Ethambutol, optic toxicity, histological changes.*

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INTRODUCTION:

Ethambutol is a water soluble heat stable compound and one of the first line antimycobacterial drug[1,2,3]. Its toxicity incidence is low[4], with no evidence of teratogenecity[5]. Its antimycobacterial activity is due to its chelating ability that disrupts the essential metal containing enzyme systems that cause bacterial cell death.[5] Its main side effect is optic neuritis[6,7]. Optic toxicity occurs in 18% of patients who receive ethambutol in doses of more than 35mg/kg/day, while it occurs in 5 to 6% and less than 1% in patients who receive ethambutol in doses of 25mg and 15mg/kg/day respectively[8]. Its bacteriostatic and bactericidal effects are achieved at daily doses of 15mg/kg and 25mg/kg,respectively[9,10]. Etambutol causes bulbar and retrobulbar neuritis, latter being more common, which manifests as painless, subacute, symmetrical, progressive visual loss, reduced visualacuity, scotomas and dyschromatopsia[4,11,12]. Ethambutol induced ocular toxicity is dose and duration dependant according to the individual susceptibility, however, it may prove to be toxic at any dose even as low as 12.3mg/kg/day. This effect is idiosyncratic[4,13]. It has been observed that Ethambutol induced ocular toxicity is reversible on discontinuation of the drug, however, a permanent damage has been reported in some cases at the therapeutic doses of 15-25 mg/kg/day[11,12]. Several factors may be responsible for irreversible damage such as old age, delayed diagnosis, alcoholism, diabetes mellitus, anemia and renal failure etc.[14] Different experimental animals treated with toxic doses of ethambutol revealed vacuolations, demyelination, axonal fragmentation and central necrosis with inflammatory changes in their optic nerves[15,16]. Vacuolation represents the main histological base of optic toxicity produced by Ethambutol by its indirect stimulation of N-methyl-D-aspartic acid (NMDA) channel, present in the retinal ganglion cells by the endogenous glutamates. Ganglion cells become more sensitive to glutamate due to an increased concentration of extracellular calcium and disturbance of energy balance in mitochondria[17]. Ethambutolis also reported to chelate intracellular Zinc which

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is essential for modulation of endonuclease. This enzyme becomes dysregulated in the absence of Zn, resulting in DNA cleavage and cell death. Other target of Zn chelation by Ethambutol is the ATPase inhibitory protein IF1 which itself is inhibited by Zn. Chelation of Zn allows the inhibition of ATPase acitivity by IF1 that results in decreased ATP synthesis. This inhibits mitochondrial dehydrogenase activity, resulting in death of ganglion cells. Ethambutol also chelates iron and copper which are essential for complex I and IV function respectively in the electron transport chain. It interferes with the process of oxidative phosphorylation and causes a decrease in ATP production and compromises the axonal transport, thus initiating the cascade of event sleading to cell death[17-21]. The exact mechanism for the formation of vacuoles is not clear, however, two theories are proposed; Ethambutol mediated increased Ca^{+2} influx activating certain intracellular enzymes including phospholipases, resulting in cell membrane damage, proteases promoting digestion of membrane and cytoskeletal proteins endonucleases associated with chromatin fragmentation and ATPases resulting in ATP depletion. The result is the failure of Na^{+} K^{+}pump leading to cellular swelling, anaerobic glycolysis, impaired protein synthesis an daccumulation of lipids inside the cell that microscopically appear like vacuoles due to processing techniques. In other mechanism, theme tabolites of Ethambutol form a complex with Zn and Cu which enters the axons and enhance the axonal dilatation (vacuolation) process.[20]Ethambutol, in toxic doses, induces the death of oligodendrocytes in optic nerve, resulting in demyelination of optic nerve. Oligodendrocytes are one of the glial cells that are responsible for deposition of myelin around the axons in central nervous system[22]. Ethambutol toxicity is mediated through the endogenous glutamates by the sustained activation of AMPA and NMDA receptors. It induces the calcium influx through the receptor channel which alters Ca^{++} homeostasis and induces a change in the mitochondrial membrane, leading to release of proapoptotic molecules such as cytochrome *c* and apoptosis-inducing factor. This results in oligodendrocyte death through caspase-dependent and independent pathways[23-25]. Ethambutol induced oligodendrocytes death through the endogenous glutamate also appears to involve a mechanism that ultimately renders the cell vulnerable to oxidative stress, probably because of depletion of cystine leading to depletion of glutathione. It was confirmed by addition of cystine that totally prevented the glutamate toxicity to oligodendroglia[26]. Aim of this study was to study and grade Ethambutol induced histological changes in optic nerves of rabbit.

MATERIAL& METHODS:

The study was conducted on 24 rabbits of either sex about 1.5 to 2.5 kg in weight in the animal house of Saidu Medical College Swat. The rabbits were divided into group A,B,C and D each containing 6 rabbits. Group A served as control group, while group B,C and D served as experimental groups. Each group was kept in a separate compartment under controlled conditions of temperature 20 ±0.5 C^{0} and humidity (50 ±10%) and 12 hours light and dark cycle. They were given standardized diet and water ad libitum. Group A served as control (No drug given). Group B, C and D were treated with ethambutol 35,50 and 100mg / kg /day respectively for 6 weeks followed by sacrifice of 3 rabbits from each group. The remaining 3 rabbits in each group were given the same drug in same doses for another 6 weeks. Finally all the 3 rabbits in each group were sacrificed at 12 weeks. Both optic nerves were harvested and removed as a single piece along with the eye balls and subjected to histopathological examination. Tissue processing was carried out for histological examination. Staining with hematoxylin and eosin was done in usual way. The slide preparations were examined under the light microscope at X40 magnification and observations were recorded. The different histological changes were recorded and graded according to a rating scale.

The slides were prepared and checked under the microscope and the histopathological changes were recorded and rated according to the scale as explained in the table No. 1. The changes recorded at 6 and 12 weeks were put in SPSS version 17 and the results were analysed. The histopathological changes were divided into two categories i.e. Category A and Category B. Category A changes are based on nuclear alignment of schwann cells, which are indirectly suggestive of demyelination. Category B changes are based on loss of eosinophilia (loss of protein content) in cytoplasm evidenced by vacuolation. The histological changes were graded as under.

Category A

1: No Change (Normal Histology)

2: Nuclear alignment of schwann cells along the nerve fiber intact with sparse clumping of nuclei at some places.

3: Nuclear alignment of schwann cells along the nerve fiber intact with frequent clumping.

4: Nuclear alignment of schwann cells along the nerve fiber lost with large clumping of nuclei.

Category B

1: No Change (Normal Histology)

2: Mild loss of eosinophilia with appearance of small vacuoles in the cytoplasm.

3: Gross loss of eosinophilia with appearance of large vacuoles in the cytoplasm.

SPSS version 17 was used for all analyses. Means of histopathological changes in each slide were computed. All data were expressed as means ± standard deviation. Paired t-test was applied to examine the differences between various experimental and control groups, the differences were examined for statistical significance using the same paired t-test. The P value of significance was set at < 0.05.

RESULTS:

Histopathological changes at ethambutol doses of 35, 50 and 100mg/kg/day in groups B, C and D at 6 weeks interval (n=6) were 3.17 ± .753, 4.00 ±.894 and 5.33±.816 respectively, as shown in table 2. These figures show that optic toxicity increased with increase in dose of ethambutol.

Histopathological changes at ethambutol doses of 35, 50 and 100mg/kg/day in groups B, C and D at 12 weeks interval (n=6) were 4.00±.894, 5.00±00 and 7.00±00 respectively, as shown in table 3. These figures show that optic toxicity increases with increase in both dose and duration from 6 to 12 weeks.

The following results were obtained after putting the above data in SPSS version 17 and applying paired t-test to compare the histopathological changes in different groups receiving the same doses at 6 and 12 weeks intervals. Group A was used as control and showed normal histology. Ethambutol toxicity did not increase significantly at 6 weeks versus 12 weeks with 35mg/kg/day (P value 0.185). There was significant increase in toxicity at ethambutol doses of 50mg/kg/day and 100mg/kg/day at 6 vs 12 weeks (P Values .041 and .004) as shown in table 4.

Table 1: Rating scale of histological changes (assessment tool)

Category A Changes :	Rating	Category B Changes	Rating
Sub Group 1	2	Sub Group 1	2
Sub Group 2	3	Sub Group 2	3
Sub Group 3	4		

Table 2: histological changes in B, C and D due to ethambutol at 6 weeks (n=6)

Groups	Dose	Rating of histological changes according to scale						Mean	S.D
B	35mg/kg/day	2	4	3	3	4	3	3.17	.753
C	50mg/kg/day	3	5	4	5	3	4	4.00	.894
D	100mg/kg/day	5	6	4	5	6	6	5.33	.816

Table 3: Histological changes B, C and D due ethambutol at 12 weeks (n=6)

Groups	Dose	Histological changes according to rating scale						Mean	S.D
B	35mg/kg/day	5	4	3	5	4	3	4	.894
C	50mg/kg/day	5	5	5	5	5	5	5	.000
D	100mg/kg/day	7	7	7	7	7	7	7	.000

Table 4: Toxicity due to ethambutol at 6 vs 12 weeks

Pairs	P value
Pair I Group B 6 weeks vs Group B 12 Weeks (35mg/kg/day)	.185
Pair II Group C 6 weeks vs Group C 12 Weeks (50mg/kg/day)	.041
Pair III Group D 6 weeks vs Group D 12 Weeks (100mg/kg/day)	.004

DISCUSSION:

The aim of the present work was to study and grade Ethambutol induced toxicity on the basis of histological changes with respect to dose and duration. Our results show that Ethambutol has a toxic effect on the optic nerve at 6 weeks at different doses of Ethambutol as shown in table 2. Optic toxicity increased with the increase in dose of Ethambutol in our study. Histological changes increased with the increase in dose from 35mg/kg/day to 100mg/kg/day both at 6 and 12 weeks duration as shown in table 2 and 3. In our study Ethambutol toxicity did not increase significantly at 6 weeks versus 12 weeks with 35mg/kg/day (P value 0.185). Ethambutol toxicity increased with 50mg/kg/day at 6 weeks versus 12 weeks (P value 0.041). While Ethambutol toxicity also significantly increased with 100mg/kg/day at 6 weeks versus 12 weeks (P value 0.004). These results show that Ethambutol toxicity increased with increase in duration except at dose of 35mg/kg/day. Leiboid[8] *et al,* and Citron[6] *et al,* studies also show that optic neuritis increases with the increase in dose of Ethambutol. According to a review performed by Donald[27] *et al,* more than 40% of patients developed toxicity at doses greater than 50 mg/ kg, and only 0-3% developed toxicity at a dose of 15 mg/kg/daily. According to Malamud[28] *et al,* the toxicity is unpredictable and may even be irreversible. Some studies show that Ethambutol toxicity may be idiosyncratic and not related to the dose and duration of Ethambutol.[29,30]. Kumar[31] *et al,* and Tsai[32] *et al,* studies show that ocular toxicity increases with increase in duration of Ethambutol treatment. In a study on rabbits, vision loss due to optic nerve toxicity was seen more in rabbits who received Ethambutol in a dose of 25mg/kg/day or more. However, visual impairment has been reported in approximately1% of rabbits receiving the drug in the recommended therapeutic dose of 15 to 25mg/kg/day. Toxicity usually occurs not earlier than 2 months of treatment with 7 months as average. Patients who have impaired renal functions showing improvement in their vision after drug discontinuation is not always a complete recovery[33].Ethambutol produces histological changes in optic nerve by interfering with the mitochondrial functions that disturb the physiology of axonal transport and axon myelininter actions, This leads to a complex sequence of events that results in damage to the axons.[18,34] The axonal damage may present itself as a change in the diameter of optic nerve. Demyelination, inflammatory changes, myelin like structure in the axoplasm and vacuolation had already been reported in rat dog, monkeys and rabbits.[15,16,35,36]Our study showed and confirmed prominent vacoulations in group B which further increased in group C and D with increase in dose and duration. Other reported histological changes like demyelination and fragmentation were also evident in our preparations, In our study Ethambutol was used in dose of 35,50 and 100mg/kg/day for 12 weeks for confirmation of its toxic effects. In our study vacuoles were observed in all preparations in experimental groups. Ethambutol produced marked toxic histological changes in all preparations of optic nerves in group B, C and D. The results of our study are supported by the earlier observations reported in many studies.[15,16,18,34-38]Ethambutol was observed to cause a bilateral stereotyped clustered or scattered vacuoles in the distal part of optic nerve and optic chiasma without any evidence of demyelination[14]. This observation has also been seen in additional experimental studies.[16,35,37]

Ethambutol, in toxic doses, induces the death of oligodendrocytes in optic nerve, resulting in demyelination of optic nerve. Oligodendrocytes are one of the glial cells that are responsible for deposition of myelin around the axons in central nervous system.[22]

CONCLUSION:

The data in our study concluded that Ethambutol in dose of 35,50 and 100mg/kg/day for 12 weeks given to experimental groups of rabbits resulted in marked vacoulation, demylination indicating optic nerve toxicity which is dose and duration dependant.

REFERENCES:

1. Sterling TR, Lehmann HP, Frieden TR. Impact of DOTS compared with DOTS-plus on multidrug resistant tuberculosis and tuberculosis deaths: decision analysis. BMJ2003;326:574:16.
2. Chan R, Kwok A. Ocular toxicity of ethambutol. Hong Kong Med J 2006;12:56-60.
3. Zoumalan CI, Agarwal M, Sadun AA. Optical coherence tomography can measure axonal loss in patients with ethambuto induced optic neuropathy. Graefe's Arch ExpOphthalmol2005;243:410-16.

4. Choi SY, Hwang JM. Optic neuropathy associated with ethambutol in Koreans. KoreansJ.Ophthalmol1997;11:106-10.
5. Lewit T, Nebel L, Terracina S, Karman .Ethambutol in pregnancy: Obesrvation on embryogenesis.Chest 1974; 66:25-6.
6. Citron Km, Thomas Go. Ocular toxicity from Ethambutol.*Thorax*1986;41:737-739.
7. Chen I, Liang Y. Optic nerve neuropathy by Ethambutoltoxicity.*ZhonghuaJieHe HeHu Xi ZaZhi*1999;22:302-4
8. Leiboid Je. The ocular toxicity of ethambutolad its relation to dose.*Ann New York AcadSci1966*;135:904-9.
9. William A, Petri Jr. Chemotherapy of tuberculosis, mycobacterium avium complex disease and leprosy. In: Brunton LL, Lazo JS, Parker KL, editors. Goodman &Gilman,s The pharmacological basis of theraputics. 11th ed. USA: Mcgraw-Hill; 2006; p: 1203-10.
10. Abernathy RA. Tuberculosis: An update. Pedeatrics in Review. 1997;18:50-8.
11. Lim SA. Ethambutol associated optic neuropathy. Ann Acad Med Singapore 2006;35:274-8.
12. Zoumalan CI, Agarwal M, Sadun AA. Optical coherence tomography can measure axonal loss in patients with ethambutolinduced optic neuropathy. Graefe's Arch ExpOphthalmol. 2005;243:410-16.
13. Chan R, Kwok A. Ocular toxicity of ethambutol. Hong Kong Med J 2006; 12:56-60.
14. Takada R, Takagi M, Oshima A, Miki A, Usui T, Hasegawa S, et al. Delayed visual recovery from severe ethambutol optic neuropathy in two patients with atypical mycobacterium infection. Neuroophthalmol 2005;29:187-93.
15. Lessell S. Histopathology of experimentalethambutol intoxication. Invest Opthalmol VisSci1976;15:765-9.
16. Matsuoka Y, Mukoyama M, and Sobue I.Histopathological study of experimentalethambutol neuropathy. Clin. Neurol1972;12:453-5.
17. Hahn JS, Aizenman E, Lipton SA. Centralmammalian neurons normally resistant toGlutamate toxicity are made sensitive byelevated extracellular Ca 2+: Toxicity is blocked by the N-methyl-D aspartateantagonist MK-801. PNAS1988;85:6556-60
18. Sadun AA. Mitochondrial Neuropathies.JNNP2002;72:424-6.
19. Heng JE, Vorwerik CK, Lessel E,Zurakowski D, Levin LA, Dreyer EB.Ethambutol is toxic to Retinal Ganglion Cellsvia an Excitotoxic Pathway. Invest. OphtalmolVisSci1999;40(1):190-6.
20. Kozak SF, Inderlied CB, Hsu HY, Heller KB,Sadun AA. The role of Copper onethambutol's antimicrobial action andimplications for ethambutol-induced opticneuropathy. DiagnMicrobiol Infect Dis1998;30:83-7.
21. Snell RS, Lemp MA, Clinical Anatomy of theEye. 2nd ed. Malden: Blackwell Science;1998
22. Zubair M, Ahmad I, Bashir A, *Sheikh HT, Samee W, Malik J*Optic Nerve Oligodendropenia, induced by EthambutolP J M H S 2013; 7(2) 906-10.
23. Matute C, Sanchez-Gomez MV, Martinez-Millan L, Miledi R. Glutamate receptor-Mediated toxicity in optic nerve oligodendrocytes. ProcNatlAcadSci 1997;94:8830-5.
24. Kolodziejczyk K, Hamilton NB, Wade A, Karadottir R, Attwell D. The effect of N-acetyl-aspartyl-glutamate and N-acetyl-aspartate on white matter oligodendrocytes. Brain 2009;132:1496-1508.
25. Sanchez-Gomez MV, Alberdi E, Perez-Navarro E, Alberch J, Matute C. Bax and calpain mediate excitotoxicoligodendrocyte death induced by activation of both AMPA and Kainate receptors. J Neurosci 2011; 31:2996-3006.
26. Oka A, Belliveau MJ, Rosenberg PA, Volpe JV. Vulnerability of oligodendroglia to glutamate: Pharmacology, Mechanisms, and Prevention. J Neurosci 1993;13:1441-53.
27. Donald, P. Ethambutol dosage for the treatment of children: literature review and recommendations [Review Article]. The International Journal of Lung Disease 2006; 10(12);1318-30.
28. Melamud A, Kosmorsky GS, Lee MS. Ocular EthambutolToxicity.*Mayo Clinic Proc. 2003;*78:1409-141
29. S Ali.Rapidly developing Optic Neuritis secondary to Ethambutol: possible mechanism of injury(case report). J Pak Med Assoc 2005;55(7)
30. Karnik. A case of ocular toxicity to ethambutol--an idiosyncratic reaction? Postgrad Med J 1985; 61:811-13.
31. Kumar A, Sandramouli S, Verma L, et al. Ocular ethambutoltoxicity: is it reversible? *J ClinNeuroopthalmol*1993;13:15-7.
32. Tsai RK, Lee YH. Reversibility of ethambutol optic neuropathy.*JOculPharmacolTher1997;*13:473-7.
33. Sivakumaran P, Harrison AC, marschner J, Martin P. Ocular toxicity from ethambutol: a review of four cases and recommended precautions.The New Zealand Medical Journal 1988;111(1077):428-430.
34. Kozak SF, Inderlied CB, Hsu HY, Heller KB,Sadun AA. The role of Copper on ethambutol'santimicrobial action andimplications forethambutol-induced optic neuropathy.DiagnMicrobiol Infect Dis. 1998;30:83-7.
35. Schmidt, I G. Central nervous system effectsof ethambutol in monkeys. Ann. N. Y. Acad.Sci. 1966;135:759-63
36. Noche RR, Nicolas MG, Biochem MS,Gonzaga NC. A Study of Optic neuritis caused by Ethambutol in Rabbits. PJMI1987;17:42-6.
37. Tateishi J. Experimental myelo-opticoneuropathydue to ethambutol. Folia Psychiatr.Neurol. Jap. 1974;29:233-42.
38. Romanes GJ. Cunningham's Manual ofPractical Anatomy. Vol. 3. 15th ed.NewYork: Oxford;2004.