



## Biochemical analysis of cerebrospinal fluid of cattle and buffaloes affected with spontaneous brain disorders

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The involvement of blood brain barrier, which stabilizes the intra-parenchymal internal environment and the absence of lymphatic drainage in combination make the brain immunologically privileged site and also render it susceptible to the development of edema (Vegad and Katiyar 1998). Considering the information on diseases of the central nervous system (CNS) in cattle and buffaloes is limited, the present study was undertaken with the objective to carry out detailed and intensive biochemical analysis of cerebrospinal fluid (CSF) to assess the biochemical alterations during the spontaneous brain disorders in both cattle and buffaloes and to establish the spontaneous disorders in the brain of cattle and buffaloes slaughtered at Tangra slaughter house, Kolkata, India.

Altogether 147 brains (85 from buffaloes and 62 from cattle) were collected by screening more than 3000 buffaloes and 2500 cattle. The cerebrospinal fluid samples were also collected from the brains showing gross lesions.

**Collection protocol:** After slaughter, the whole brain was removed carefully from the cranial cavity as per the standard procedures and examined for gross changes. Altogether, 147 brain samples (85 samples from buffaloes and 62 from cattle) and CSF samples from 30 cattle and 45 buffaloes suspected for infection were collected. The CSF from 12 each of healthy cattle and buffaloes of the same age group were also collected. CSF samples (15 ml) were aseptically collected in sterile test tubes, individually from each animal, directly by sub-occipital approach, utilizing 3 to 4 inch 16 gauge needle, after cutting the skin and muscles around the occipital area during slaughter of the animals. The collected samples were

kept in refrigerated condition at 4°C until use. Samples were processed within 2 h after collection.

**Analysis of cerebrospinal fluid:** The CSF samples collected from 30 cattle and 45 buffaloes suspected for CNS lesions at necropsy were examined physically, biochemically, cytologically and microbiologically. The CSF from 12 each of healthy cattle and buffaloes of the same age group were also evaluated for comparison.

**Physical examination:** Immediately after collection, gross appearance, turbidity and clot formation of each of the CSF samples were observed and recorded. Clot formation was graded as absent (0), very slow (1+), slow (2+), rapid (3+) and immediate (4+). Variation in color, turbidity and the presence of flocculent materials were observed in CSF samples from both infected cattle and buffaloes having meningitis.

### Cytological examination

**Total leukocyte count:** The mean total leukocytic count in CSF by standard hemocytometer in infected cattle was  $26.06 \pm 0.513 \times 10^3 / \text{mm}^3$  which was significantly ( $P < 0.05$ ) higher than in control group ( $2.85 \pm 0.041 \times 10^3 / \text{mm}^3$ ). The TLC of infected buffalo group ( $29.46 \pm 0.533 \times 10^3 / \text{mm}^3$ ) was also significantly ( $P < 0.05$ ) higher than that of control group buffaloes ( $2.925 \pm 0.04 \times 10^3 / \text{mm}^3$ ). The increased levels of leucocytes were mainly due to the absolute increase of neutrophils. Increased total leukocyte counts were generally encountered in yellow and turbid CSF having some flocculent materials. Higher mean absolute number of neutrophils reflected exudative properties of CSF from the bacterial meningitis of cattle and buffaloes (Coles 1986).

### Biochemical analyses

**Total protein estimation:** The CSF protein levels in infected cattle and buffaloes estimated by Lowry's method (Plummer 1988) were  $76.69 \pm 2.12$  mg/dl and  $89.55 \pm 3.767$  mg/dl respectively which were significantly ( $P < 0.05$ ) higher when compared with the values of the respective control groups ( $30.10 \pm 0.419$  mg/dl and  $36.83 \pm 1.152$  mg/dl). This corroborated the results of Nazifi *et al.* (1997).

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Increased CSF protein values in infected cattle and buffaloes were likely due to increased capillary permeability that permits higher molecular weight protein fractions, mainly globulin to enter the CSF. Inflammation of the brain perhaps allows the passage and retention of the proteins of high molecular weight as a result of reduced quality of HA or depolymerization or both. Globulin may also be observed in CSF as a result of hemorrhages into the subarachnoid space. If there is considerable tissue destruction, the quantity of protein in CSF is increased (Coles 1986, Wells *et al.* 1994).

**Glucose estimation:** The mean CSF glucose concentration in the infected cattle and buffaloes were observed as  $32.38 \pm 0.493$  mg/dl and  $33.85 \pm 0.543$  mg/dl respectively, which were significantly ( $P < 0.05$ ) lower than that of the control groups ( $44.36 \pm 0.667$  mg/dl and  $49.82 \pm 0.906$  mg/dl). The reduction of CSF sugar content in the infected animals might be due to the alteration of normal equilibrium between entrance and utilization of sugar as a result of marked inflammatory processes in the meninges caused by *E. coli*. It may be considered that cell-free CSF had practically no glycolytic activity in comparison to that of infected CSF with absolute high number of neutrophils, the presence of glycolytic enzymes being almost entirely confined to the neutrophils. It is also possible that the presence of bacterial infection with increased number of neutrophils in the CSF might result to significant reduction of CSF sugar in the infected cattle and buffaloes (Finegold and Martin 1982).

**Free radical activity in CSF:** The deleterious effects are initiated by free radicals ( $\text{ROO}^\cdot$ ,  $\text{RO}^\cdot$ ,  $\text{OH}^\cdot$ ) produced during peroxide formation from fatty acids containing methylene interrupted double bonds, i.e. those found in the naturally occurring polyunsaturated fatty acids. Lipid peroxidation is a chain reaction providing a continuous supply of free radicals that initiate further peroxidation (Murray *et al.* 1999).

**Malondialdehyde (MDA) assay:** The average CSF lipid peroxidation status (malondialdehyde levels) of infected cattle and buffaloes estimated by thiobarbituric acid (TBA) method using MDA and TBARS were  $4.744 \pm 0.63$  mmol/l, and  $4.995 \pm 0.308$  mmol/l, respectively. The determined levels were significantly ( $P < 0.05$ ) higher when compared with the values of control groups ( $1.638 \pm 0.89$  mmol/l and  $2.729 \pm 0.45$  mmol). Increased CSF lipid peroxidation might be due to the oxidative free radicals attack, methylene interrupted double bonds in poly-unsaturated fatty acids (PUFA) of cell membrane, producing lipid peroxides (Murray *et al.* 1999). Reduction in antioxidant defense during disease conditions and inflammatory progress might also be the cause for the increased lipid peroxides and their propagation products (MDA etc.). Glucose auto-oxidation also produced free radicals leading to lipid peroxidation (Surya Prabha *et al.* 1990, Yagi 1994, Cariello *et al.* 1997).

### SUMMARY

The present study was conducted to analyze the various

biochemical parameters in spontaneous brain disorders of cattle and buffaloes which are being slaughtered at Kolkata, India. The mean CSF glucose concentrations in infected cattle and buffaloes were significantly lower than the values of control groups which indicated bacterial infection. The CSF protein levels of the infected cattle and buffaloes were significantly higher when compared with the values of control groups. The MDA assay showed that the average CSF status of the infected cattle and buffaloes were significantly higher when compared to that of control groups. The mean total leukocyte count of infected cattle and buffaloes were significantly higher than the values of control groups, which suggested the exudative properties of CSF due to bacterial meningitis.

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