EFFECT OF IRON CHELATING AGENTS DESFERRIOXAMINE, DEFERIPRONE AND THEIR COMBINATION ON BRAINSTEM AUDITORY EVOKED POTENTIAL (BAEP)

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Abstract

The purpose of this study was to detect sub clinical lesions of auditory pathways in thalassemic patients undergoing iron chelation therapy non invasively by doing brain stem auditory evoked potential. 45 patients of thalassemia receiving iron chelation therapy were divided into three groups of 15 patients each. First group received desferrioxamine, second group received both desferrioxamine and deferiprone and third group received deferiprone only Brainstem auditory evoked potentials were recorded in patients by using RMS EMG EP Mk2. Absolute latencies of waves I, III & IV were significantly prolonged (p<0.05) in patients receiving desferrioxamine when compared with patients on deferiprone & combination therapy. Also inter-peak latency III-V was significantly increased (p<0.01) in patients on desferrioxamine. From our study we also concluded that the toxicity exerted by desferrioxamine is dose dependent. Our findings indicate that it should be imperative to monitor hearing functions in patients receiving DFO. The subjective tailoring of optimal safe dosage of DFO must be done on the basis of degree of iron overload at that time. It is advocated to maintain patients with the lowest effective dose of DFO or combination of DFO and deferiprone with regular monitoring of their audiologic performance for sub clinical diagnosis of effect on auditory nerve so as to prevent the impending sensorineural hearing loss.

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Key Words: thalassemia, desferrioxamine, deferiprone, ototoxicity,
INTRODUCTION

Thalassemias are a group of inherited hematologic diseases that present early in life with hypochromic anaemia of varying degree of severity. The genetic defects underlying this disease include partial or total deletion of globin chain genes and nucleotide substitution, deletion and insertions resulting in decreased or suppressed synthesis of Hb polypeptide chain. Thalassemia major describes the severe transfusion dependent anaemias. These children generally become symptomatic as severe progressive anaemia during 6th to 9th month of life. The children suffering from thalassemia require regular blood transfusion to maintain hemoglobin levels in blood. Although transfusion therapy improves the quality of life and also survival of these patients is prolonged\(^1,2\) there occurs accumulation of iron in the body because of lack of physiological mechanism for excretion of iron. Therefore, iron chelation therapy has become an essential facet of better clinical management of this disease.\(^3\) Various drugs such as desferrioxamine (DFO), deferiprone and combination of both these drugs are being used as iron chelating agents. Inspite of benefits of DFO as iron chelating agent many side effects after chronic use of this drug have been reported.\(^4\) Some of these include clinical and subclinical auditory neurotoxicity. The observed auditory neurotoxicity include high frequency sensorineural hearing loss.\(^5\) Porter et al reported that patients receiving DFO had abnormal audiograms and displayed bilateral symmetrical high frequency sensorineural hearing loss.\(^6\) Gallent et al documented auditory neurotoxicity in the form of defective audiograms in the range of 4000-8000 Hz with the conclusion that ototoxicity of DFO was reversible.\(^7\) Abnormal BAEPs have been recorded in a study with thalassemia patients undergoing DFO treatment.\(^8\) Desferrioxamine has been reported to cause ototoxicity with wide variations in different studies; De Virgiliis et al 57%, Olivieri et al 24.7%, Cohen et al 6%, Kontzoglou et al 27%, Chiiodo et al 54%, Passet et al 29.2%.\(^9\)

In view of the above information the present study was planned to detect subclinical lesion of auditory pathways in thalassemic patients undergoing DFO therapy non invasively by doing Brainstem Auditory evoked potential.

MATERIAL & METHODS

The present study was conducted in the Department of Physiology & in the Department of Paediatrics, Pt. B. D. Sharma Post Graduate Institute of Medical Sciences, Rohtak, in 45 patients of thalassemia. All the patients of thalassemia were more than 5 years of age clinically diagnosed and confirmed by Hb Electrophoresis attending the Thalassemia Day Care centre. Patients with history of neurological disease, intake of drugs with known auditory neurotoxicity, patients of diabetes mellitus and with conductive deafness were excluded from the study. The patients receiving iron chelation therapy for more than 6 months were enrolled in the study. They were divided into three groups of 15 patients each. The first group (Group I) included patients of thalassemia receiving DFO therapy (Injection Desferrioxamine) for more than 6 months. The second group (Group II) received DFO and Deferiprone on every alternate day. The third group (Group III) included patients receiving Deferiprone alone (oral chelating agent). In both groups receiving desferrioxamine the dosage ranged from 22- 58 mg/kg/day. DFO was administered by subcutaneous infusion pump over a period of 12 hours. The dose of deferiprone was 20-168 mg/kg/day. The data was collected which included name, age, sex, Hb, duration of chelation therapy and dose of chelation therapy at the time of audiologic test.

BAEP recording was done by using RMS EMG EP MK2 machine. Hearing threshold of 25 dB was considered as normal according to WHO classification of hearing impairment. Click stimuli at the rate of 11.1/sec with intensity of 70 dB above normal hearing threshold were presented to both ears monaurally, in the form of 0.1 ms square pulses through shielded headphones with alternating polarity.\(^14,15\) During stimulation, other ear was masked by 40 dB sound. Total 2000 stimulations were applied. Comparisons were made between all the three above mentioned groups of thalassemic patients with regard to clinical and laboratory data. Statistical analysis was done by using Student's t test.

RESULTS

The three groups were age and sex matched. The male and female ratio was same in three groups (4:1). The children belonged to all socioeconomic strata from very poor to affluent families. The mean height of patients on desferrioxamine was significantly more than the combination group and deferiprone group. There was also significant difference between the weight of patients on DFO (35.93 ± 9.64 kg) and on deferiprone (28.13± 6.66 kg). When the patients on DFO and combination therapy were compared, in the right ear, there was significant increase in the absolute latency of wave I on DFO whereas there was no significant difference in absolute latencies of other waves and interpeak latencies. There was significant increase in the absolute latencies of wave I, III & V between the patients on DFO and oral deferiprone. No significant difference was observed in absolute latencies and interpeak latencies between the patients on deferiprone and combination group. (Table 1) In the left ear, there was significant increase in the absolute latency of wave V and interpeak latency III-V of DFO group than deferiprone group. On comparing combination group and deferiprone group, there was significant increase in the absolute latency of wave V and interpeak latency III-V of combination group. There was no statistically significant difference in the absolute latencies and interpeak latencies between combination group and desferrioxamine group. (Table 1)
Effect of dose of DFO on BAEP

In our study DFO was found to cause changes in the waves of BAEP, we tried to establish any relationship between dose of DFO and prolongation of absolute peripheral hemolysis and anaemia. This condition requires regular blood transfusions leading to secondary iron overload and its consequencies. A major problem in the management of thalassemia patients is the control of transfusional iron overload which is obtained by intensive iron chelation therapy with DFO, deferiprone and a combination of both. Desferrioxamine was introduced in 1960. Desferrioxamine is a member of the hydroxamic acid class of iron chelators which is produced by the microbe streptomyces pilosis. The three hydroxamic acid groups form six coordination ligands with ferric ions with a very high affinity. Barry et al reported that persistent use of DFO over a period of

**Table 1:**

Comparison of absolute latencies and interpeak latencies between thalassemia patients on desferrioxamine, combination of deferiprone and desferrioxamine and deferiprone.

<table>
<thead>
<tr>
<th>Latency (msec)</th>
<th>RIGHT EAR</th>
<th>LEFT EAR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group I</td>
<td>Group II</td>
</tr>
<tr>
<td>Wave I</td>
<td>1.96 ± 0.58*</td>
<td>1.52 ± 0.18</td>
</tr>
<tr>
<td>Wave II</td>
<td>2.72 ± 0.36</td>
<td>2.57 ± 0.23</td>
</tr>
<tr>
<td>Wave III</td>
<td>3.75 ± 0.38</td>
<td>3.52 ± 0.29</td>
</tr>
<tr>
<td>Wave IV</td>
<td>4.79 ± 0.34</td>
<td>4.74 ± 0.34</td>
</tr>
<tr>
<td>Wave V</td>
<td>5.70 ± 0.49†</td>
<td>5.49 ± 0.46</td>
</tr>
<tr>
<td>I – III</td>
<td>2.04 ± 0.21</td>
<td>1.99 ± 0.29</td>
</tr>
<tr>
<td>I – V</td>
<td>3.99 ± 0.60</td>
<td>3.96 ± 0.39</td>
</tr>
<tr>
<td>III - V</td>
<td>1.95 ± 0.67</td>
<td>1.95 ± 0.51</td>
</tr>
</tbody>
</table>

* p <0.01 as compared to group II, † p<0.01 as compared to group III, ‡ p<0.01 as compared to group III

**Table 2:**

Effect of dose of desferrioxamine on absolute latencies and interpeak latencies of BAEP

<table>
<thead>
<tr>
<th>Latency (msec)</th>
<th>RIGHT EAR</th>
<th>LEFT EAR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I a &lt;40 mg/kg/d (mean ± SD)</td>
<td>I b 41-50 mg/kg/d (mean ± SD)</td>
</tr>
<tr>
<td>Wave I</td>
<td>2.09 ± 0.69</td>
<td>1.62 ± 0.15</td>
</tr>
<tr>
<td>Wave II</td>
<td>2.79 ± 0.5</td>
<td>2.65 ± 0.21</td>
</tr>
<tr>
<td>Wave III</td>
<td>3.9 ± 0.48</td>
<td>3.58 ± 0.21</td>
</tr>
<tr>
<td>Wave IV</td>
<td>4.72 ± 0.42</td>
<td>4.60 ± 0.25</td>
</tr>
<tr>
<td>Wave V</td>
<td>5.33 ± 0.27</td>
<td>5.74 ± 0.38</td>
</tr>
<tr>
<td>I – III</td>
<td>2.10 ± 0.26</td>
<td>1.97 ± 0.25</td>
</tr>
<tr>
<td>I – V</td>
<td>3.53 ± 0.27</td>
<td>4.13 ± 0.43</td>
</tr>
<tr>
<td>III - V</td>
<td>1.43 ± 0.3</td>
<td>2.15 ± 0.45*</td>
</tr>
</tbody>
</table>

* p< 0.05 as compared to group Ia, † p<0.05 as compared to group Ia, ‡ p<0.05 as compared to group Ib

Effect of dose of DFO on BAEP

In our study DFO was found to cause changes in the waves of BAEP, we tried to establish any relationship between dose of DFO and prolongation of absolute latencies and interpeak latencies. The patients receiving DFO were divided into three groups- less than 40 mg/kg/d, 41-50 mg/kg/d and more than 50 mg/kg/d. It was seen that absolute latency of wave V with dose >50mg/kg/d was significantly increased than with dose < 40 mg/kg/d of DFO in the right ear. Also there was significant change in latency of Wave IV in patients on DFO dose 41-50 mg/kg/d and those on dose >50 mg/kg/d. It was also seen that the inter peak latencies I-V in patients on dose >50mg/kg/d than on dose <40mg/kg/d was significantly increased. IPL III-V was also significantly increased in the higher dose. (Table 2)

**DISCUSSION**

Thalassemia is a chronic genetically determined disorder characterized by ineffective erythropoiesis, peripheral hemolysis and anaemia. This condition requires regular blood transfusions leading to secondary iron overload and its consequencies. A major problem in the management of thalassemia patients is the control of transfusional iron overload which is obtained by intensive iron chelation therapy with DFO, deferiprone and a combination of both. Desferrioxamine was introduced in 1960. Desferrioxamine is a member of the hydroxamic acid class of iron chelators which is produced by the microbe streptomyces pilosis. The three hydroxamic acid groups form six coordination ligands with ferric ions with a very high affinity. Barry et al reported that persistent use of DFO over a period of
Deferiprone:

than occasional episodes of hypotension. The slow prevents growth retardation due to iron deposition.

thalasemic patients without any adverse toxicity other than DFO is a better chelating agent than deferiprone and

It is an orally active iron chelator but efficacy still remains to be established. Tafer et al compared efficacy of desferrioxamine and deferiprone and found that deferiprone had comparable efficacy with minimal side effects and better compliance. Deferiprone is especially suitable for patients who can't take desferrioxamine due to side effects or poor compliance or cost of treatment.

Balfour found that deferiprone appears to be less effective than desferrioxamine but compliance is superior. The most important adverse effects are arthropathy and neutropenia/agranulocytosis, other adverse effects include gastrointestinal disturbances, ALT elevation and development of antinuclear antibodies. There is no available literature on ototoxic effects of deferiprone.

Combination of Desferrioxamine and Deferiprone:- Combination of two iron chelators have been shown to produce additive and synergistic effects.

A study found that combination of desferrioxamine and deferiprone results in more iron excretion than when used alone. Moreover a combination of both drugs led to decrease in deferiprone mediated toxicity.

DFO is generally considered as having minimum side effects though cases of hearing impairment due to acoustic neurotoxicity have been reported. Various diagnostic tools are used to detect hearing impairment, one of them is BAEP. BAEP is a neurophysiologic diagnostic tool which can detect hearing impairment precisely and accurately. BAEP advantages are not requiring patients’ cooperation, objective and non-invasive.

The greater number of males in three groups of thalassemia patients may be due to the fact that in a country like ours more males with chronic disease like thalassemia will come for medical attention. There was significant difference between the height of the patients on DFO and those on the combination therapy but the difference in their weight was not significant. There was highly significant difference in the height and weight of the patients on DFO and on deferiprone. This suggests that DFO is a better chelating agent than deferiprone and prevents growth retardation due to iron deposition.

BAEP has been performed in thalassemic patients on DFO as iron chelation therapy to detect early diagnosis and to monitor hearing impairment as reported by certain authors to demonstrate sensorineural hearing impairment with prolonged latencies and interpeak latency. There is no literature on the ototoxicity of deferiprone and no acoustic symptoms have been reported to be shown by patients on deferiprone therapy. Waves of BAEP arise from different sites on the stimulation of auditory neural pathway.

Wave I arises from peripheral auditory nerve close to cochlea, Wave II arises from the same generator as Wave I, Wave III from superior olivary nucleus, IV and V from lateral leminiscus and superior colliculus respectively. IPL I-III measures neuronal conduction from acoustic nerve across subarachnoid space into core of lower pons. IPL I-V measures central neuronal conduction from proximal acoustic nerve through pons to midbrain. The latencies of waves in evoked potential are a function of the state of myelination of the pathway. Prolongation of a wave possibly indicates that the timing of neuronal activity is delayed uniformly across the cell population. It indicates dysfunction but not complete loss of activity; in a part of infratentorial auditory pathways.

Prolongation of absolute latency of wave I reflect peripheral auditory dysfunction due to cochlear involvement due to demyelination. From our study we infer that DFO exerts dose dependent toxicity in agreement with the studies of Porter et al who reported that all the patients with severe sensorineural hearing loss have received a maximum DFO dose >35mg/kg/d. Similar findings were reported by Giardiana et al that high frequency, sensorineural hearing loss occurs at dose >50mg/kg/d. Dose reduction or temporary cessation has generally resulted in clinical improvement of auditory changes. We also tried to see the effect of duration of dose of DFO with the prolongation of waves of BAEP. It was observed that the absolute latency of wave IV was more in the patients who were on DFO therapy for longer duration. There was no significant increase in the interpeak latencies. Our study did not match with the other studies which found no correlation between duration of DFO and sensorineural deficit.

The exact role of DFO in the neurotoxicity has not been defined but different mechanisms have been proposed by other authors for the toxic effects. One mechanism could be trace metal chelation as a potential cause, the most commonly affected being zinc. In patients who are better chelated with high dose of DFO, there is more availability of the iron free drug which also chelates the trace metals in the cochlea and inhibits important metalloenzymes such as tyrosinase or lipoxygenase. It may also be possible that in patients with neurotoxicity DFO may have interfered with critical iron-dependent enzyme activity as has been shown to be potent inhibitor
of DNA synthesis of human B and T lymphocytes in vitro. By blocking the iron dependent enzyme ribonucleotide reductase, which is essential for DNA synthesis, it prevented the lymphocytes from completing the S phase of the cell proliferation cycle. Alternatively there may be a direct toxic effect of DFO. In a study done in USA, primary neuron cultures were treated with DFO for 24 hours and then analyzed for viability, mitochondrial mass, mitochondrial function and pro-apoptosis and sprouting gene expression. It was observed that DFO causes hypoxia type injury without free radical generation. DFO treatment resulted in dose dependent neuronal loss associated with impaired mitochondrial function, proliferation of neurites and reduced expression of GAP-43, which has a role in path finding during neurite outgrowth.

Our findings indicate that it should be imperative to monitor hearing functions in patients receiving DFO. A combination of DFO and deferiprone is favorable for prevention of toxicity of auditory nerve (Prolongation of absolute latency of wave I in patients on DFO alone). The subjective tailoring of optimal safe dosage of DFO must be done on the basis of degree of iron overload at that time. D It is advocated to maintain patients with the lowest effective dose of DFO with regular monitoring of their audiologic performance for subclinical diagnosis of effect on auditory nerve so as to prevent the impending sensorineural hearing loss. Also an indispensable requirement is felt for prospective studies of risk factors and also other neurological involvement. Controlled studies in animal models could be the answer.

References