Determination of potential role of antioxidative status and circulating biochemical markers in the pathogenesis of ethambutol induced toxic optic neuropathy among diabetic and non-diabetic patients

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Abstract The present study was designed to explore the antioxidative status and circulating biochemical markers having a potential role in the pathogenesis of ethambutol (EMB) induced toxic optic neuropathy (TON) among diabetic and non-diabetic patients.
Fifty patients under complete therapy of EMB for tuberculosis were included in the present study. Inclusion criteria for patients were to receive EMB everyday during treatment, a dose of

Abbreviations: TON, toxic optic neuropathy; EMB, ethambutol; Vit, vitamins; ROS, reactive oxygen species; SOD, superoxide dismutase; CAT, catalase; GSH, glutathione; MDA, malondialdehyde; ALT, alanine transaminase aspartate; ALP, alkaline phosphatase.
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1. Introduction

Ethambutol is a highly specific chemical drug (PubChem CID = 14052) (Fig. 1). It is specifically active against Mycobacterium, thus used for a treatment of tuberculosis and is considered to be very important drug. Like all other drugs EMB also has few adverse side effects such as visual acuity, reduction of visual fields, scotomas, and problem in discriminating between red and green color. About 2–6% patients of tuberculosis receiving EMB suffer from its serious side effect, toxic optic neuropathy, which is dose as well as duration dependent (Leibold, 1966; Kahana, 1987; Kho, 2004). Involvement of optic neuropathy, which is dose as well as duration dependent loss is receiving EMB suffer from its serious side effect, toxic optic neuropathy, which is dose as well as duration dependent (Leibold, 1966; Kahana, 1987; Kho, 2004). Involvement of optic nerve is not common during treatment of less than 2 months and visual function can be improved by discontinuation of the drug while in some cases irreversibility has been observed (Schild and Fox, 1991; Alvarez and Krop, 1993; Cruz et al., 2010). Several studies indicated and confirmed toxic outcomes of EMB and a number of pathophysiological mechanisms have been explained like disruption of mitochondrial complex IV, glutamate excitotoxicity and Zinc-associated lysosomal membrane permeabilization.

The present study was designed to explore the antioxidative status and circulating biochemical markers having a potential role in the pathogenesis of EMB induced toxic optic neuropathy.

2. Materials and methods

A total of fifty patients taking complete therapy of EMB for tuberculosis were eligible for inclusion in the study at the Mayo Hospital, Lahore during March–September 2013. Informed consent form was taken before the start of the study. Detailed history, including clinical complications, smoking habits and tobacco chewing was collected in a detailed questionnaire. Clinical diagnosis of the patient was also being taken into consideration. Twenty controls, age matched healthy individuals, were included in the study. The study was approved by local ethics committee of the University of Lahore, Pakistan.

2.1. Biochemical assays

Hepatic profile [alanine transaminase (ALT), aspartate aminotransferase, and alkaline phosphatase (ALP)] was estimated by using the commercial Randox kits (Randox Laboratories, Northern Ireland, UK). Serum bilirubin levels were measured by the method of Jendrassik and Grof (1938). Hematological profile (Hemoglobin and RBC) was determined using cyanmeth reagent (Van-Kampen and Zijlstra, 1965). Blood urea was estimated by the kinetic method (Tiffany et al., 1972) and creatinine levels by the rate of change in absorbance using alkaline picrate (Larsen, 1972). Glutathione (GSH), catalase (CAT), superoxide dismutase (SOD) and MDA levels were estimated by the method of Ellman (Ellman, 1959), Aebi (Aebi, 1974), Kakkar (Kakkar et al., 1984) and Ohkawa (Ohkawa et al., 1979), respectively. Lipid profile (total cholesterol, low density lipoprotein, high density lipoprotein, and total triglycerides) was determined using Friedewald’s formula (Friedewald et al., 1972).

2.2. Statistical analysis

Results have been expressed as mean ± standard deviation. The level of significance was determined by one way analysis
of variance and spearman two tailed correlation. The values of significant difference were evaluated with “p” values and considered significant at p < 0.05.

3. Results

Fifteen out of fifty patients were found to be diabetic. The visual acuity and color vision testing were explored in the both groups including age matched controls (Table 1). The mean age of evaluated patients was 20–41 years (control), 21–65 years (non-diabetics) and 20–65 years (diabetics).

Statistically significant differences and consistent decreasing pattern in Hemoglobin and RBC count between and within the diabetic and non diabetic subjects (p = 0.001 and 0.021 respectively) were noted. The lowest value of RBC count (3.80 × 10⁶/mm³) was found in diabetic patients, however minor reductions were reported for non-diabetic (5.4 × 10⁶/mm³) in comparison to control (5.97 × 10⁶/mm³). The highest values of ALT, aspartate aminotransferase, ALP and total bilirubin (65.73, 49.40, 376.19 and 2.10 IU/L respectively) were observed in diabetic in comparison with that of control (Fig. 2). The higher values of ALP (376.19 IU/L) compared to ALT (65.73 IU/L) were also recorded in diabetic patients and the ratio of ALP/ALT in the studied groups reflects the progression of optic neurotoxicity in preponderance diabetics.

A decreasing trend of high-density lipoprotein levels was recorded (control = 1.73 mg/dl; non-diabetic = 1.27 mg/dl and diabetic = 1.17 mg/dl), however a reverse trend was found for total cholesterol (control = 4.44 mg/dl; non-diabetic = 4.91 mg/dl and diabetic = 6.37 mg/dl), and low-density lipoprotein (control = 2.31 mg/dl; non-diabetic = 2.83 mg/dl and diabetic = 3.05 mg/dl) blood levels. Inconsistent pattern, an increase in non-diabetic and a decrease in diabetic, of blood triglycerides, creatinine, and urea levels was recorded within the studied groups (Fig. 2).

Stress biochemical markers MDA, SOD, GSH and CAT show a significant pattern between and within the studied groups. The consistent increasing trend in MDA levels (1.36, 5.28, 9.24 mmol/ml) was recorded in control, non diabetic and diabetic groups respectively. However a clear consistent decreasing trend in both SOD (0.73, 0.16 and 0.04 nmol/ml) and CAT (4.27, 0.79 and 0.36 nmol/ml) was observed in the studied groups. However, GSH showed inconsistent pattern.

Among vitamins and minerals, a consistent decreasing pattern was reported between and within the studied groups. The optimum values recorded for control, non-diabetic and diabetic patients were 686.45, 533.63 and 285.13 mmol/ml for Vit-B12; 9.60, 8.20 and 6.47 mmol/ml for Vit-E; 1.58, 1.15, and 0.42 mmol/ml for Vit-B1; 1.16, 0.88 and 0.67 mmol/ml for Vit-A; and 12.30, 8.37 and 6.77 mmol/ml for Zinc.

4. Discussion

Reactive oxygen species (ROS) under pathological conditions is generated through mitochondrial respiratory chain and its level increases intensely during environmental stress (Devasagayam et al., 2004). The mechanisms of ROS mediated damage and its involvement in disease progression are evident. ROS production contributes to mitochondrial damage in various diseases. Superoxide anion is the precursor of most reactive oxygen species. Superoxide anion (O₂⁻) is produced by the one-electron reduction of molecular oxygen (O₂) inside the mitochondrion. Dismutation of superoxide anion generates hydrogen peroxide (H₂O₂), which may reduce to water or to hydroxyl radical (OH⁻) (Liochev and Fridovich, 1999).

Most of the ROS mediated diseases have a mitochondrial component. Mitochondrial DNA translates few proteins which are related to the electron transport chain, the main source of ROS synthesis in cells.

Ethambutol is a vital medicine for the treatment of tuberculosis, however, a serious duration and dose-dependent side effect named “toxic optic neuropathy” has been reported to develop in 2–6% of patients receiving EMB (Citron and Thomas, 1986). Short treatment duration i.e., less than 2 months is recommended to avoid any damage to optic nerve, and discontinuation of the drug might help to improve visual function, however in some cases, it is irreversible. Toxic effect of EMB has been established in many studies and few pathophysiological mechanisms like disruption of mitochondrial complex IV, lysosomal membrane permeabilization and glutamate excitotoxicity have been proposed to explain the EMB toxicity (Pierce and Denison, 1994). Because of better patient tolerance and easy administration EMB became preferred choice for tuberculosis therapy since last 25 years (Russo and Chaglasian, 1994; Blumberg et al., 2003; Benson et al., 2003). It targets specifically on propagating mycobacterial cells, and seems to change the RNA synthesis pattern by stopping the mycolic acid integration into the cell wall. Optic neuritis, the most important side effect of this drug is, resulting in reduced visual perception and color blindness (Kumar et al., 1993; Chan and Kwok, 2006).

The results of the present study demonstrated a clear cut picture regarding the circulating biochemical markers between the studied groups i.e., control and patients with EMB induced TON. Data regarding hematological, hepatic, renal, lipid and antioxidative profile show a highly significant difference among the studied groups specially patients with diabetes (Chatterjee et al., 1986). Vitamins A, E, B₁₂ and Zinc seem to be play a major role in the pathogenesis of TON compared to superoxide dismutase, catalase and glutathione but Vit-E

Table 1  Experimental design of patients with ethambutol induced toxic optic neuropathy.

| Groups          | Age (years) | Baseline visual acuity | Worst visual acuity | Baseline color vision | Worst color vision Ocular symptoms |
|-----------------|-------------|------------------------|---------------------|-----------------------|-----------------------------------|
| Control (n = 20)| 20–41       | 20/15                  | Nil                 | 14/14                 | Nil                               |
| Non-diabetic (n = 35) | 21–65     | 20/30                  | 20/150              | –                     | 4/14                              | Blurred vision/disturbance |
| Diabetic (n = 15) | 20–65      | 20/40                  | 20/200              | –                     | 0/14                              | Worst blurred vision     |
and Vit-B$_1$ surpassed all the antioxidants as they have a highly significant inverse relationships with malondialdehyde (MDA vs Vit-E, $r = -0.676^{**}$ and MDA vs Vit-B$_1$, $r = -0.724^{**}$ respectively).

Figure 2  Assessment of hematological profile in ethambutol induced toxic optic neuropathy in non-diabetic and diabetic patients: (a) showing increasing and decreasing levels of ALP and vitamin B$_12$ in diabetic TON patients respectively, (b) showing increasing trend for ALT and alkaline aminotransferase and decreasing trend for myoglobin and Zinc while urea level was inconsistent, (c) showing increased value for total bilirubin, total cholesterol, total triglycerides, low-density lipoprotein, creatinine, and malondialdehyde while reduced value for Hemoglobin, high-density lipoprotein, superoxide dismutase, catalase, vitamin-A, -E and -B$_1$.

5. Conclusion

In conclusion, prolonged ethambutol therapy decreases Vit-E and Vit-B$_1$ levels and possibly plays a role in the development...
of TON. Thus Vit-E and Vit-B₄ may be used as therapeutic agents to lessen the deleterious effects of EMB after confirmation. Data regarding hematological, hepatic, renal, lipid and antioxidative profile further revealed a highly significant difference among the studied groups specially patients with diabetes.

Conflict of Interest

The authors declare no conflict of interest.

Authors’ contribution

MR, AM and MHQ designed the study and drafted the paper. AM, KA and AMA performed the experiments. SZ and NS helped to collect data. MAK and SK analyzed the data and critically reviewed the manuscript.

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