A cross-sectional study was conducted among 429 children aged 6 – 12 years, selected by stratified random sampling from two different areas with different levels of fluoride in drinking water in and around Lucknow district. Dental fluorosis was measured using Dean’s Fluorosis Index. Intelligence Quotient was measured using Raven’s Coloured Progressive Matrices (1998 edition).

Results: Majority of the fluorosis free children (76.3%) had an IQ grade 2 (definitely above the average). Majority of the children suffering from very mild and mild dental fluorosis were found to have IQ grade 3 (Intellectually average). Children with moderate cases of dental fluorosis were found to have IQ grade 4 (Definitely below average). Only 5 children with severe fluorosis were included in the study and they all were found to have an IQ grade 5. Hence, a trend of increase in the IQ grade (decrease in intellectual capacity) was observed indicating a strong correlation between fluorosis grade and IQ grade.

Conclusion: Findings of this study suggest that the overall IQ of the children exposed to high fluoride levels in drinking water and hence suffering from dental fluorosis were significantly lower than those of the low fluoride area.

Keywords: Cognitive dysfunction, Dean’s fluorosis index, Intellectual deficits, Neurotoxicity, Raven’s coloured progressive matrices

INTRODUCTION
Water is one of the most valuable natural resources for sustaining life. Though, its suitability to be used for domestic, industrial or agricultural purposes is majorly decided by its chemical composition. Fluoride is a valuable component of water when it is present in small quantity. However, in excess amount it may produce several health problems such as dental fluorosis, skeletal fluorosis and various neurological manifestations [1-3].

Fluoride ion in drinking water is known to have beneficial effects on teeth at low concentrations [4]. Fluoride has been added to the public water supplies to reduce the incidence of dental caries [5]. Dean and others [6] determined 1ppm to be the optimum level of fluoridation for caries reduction while minimizing levels of dental fluorosis [7].

In India fluorosis was first reported by Shortt et al., in Nellore district of Madras state [8]. An estimated 62 million people, including 6 million children below the age of 14 years, suffer from fluorosis in India, due to consumption of fluoridated water [9].

Fluoride can attack mercilessly, against any age group, but its effects are especially harmful to pregnant women & developing children [1,10]. Its adverse effects are irreversible, ranging from mild dental fluorosis to crippling skeletal fluorosis [1,2]. The severity of fluorosis depends upon the concentration of fluoride in the drinking water, daily intake, duration of exposure and climatic conditions [11].

Fluoride ingestion in excess amounts also has detrimental neurological effects. Fluoride has the ability to form lipid soluble complexes which can penetrate the foetal blood brain barrier and accumulate in cerebral tissues before birth thereby affecting intelligence [12]. Some studies reveal that fluoride can induce changes in the brain’s physical structure and biochemistry which affect the mental development of children during cognitive processes, such as learning and memory specifically in the foetal period and first 8 years of life [1,10,13,14].

In the light of above reports, the present study is aimed at investigating a link between the intelligence ability and level of fluoride exposure of the children by examining the intelligence quotient (IQ) scores and dental fluorosis status of the children falling in the age group of 6-11 years from areas in and around Lucknow district with low (0.19 ppm) and high (2.41 ppm) concentrations of fluoride in drinking water.

MATERIALS AND METHODS
Data collection: Ethical Clearance and Informed Consent: This study was conducted under the surveillance of Department of Pedodontics and Preventive Dentistry in Saraswati Dental College and Hospital, Lucknow. The study protocol was approved by the ethical committee of Saraswati Dental College. Informed consent was obtained from the parents and respective school authorities.

Study Design: The present study was conducted to compare the IQ levels of children aged 6-11 years, of two different locations having different fluoride levels in water and to establish a relationship between fluoride levels, prevalence of fluorosis and its effect on IQ levels. The duration of the study was 6 months and the sample collection was carried out during the school hours.
Study Population: A cross-sectional study was carried out among 6-11-year-old children in high fluoride area of Asoha block in district Unnao & low fluoride area of Tiwariganj block in district Lucknow of Uttar Pradesh, India. A total of 429 children fulfilling the inclusion criteria, were selected through stratified random sampling and enrolled in the study. Out of these 429 children, 214 (49.9%) belonged to Tiwariganj where fluoride levels were found to be 0.19 ppm and rest 215 (50.1%) belonged to Unnao where fluoride levels were found to be 2.41 ppm.

Inclusion Criteria: Children included in the study were 6-11 years of age and had –
- Similar socio-economic status.
- Normal birth history.
- Continuous residents of the study area since birth and drinking water from the same source.
- No history of chronic illness, trauma to the head and not on any medication.

Exclusion Criteria: Children excluded from the study had –
- History of trauma to the head or other neurological disorder.
- Any congenital or acquired diseases affecting intelligence.
- Children who had a change in the source of drinking water since birth.
- Severe extrinsic stains on their teeth due to which assessment of dental fluorosis status was not possible.

Collection and analysis of water sample: Prior to the instigation of the study, the selection of the areas for the study was done in accordance with the findings of the fluoride mapping survey conducted by Srivastava et al., in villages of Uttar Pradesh [15]. The areas chosen were village Moradabad in district Barabanki, villages Kandharapur and Gosainkhera of Asoha block and Maheshkhera of Sumerpur block in district Unnao and village Tiwariganj located in district Lucknow. Water samples were collected from bore-well and stored in sterilized plastic cans of 2 litres capacity each. After collection of water samples, proper labelling of bottles was done depicting the area of water sample from where it was collected and within 24 hours of collection of samples, they were sent to the laboratory for the estimation of fluoride using IS 3025 (Part 60): 2008 (Ion Selective Electrode Method) [16]. Based on the water fluoride analysis reports, areas finally selected for the study were - Tiwariganj block in District Lucknow (0.19ppm) as the low fluoride area and Asoha block in District Unnao (2.41ppm) as the high fluoride area.

Selection of study area: The areas selected were rural areas having similar climatic conditions, with majority of the population belonging to lower socio-economic status with similar standards of living, quality of education, medical facilities and cultural status. The children included in the study had comparable physical health and nutrition. Each of the two selected areas had only one government secondary school. All the children studying in these schools were residing within a radius of 1-2 kms.

Study Tools: Data was collected by clinical examination for assessment of dental fluorosis using Dean’s fluorosis index [17] and the IQ levels were tested by means of Raven’s Coloured Progressive Matrices (1998 edition) [18]. A self-administered questionnaire proforma was attached consisting of personal information, source of drinking water and duration of use of present source of drinking water.

Clinical examination for the assessment of dental fluorosis –
Clinical examination of severity and grades of dental fluorosis was done

<table>
<thead>
<tr>
<th>Fluorosis Level</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0</td>
</tr>
<tr>
<td>Questionable</td>
<td>0.5</td>
</tr>
<tr>
<td>Very mild</td>
<td>1.0</td>
</tr>
<tr>
<td>Mild</td>
<td>2.0</td>
</tr>
<tr>
<td>Moderate</td>
<td>3.0</td>
</tr>
<tr>
<td>Moderately severe</td>
<td>3.0</td>
</tr>
<tr>
<td>Severe</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Standardization of the recording procedure: The examination of all subjects was carried out by a single examiner (i.e., the investigator himself) and recording of the scores was also done by the same individual. Calibration of the examiner was done before the study was conducted as well as during the study by doing duplicate examination of 5% (1 in 20) of the total population.

Statistical Analysis
The statistical analysis was done using SPSS (Statistical Package for Social Sciences) Version 15.0 statistical Analysis Software. The statistical tools used include – Mean, Standard Deviation, Chi-square test, ANOVA and Post-Hoc Analysis. Spearman’s rank correlation was used for measuring the relationship between the two variables.

Results
[Table/Fig-3] shows the demographic profile of children at two locations, including the number of children and their distribution according to age and gender. Females were found to be higher in the high fluoride area as compared to the low fluoride area and...
CPM score 29.67±4.05 (Mean IQ 110.1±9.0). IQ score of normal fluorosis index level children was found to be higher than that of children with other grades of fluorosis, and this difference was found to be statistically significant (p<0.001)

[Table/Fig-6] shows the correlation between fluorosis grades and IQ grades. With increase in fluorosis grade, an increase in grade of IQ was observed. Amongst children with IQ grade 1, majority of subjects had normal and very mild fluorosis index (n=190; 78.8%), whereas among subjects with IQ grade 5, all the subjects had severe fluorosis. A strong correlation between fluorosis grade and IQ scores was found (Spearman’s p=0.766).

[Table/Fig-7] shows the comparison of IQ grades between two genders. In overall population, difference in IQ Grade of both the genders was not found to be statistically significant (p=0.724).

[Table/Fig-8] shows the comparison of IQ grades between two age groups. In overall population proportional difference in IQ Grade of children from different age groups was found, however this difference was not found to be statistically significant (p=0.105). Since a lot of studies have been conducted in India & worldwide, a comparison of the results of similar studies, in literature, with present study are tabulated [Table/Fig-9].

**DISCUSSION**

The present study was a cross-sectional study conducted on a stratified random sample of 429 school going children selected from 2 different areas in Lucknow and Unnao with different water fluoride concentrations in their drinking water supply. In the present study, for the estimation of fluoride in drinking water, IS 3025 (Part 60): 2008 (Ion Selective Electrode Method), originally developed by Frant & Ross has been used [16]. This method has been chosen for the reason that it is an automatic analysing system & can be used for estimation of both ionic & non ionic forms of fluoride in contrast to other methods which cannot distinguish between organic and inorganic fluoride [16]. Also, it has tremendous tolerance for extraneous ions like sulfates & phosphates and does calibrations to give final readings in parts per million & is more accurate than the other methods [16].

In this study, on comparison of children at two locations according to IQ grades [Table/Fig-4], majority of the children (74.8%) living in high fluoride area fall under IQ grade 3 (intellectually average). None of the children from the low fluoride area had an IQ grade 2 (definitely above the average in intellectual capacity). None of the children from the low fluoride area had an IQ grade 4 and 5 (definitely below average and intellectually impaired). On the other hand, majority of children (58.1%) from high fluoride area fall under IQ grade 3 (intellectually average). None of the children from high fluoride area had an IQ grade 1 (intellectually impaired) this difference was found to be statistically significant (p=0.008). No statistically significant differences (p=0.151) were found between ages of children residing in the two areas.

[Table/Fig-4] shows the comparison of children at two locations according to IQ grades. Majority of children from Tiwariganj (74.8%) had IQ Grade 2 followed by Grade 3 (22.4%) and Grade 1 (2.8%). None of the children from Tiwariganj had IQ Grade lower than 3, while majority of children from Unnao (58.1%) had IQ Grade 3, followed by Grade 2 (16.7%), Grade 4 (14.0%) and Grade 5 (11.2%) and none of the children from Unnao had IQ Grade 1. Difference in IQ grade of children from different habitats was found to be statistically significant (p<0.001).

[Table/Fig-5] shows the association between dental fluorosis and Intelligence Quotient (IQ). Out of 429 children enrolled in the study, 241 individuals whose fluorosis level was graded as normal had IQ grade 1 and 2 (intellectually impaired). On the other hand, majority of children (58.1%) from high fluoride area fall under IQ grade 3 (intellectually average). None of the children from high fluoride area had an IQ grade 1 (intellectually impaired)
superior). This difference in IQ grades of children amongst the two areas was found to be statistically significant (p<0.001). Sudhir et al., has also reported only 7.8% children with IQ grade 2 and 3 (definitely above the average and intellectually average) in the high fluoride area as compared to 29.9% children with IQ grade 2 and 3 in the low fluoride area [24].

In this study, on analysing the association between different grades of dental fluorosis and IQ grades in the overall study population [Table/Fig-6], it was observed that majority of the fluorosis free children (76.3%) had an IQ grade 2. Only 6 out of 429 children with IQ grade 1 were found in our study, and they were all belonging to the fluorosis free population. No IQ grade 5 children were reported amongst normal population. Majority of the children (82.3%) suffering from very mild dental fluorosis were found to have IQ grade 3. Most of the mild fluorosis index children (65.9%) were also found to have IQ grade 3 followed by IQ grade 4 (15.9%). None of the children with moderate fluorosis were found to have IQ grade 1 and 2. Only five children suffering from severe fluorosis were observed in our study sample and each one of them had an IQ grade 5. Thus it is clearly evident that with increase in the grade of fluorosis, a trend of increase in the IQ grade (decrease in intellectual capacity) was observed indicating a strong correlation fluorosis grade and IQ grade (Spearman’s p=0.766). Sudhir et al., and Shivaparaksh et al., in their study have also demonstrated that intellectual capacity of children decreases with increase in the fluorosis grade [4,24]. Possible mechanisms for the neurotoxic effects of fluoride may be explained by several animal studies. Controlled animal studies have shown that rats exposed to drinking water with high concentration of fluoride at weaning were found to have elevated fluoride levels in 6 of 7 brain regions and plasma fluoride levels 7 to 42 times higher than those found in control animals [14]. These were associated with deterioration of learning and memory ability, decreased thickness of post-synaptic density, increased width of synaptic cleft and high cholinesterase activity in brain tissues [30,31].

**Table/Fig-9:** Comparison of results of similar studies conducted in various parts of the world with the present study

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Authors’ Name &amp; Year of Study</th>
<th>Place of Study</th>
<th>Sample Size</th>
<th>Results</th>
<th>Results of the present study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Karmazade S et al., [21] (2014)</td>
<td>Azerbaijan, Iran</td>
<td>39</td>
<td>The results showed that the mean IQ of children living in high drinking water fluoride region (81.2±11.17) was lower than that of children living in low drinking water fluoride region (104.2±20.73).</td>
<td>The present study showed that the mean IQ scores of fluorosis free children found to be 110.1±9.0 which is much higher than the mean IQ score of 62.4±2 for severe fluorosis children.</td>
</tr>
<tr>
<td>2</td>
<td>Nagarajappa R et al., [1] (2013)</td>
<td>Gujrat, India</td>
<td>100</td>
<td>This study concluded that chronic exposure to high levels of fluoride and water is associated with lower IQ.</td>
<td>The present study showed that children living in high fluoride area had lower IQ grades than the children living in low fluoride area.</td>
</tr>
<tr>
<td>3</td>
<td>Seraj B et al., [10] (2012)</td>
<td>Makoo, Iran</td>
<td>293</td>
<td>Results showed that the average IQ scores decreased from the normal fluoride group (97.77±18.91) to medium fluoride group (89.03±12.99) to high fluoride group (88.9±16.01).</td>
<td>Results from the present study showed that the mean IQ scores decreased from fluorosis free children (110.1±9.0) to children having mild fluorosis (85.9±9.2) to children having severe fluorosis (62.4±2.4).</td>
</tr>
<tr>
<td>4</td>
<td>Shivaparakash PK et al., [4] (2011)</td>
<td>Karnataka, India</td>
<td>160</td>
<td>Results of this study revealed that children with dental fluorosis had lower IQ (86.6±18.09) than those without dental fluorosis (76.8±20.84).</td>
<td>In the present study, with increase in fluorosis grade, a statistically significant increase in grade of IQ (decrease in intelligence) was observed.</td>
</tr>
<tr>
<td>5</td>
<td>Pourlesami HR et al., [22] (2011)</td>
<td>Kerman, Iran</td>
<td>120</td>
<td>Results of this study confirmed that average IQ of high fluoride group (91.37±16.63) was significantly lower than the average IQ of low fluoride group (97.80±15.59).</td>
<td>In the present study, IQ score of normal fluorosis index level children was found to be higher than that of children with other grades of fluorosis, and this difference was found to be statistically significant.</td>
</tr>
<tr>
<td>6</td>
<td>Eswar P et al., [23] (2011)</td>
<td>Karnataka, India</td>
<td>133</td>
<td>They arrived at the result that 63.2% of children in high fluoride village had IQ less than 90, versus 47.7% of children in low fluoride village but this difference was not found to be statistically significant.</td>
<td>Statistically significant differences were found between the IQ grades of children living in high &amp; low fluoride areas.</td>
</tr>
<tr>
<td>7</td>
<td>Sudhir KM et al., [24] (2009)</td>
<td>Andhra Pradesh, India</td>
<td>1000</td>
<td>Findings of the study suggested that the overall IQ levels in children exposed to high fluoride level were significantly lower than the low fluoride areas.</td>
<td>The present study showed that 100% children with severe fluorosis had IQ Grade 5 whereas all the children with IQ Grade 1 were fluorosis free.</td>
</tr>
<tr>
<td>8</td>
<td>Amador DR, et al., [25] (2007)</td>
<td>Durango, Mexico</td>
<td>132</td>
<td>The results showed that both fluoride in urine and fluoride in water were significantly correlated with lower IQ in children.</td>
<td>The present study found that fluorosis and IQ levels were affected by drinking fluoride concentrations as suggested by the significant deficit in IQ of children living in the high fluoride area as compared to those living in low fluoride area.</td>
</tr>
<tr>
<td>9</td>
<td>Xiang Q et al., [26] (2003)</td>
<td>Jiangsu, China</td>
<td>512</td>
<td>The mean IQ score of high F village (82.02±13.00) was found to be lower than the mean IQ score of low F village (100.41±13.21). It was therefore concluded that higher drinking water fluoride is significantly associated with higher rates of mental retardation and borderline intelligence, thus lower IQ.</td>
<td>The present study demonstrated that the children living in high fluoride area had lower IQ grades than the children living in low fluoride area.</td>
</tr>
<tr>
<td>10</td>
<td>Lu Y et al., [27] (2000)</td>
<td>Tianjin Xiqing, China</td>
<td>118</td>
<td>The mean IQ scores were found to be significantly low in high fluoride area (3.15±0.61mg/l) than in the low fluoride area (0.37±0.04mg/l) and hence it was concluded that a real relationship exists between fluoride exposure and intelligence.</td>
<td>The results from the present study confirm that children living in high fluoride area had lower IQ Grades than the children living in low fluoride area.</td>
</tr>
<tr>
<td>11</td>
<td>Wang G et al., [28] (1996)</td>
<td>Xinjiang, China</td>
<td>230</td>
<td>Results showed that the average IQ of high F group (85.6±14.34) was lower than in control group (101.2±23.54).</td>
<td>Results from the present study also showed that IQ grades of children with severe fluorosis were higher than those of children with moderate, mild &amp; no fluorosis.</td>
</tr>
<tr>
<td>12</td>
<td>Li XS et al., [29] (1995)</td>
<td>Guzhou, China</td>
<td>907</td>
<td>The results demonstrated that the IQ of children living in areas with a medium (79.7±12.7) or severe (80.3±12.9) prevalence of fluorosis was lower than that of children living in areas with slight fluorosis (89.7±12.7) or low fluorosis (89.9±10.4).</td>
<td>Results of the present study showed that majority of the fluorosis free children (78.3%) had Grade 2 IQ scores, majority of children with mild fluorosis (85.9%) had Grade 3 IQ scores, Majority of children with moderate fluorosis (37.2%) had Grade 4 IQ scores whereas all the children with severe fluorosis (100%) had Grade 5 IQ scores.</td>
</tr>
</tbody>
</table>
Once absorbed in the blood through diet, fluoride forms lipid soluble complexes which cross the blood brain barrier to accumulate in the cerebral tissues [32,33]. The penetrated fluoride complexes adversely affect the CNS development by different neurotoxic mechanisms, such as free radical generation, inhibition of anti-oxidants and mitochondrial energy enzymes and inhibition of glutamate transporters [13]. Studies in absorbed human foetuses have shown disruption of neurotransmitters, receptors in nerve cells, increased density of neurons & undifferentiated neuroblasts [34]. There has also been evidence of reduction in number of mitochondria, rough endoplasmic reticulum (RER) and free ribosomes. The structural and functional changes in the CNS, specifically in the foetal period and the first 8 years of life, may lead to learning and intellectual deficits and cognitive dysfunction [14,32,34,35].

On comparison of IQ grades between the two genders [Table/Fig-7], no correlation was found (p=0.724) in the present study. These findings were further supported by the studies conducted by Li et al., Zhao et al., Sudhir et al., and Seraj et al., [10,24,29,36]. Nonetheless, in the research of Xiang et al., a significant relationship between IQ and gender was found in one of the two study villages [26]. However, in psychological literatures, gender is mentioned as a non-related factor to IQ [37].

Statistically no significant difference in the IQ of children from different age groups was found [Table/Fig-8]. Thus no correlation between age and IQ was established in the present study that included 6-11-year-old children with 429 subjects. Similar findings were reported by Li et al., and Sudhir et al., [24,29].

A host of many genetic, socio-economical and geographical factors have been known to influence the overall neuro-behavioural development of an individual [19]. Therefore, we have recruited samples from a homogenous rural population of Unnao and Tiwariganj, thus diminishing the effect of some environmental and inherited factors, yet it is obvious that complete exclusion of such factors is impossible.

Certain trace elements in water such as Arsenic and Lead as well as iodine deficiency, may also affect the intellectual ability of children. Many researchers such as Amador et al., and Wang et al., have shown in their studies that children intelligence can be affected by high levels of Arsenic [25,38]. However, Amador et al., have revealed in their study that in comparison to fluoride, the effect attributable to Arsenic in the IQ reduction is smaller [25]. They have also confirmed through their study that observed deficits in IQ scores cannot be attributed to Lead exposure. Susheela et al., in their study revealed that since fluoride interferes with the activity of thyroid gland and secretion of its hormones, elevated fluoride uptake may cause iodine deficiency in fluorosed individuals, even when they reside in non-iodine deficient areas, hereby affecting the developing brain [39]. Nevertheless, further researches are required to investigate the effect of other environmental or geological contaminants on Intelligence.

LIMITATIONS

In the present study, assessment of the effect of fluoride concentration in drinking water on child’s IQ has been done, but it is possible that some other trace elements in drinking water like Arsenic, Lead, iodine etc. may also have neurological side effects. Thus, further researches are required to investigate the effect of other environmental or geological contaminants on Intelligence.

In the present study, samples from a homogenous rural population of Unnao and Tiwariganj have been recruited to diminish the effect of some environmental and inherited factors, yet it is obvious that complete exclusion of such factors is impossible. Although, children who had a change in the source of drinking water since birth, were excluded in the present study, we could not completely exclude the influence of recall bias. Moreover, fluoride level in a particular source of water may change over a period of time. Therefore, the need for a more careful evaluation of the effect of fluoride on intelligence is emphasized.

CONCLUSION

In the light of all the facts discussed above, the present study that encompasses 429 subjects falling within the age group of 6-11 years, concludes that fluorosis and IQ levels were unaffected by age and gender, however, they were both affected by drinking water fluoride concentrations as suggested by the significant deficit in IQ of children living in the high fluoride area as compared to those living in low fluoride area. The data from this research may support the hypothesis that excess fluoride in drinking water has toxic effects on the nervous system. Millions of children around the world are exposed to high concentrations of fluoride in water and are potentially at risk. Therefore, a close monitoring of fluoride levels in local water supplies from areas with endemic fluorosis and implementing public health measures, such as defluoridation, to reduce the fluoride exposure levels in high fluoride regions seem necessary.

ACKNOWLEDGEMENTS

We concede the help and support of the Department of Pedodontics and Pediatric dentistry Saraswati Dental College and Hospital, Lucknow. We also thank the children and their parents who participated in the study for their immense contribution and time.

REFERENCES

### ORAL HEALTH ASSESSMENT FORM (Questionnaire)

<table>
<thead>
<tr>
<th>Name-</th>
<th>Date-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age-</td>
<td>Examiner-</td>
</tr>
<tr>
<td>Sex-</td>
<td></td>
</tr>
<tr>
<td>Name of School-</td>
<td>Village-</td>
</tr>
<tr>
<td>Residence Since-</td>
<td>Any Immigration-</td>
</tr>
<tr>
<td>Socioeconomic Status-</td>
<td>Source of Drinking Water-</td>
</tr>
<tr>
<td>Duration of Use-</td>
<td>Oral Hygiene Habits –</td>
</tr>
<tr>
<td>Never Clean-</td>
<td>Brush-</td>
</tr>
<tr>
<td>Dattan-</td>
<td>Any Other-</td>
</tr>
<tr>
<td>Frequency –</td>
<td></td>
</tr>
</tbody>
</table>

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### FINANCIAL OR OTHER COMPETING INTERESTS:
None.

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