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A Comprehensive Review on the Techniques and Indexes Used for the Analysis of Fluorosis in Humans and Cattle

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ABSTRACT

Fluoride is known to play a significant role in dental formation. High fluoride intake leads to different symptoms one of them is dental fluorosis, which is chronic dental toxicity. Various indexes have been introduced to measure the intensity and severity of dental fluorosis. Some of these indexes are fluoride specific, such as Dean's index, Thylstrup and Fejerskov index, the Tooth Surface Index of Fluorosis index, ICMR index. While others are non-fluoride descriptive indexes such as the Developmental Defects of enamel index. Dental fluorosis is most commonly assessed by clinical examination by experts in these indexes, but nowadays, technical assistance such as photographs is used for diagnosis. Recent advancements have also witnessed the development of Visual analog scales and quantitative light fluorescence methods for dental fluorosis assessments. This review article focuses on important techniques and indexes used in the evaluation and characterization of dental fluorosis. A comparative review analysis of available indexes and the scope of future advancements have also been compiled.

Keywords: Cattle, Dental, Skeletal, Fluorosis, Indexes.

INTRODUCTION

The health of the population of an area is significantly determined by the quality of water consumed. Groundwater is the most crucial water source for any nation and has a critical role in the community's health[1]. While the quality of water is determined by several parameters like pH, turbidity, total hardness[2-3], heavy metals[4-8], and ions such as chloride, nitrate and fluoride. However, different parameters have different types of effects on living beings, so is the case with fluoride. It is highly reactive[9]. Fluoride-containing minerals such as fluorspar, cryolite, sellaite, and fluorapatite are the primary source of it in the earth's crust[10]. Secondary sources are granite, gneissic rocks and sediments of marine origin in mountainous areas[11]. Whereas anthropogenic sources of fluoride toxicity include mining activities, industrial processes, pesticides, agrochemicals, industrial effluents, and brick kiln[12-13]. WHO has set the permissible limit of fluoride up to 1.5 mg/L[14] in drinking water, whereas US Public Health Service suggested a fluoride limit of 0.7 mg/L in drinking water[15]; as it plays a significant role in healthy tooth growth at a low level of 0.7^{-1} mg/L[16-17]. Worldwide 25 countries are affected by the high concentration of fluoride[18-20], including China[21-22], India[23-27], Africa[28-29], Korea[30], Kenya[31], Nigeria[32]. In India alone, 177 districts in 21 states are affected by high fluoride contamination in water[33-35].

The research trends since the 1930s started visualizing the toxic effects of high concentrations of fluoride not only on humans but on animals also[36]. The fluoride acts by forming hydrofluoric acid that is very corrosive to the bones and acts on the carbonated hydroxyapatite, forming an insoluble salt (CaF_2), which weakens the bones[37-38]. As a result, it significantly affects calcified tissues resulting in dental or skeletal fluorosis[39-40]. Fluoride toxicity can be acute or chronic depending on fluoride exposure[41]. Symptoms of skeletal fluorosis in humans include chronic joint pains, stiffness in joints, calcification of ligaments, and osteosclerosis[42] while in livestock, hoof deformity, lessons in mandible, enlargement of bones and joints, extra bone development, calcification of ligaments, stiffness, and lameness[43-44]. Dental fluorosis is characterized by hypo-mineralization, resulting in loss of luster in enamel, white chalky patches, and brownish striations, which results in pitting, mottling enamel, and finally loss of teeth in severe cases[45-49]. These fluoride-related enamel alterations start during the enamel development stage as deciduous teeth mineralize before birth[50]. Thus, the placenta serves as a passive barrier for passing high fluoride concentration from the maternal plasma to the fetus[51-52]. In addition to these, recent studies have found that fluoride toxicity is also related to other diseases such as Alzheimer's, neurological problems, hypertension[53-55].

History of Dental Fluorosis Indexes

Dental Fluorosis in Human

Dental fluorosis studies gained wide recognition with the work of HT Dean in the 1930s. The relation between fluoride contamination and its dental effects was precisely understood and outlined by HT Dean and his colleagues at the US Public Health Service. While conducting surveys of dental fluorosis severity in fluoride-stricken areas, Dean felt the need to quantify and measure the degree of severity of enamel mottling in people[56]. As a result, an ordinal scale of 0-7 was introduced by Dean, widely accepted and popularly known as the Dean's Index. After the work of Dean, several researchers came forward to introduced different indexes to quantify and classify dental fluorosis in humans and animals. These indexes were laterally categorized into two groups, fluoride-specific indexes and descriptive indexes. This paper deals with a detailed literature review of different dental fluorosis indexes in humans and cattle. A comparative analysis of these indexes is helpful for examiners to select the correct index for the study in the field.

Dean's Index

Dean developed a characterizing and grading criterion that can assess dental fluorotic lesions. Dean proposed an index on an ordinal scale of 0-7 in 1934 with seven classifying groups "Normal, Questionable, very mild, mild, moderate, moderately severe and severe"[56]. Later in 1942, this index was modified by converging the moderately severe and severe[57], as shown in Table 1. After that, this index became widely accepted. The scale 0 to 4 covers all the classifications, "normal (0), questionable (0.5), very mild (1), mild (2), moderate (3) and severe (4)". According to the diagnostic procedure used by Dean, teeth are to be diagnosed under good natural light with mirrors and probes needed. A person was characterized based upon the two most affected teeth. This index has been recommended by WHO in the Basic survey manual, 4th edition, 1997[58], since it is easy and straightforward to use. However, Dean's index has been questioned because it does not include details on fluorosis distribution within the dentition, its lowest score "questionable" is too ambiguous, and its higher scores are not sensitive enough[59].

Table 1: Criteria for Dean's system of classification for fluorosis

Score	Criteria
0 (Normal)	Enamel surface- smooth, shiny, and generally pale, creamy white color Enamel Structure-Translucent semi vitri form type
0.5 (Questionable)	Enamel-Slight aberrations, few white flecks to occasional white spots.
1 (Very Mild)	Twenty-five percent area of the tooth has irregularly scattered small, opaque, paperwhite areas. Teeth show white opacity of approximately 1-2mm at the tip of their summit of cusps of the bicuspid or second molar
2 (Mild)	White opacities are more extensive but present in less than fifty percent of too
3 (Moderate)	Brown stains are frequently observed. All enamel surfaces show wear and are affected
4 (Severe)	The general form of the tooth is affected and hypoplasia is observed. Discrete and confluent pitting; widely spread brown stains and often corroded-like appearance

Thylstrup and Fejerskov Index (T-F)

A new criterion formulated for measuring fluoride origin defects was proposed by Thylstrup and Fejerskov (1978)[60]. This index suggested classifying a 10-point scale to categorize degrees of fluorosis in each tooth on buccal, lingual, and occlusal surfaces Table 2. Fig. 1 represents different scores given to teeth using the TF index. Drying teeth with the cotton wool roll was recommended under the methodology. This index attempts to validate visual appearance against histological defects.

Table 2: Classification of Thalystrup and Fejerskov index

Score	Criteria
0	After prolonged drying, normal translucency of enamel is observed
1	Narrow white lines are observed, which are located corresponding to the perikymata.
2	Occasionally confluence of adjacent lines and more pronounced lines of opacity are observed on smooth surfaces. These lines of opacities follow the perikymata. The occlusal surface is marked with opacity<2mm in diameter scattered on surface areas and pronounces opacity of cuspal ridges.
3	Cloudy areas of opacities that merging and irregular are observed on the smooth surface. Accentuated drawing of perikymata is often seen in between opacities. Occlusal Surfaces are observed with confluent areas of marked opacity. However, worn areas appear normal but are generally circumscribed by a rim of the opaque enamel.
4	The entire smooth surface appears chalky white and also exhibits marked opacities. However, those parts of the surface that are exposed to attrition look less affected. The entire occlusal surface exhibits marked opacities. Attrition is often pronounced shortly after the eruption.
5	Marked opacities are observed over entire smooth and occlusal surfaces. These opacities are with pits < 2mm in diameter.
6	Smooth surfaces have pits that are regularly arranged in horizontal bands <2 mm in vertical extensions. Occlusal surface Confluent areas <3mm in diameter exhibit loss of enamel. Marked attrition
7	Loss of outermost enamel in irregular areas involving<1/2 of the entire smooth surface Changes in the morphology caused by merging pits and marked attrition in occlusal surfaces
8	Loss of outer enamel involving>1/2 of the smooth and occlusal surface.
9	Loss of the main part of tooth enamel and change in the anatomic appearance of smooth and occlusal surfaces. The cervical rim of nearly unaffected enamel is generally observed.

The only index which tries to associate the clinical appearance to the pathological variations inside the tissue was the TF index, and therefore, it is a suitable tool while assessing dental fluorosis severity in epidemiologic studies. Granath *et al.,* (1985) conducted a study to compare Dean's and TF indexes[61]. He discovered that the TF index was more detailed and sensitive as this was based upon histological changes of the tooth with hypomineralisation. Fig. 1 represents various teeth affected by excessive fluoride and scoring given according to Thylstrup-Fejerskov index. One of the drawbacks of the TF index is that replicate examination in field surveys is not possible using this index[59].

In a comparative study of the TF index and Dean's index, Burger *et al.,* (1987) found that both the indexes resulted in similar prevalence values, but the severity values differed across the two scales; in general, the TF index showed higher scores[62]. Further, the authors recommended using the TF index for field studies considering its ease and well-defined parameters.

Fig. 1. various teeth examined using T-F index and scoring (Source: Fejerskov *et al.,*1994)

Tooth Surface Index of Fluorosis (TSIF)

This index was recommended by Horowitz *et al.,* (1984) to diminish the DEAN and TF index shortcomings[63]. This index assesses the fluorosis prevalence from a tooth surface perspective. The Tooth Surface Index of Fluorosis (TSIF) gives different scores to each tooth surface where anterior teeth get two scores while three were assigned to posterior teeth. When more than two scores were assigned to one tooth, a higher score was considered for that tooth. The teeth were classified into eight categories from 0-7 Table 3. Artificial lights and wet examination were recommended to be used and to get a score, at least one tooth surface should have erupted.

Table 3: TSIF classification and identification criteria

Score	Descriptive Criteria
0	Normal tooth appearance and no evidence of fluorosis is observed
1	Definite fluorosis can be seen. Less than one-third of enamel is observed with parchment-white color. The fluorosis is confined only to incisal edges of anterior teeth and cusp tips of posterior teeth ("snow capping") is considered under this category.
2	At least one-third of the visible surface is covered with Parchment-white fluorosis, but less than two-thirds.
3	At least two-thirds of the visible surface is covered with Parchment-white fluorosis.
4	Staining in addition to any of the earlier mentioned effects may be observed on enamel. Staining is defined as "an area of definite discoloration that may range from light to very dark brown."
5	Discrete enamel pitting is present, but there is no evidence of staining of intact enamel. A pit can be defined as "a definite physical defect in the enamel surface with a rough floor surrounded by a wall of intact enamel. The pitted area is generally stained or of a different color from the surrounding enamel."
6	Both discrete pitting and staining of the intact enamel are observed.
7	Enamel surface with confluent pitting is seen. The anatomy of the tooth may be changed because large enamel areas are missing. A dark brown stain is usually present.

Cleaton-Jones and Hargreaves (1990) examined the three indexes (DEAN, T-F, and TSIF) in deciduous dentition, discovering that the TF index detected fluorosis more commonly in individual teeth[64]. They concluded that the T-F index was most appropriate for the work that requires comprehensive knowledge about the issue. This index improves the sensitivity of diagnosis in severe cases of fluoride exposure. A distinction between discrete pitting and confluent pitting has been provided under this index. At the same time, Rozier (1994) pointed out some of the pitfalls in the index. This index scores each surface of the tooth, which increases inter-examiner variability[59].

Indian Council of Medical Research (ICMR) Index

In order to address fluorosis, ICMR formed a task force with four subgroups, one of which was on dental fluorosis[65]. The subgroup recommended formulation of dental fluorosis grading and identification criteria that the health professional could use with basic training. As per that, ICMR index for dental fluorosis' was introduced in 2013 (Table 4). The index characterizes dental fluorosis on an ordinal scale of 0-3. In addition, the guidelines for the diagnosis of teeth were also given by ICMR[65]:

1. All 28 permanent teeth are to be examined except the third molar using natural light and probe.
2. All surfaces of teeth should be examined with special attention to the labial surface of anterior teeth and buccal of posterior.
3. A dried examination is recommended.
4. Each tooth should be examined and graded individually and an individual must be graded based upon two teeth having the most severity grade.

Table 4: ICMR Index for Dental Fluorosis

Grade	Description
0(normal)	Enamel surface appears smooth, glossy, translucent, creamy white/pale in color.
1(mild)	Enamel surface showing extensive chalky white opaque areas in two or more teeth.
2(moderate)	Enamel surface showing extensive chalky white opaque areas in two or more teeth.
3(severe)	Enamel surfaces showing brown color with the pitted, discrete or confluent, eroded or destroyed structure of two or more teeth.

The pilot study was conducted by Goyal *et al.,* (2016), to authenticate the ICMR index[65]. The study indicated that the inter-examiner agreement was nearly perfect. It was also concluded that non-dental personals could use ICMR Index in field studies since it is more straightforward and reliable. Kumar *et al.,* (2018), after comparing Dean, TSIF, and ICMR index, found that the cases that were classified as "moderate" using Dean's index were dispersed into "mild (white opacities alone) and moderate category (brown stains also present)" by TSIF index and ICMR index respectively[66]. There was an apparent discrepancy between teeth with white opacities (mild fluorosis) and brown stains (moderate fluorosis), according to TSIF and the ICMR index. This discrepancy highlights the advantage of using the TSIF or ICMR index in dental fluorosis studies compared to Dean's index, which sometimes overvalues the severity of fluorosis, particularly in the moderate category. The ICMR index is easy to use and less time-consuming than Dean's index, which may overestimate the severity of fluorosis; the ICMR index gives the actual severity of dental fluorosis.

Developmental Defects of Enamel (DDE) Index

In 1982, a working group of FDI group recommended using descriptive criteria for analysis of fluorosis and introduced the "Developmental Defects of Enamel" index. In the DDE index, type, number, demarcation and location of defects on the buccal and lingual surfaces of teeth were recorded. Clarkson and Mullane later recommended the modified form of the DDE index in 1989[67]. The modified index classifies defects into three main categories, namely: demarcated, diffuse and hypoplastic. The degree of tooth surface covered by a defect was also scored. This index recommends a wet examination of lingual and buccal surfaces of all erupted permanent teeth except third molars. Maxillary central and lateral incisor; maxillary first premolar and mandibular first molars were to examined. Natural light was used for examining index teeth, and fibre optic source was used for full mouth (Table 5).

Table 5: Modified DDE index

Code	Description
0	Normal
	Demarcated opacities:
1	White/ Cream
2	Yellow/brown Diffuse Opacities:
3	Diffuse-lines
4	Diffuse-confluent
6	Confluent/patchy+staining+loss of enamel Hypoplasia:
7	Pits
8	Missing Enamel
9	Any other defects Extent of Defects:
0	Normal
1	<1/3
2	At least 1/3<2/3
3	At least 2/3

This index gives a detailed measurement including a wide range of defects with information on the spreading and locations of defects. However, since this index is non-fluoride specific, it cannot access fluoride-induced effects.

Several researchers have introduced indexes of dental fluorosis apart from the indexes mentioned above. Fluoride-specific indexes were recommended by Zimmerman (1954) and Nevitt *et al.,* (1963)[68,69]. Many other researchers stated that many dental lesions are aesthetically similar to fluorotic lesions but are caused by reasons other than fluoride. Indexes depending on the clinical appearance of defects were also recommended by Alousi *et al.,* (1975), Jackson *et al.,* (1975), and Murray and Shaw (1979)[70-72]. These indexes although did not gain wide acceptance and recognition.

Dental Fluorosis Indexes in Cattle

Dental fluorosis is commonly observed in cattle in fluoride-contaminated areas due to fluoride-contaminated water and fodder consumption. Choubsia *et al.,* (2011) worked on Dental fluorosis in different domestic animals in the Rajasthan state of India[73]. The research found that the severity of dental fluorosis in grass eaters was higher than in plant parts eaters. They concluded that edible parts of the plant (such as leaves, pods, small fruits) are rich in calcium and vitamin C, which can counteract fluoride toxicity. Sometimes, the index introduced by Dean is used for characterizing dental fluorosis in cattle. However, many fluoride-specific indexes for cattle have been recommended by Shupe and colleagues, the National Academy of Sciences, and L. Krook. For example, a classification and characterization criteria for Dental fluorosis in cattle was recommended by Shupe *et al.,* (1972)[74]. This classification classified the dental lesions on a scale of 0 (normal) to 5 (excessive effects). This classification criterion was widely accepted and is still used by examiners in field surveys for cattle dental fluorosis studies.

The Committee on Animal Nutrition,

National Research Council (NRC) in 1974 published a report on fluoride effect on animals[75]. That study found that fluoride sensitivity occurs in the growing teeth between the ages of six months and eight years. Gross fluorotic lesions of the tooth enamel are usually termed as "mottling (white, chalk-like patches or strains in the enamel), chalkiness (dull-white, chalk-like appearance), hypoplasia (defective enamel), hypo calcification (defective calcification)". As cheek teeth are hard to inspect in live animals, dental fluorosis is generally diagnosed by incisor teeth. Characterization criteria given by National Academy divides animals into six classes and an ordinal scale of 0-5, which is very similar to the classification recommended by Shupe and colleagues (Table 6).

Table 6: Fluorosis classification criteria given by the Shupe *et al.,* (1972), NRC (1974) and Shupe *et al.,* (1979)[74-76]

Score	Criteria
Normal (0)	The normal shape of the tooth having a translucent, smooth, and glossy white appearance.
Questionable effects (1)	Teeth show little deviation from normal; the cause is not determined exactly; enamel flecks may be present but not mottles.
Slight effects (2)	There is a little mottling of enamel that is seen as horizontal striations; may have light staining but no significant increase in the normal rate of wear.
Moderate effects (3)	Teeth show definite mottling of the entire tooth and a large area of chalky enamel; the tooth has a little higher rate of wear and may be stained.
Marked effect (4)	Hypoplasia and hypocalcification are observed along with definite mottling and pitting of enamel; the rate of wear and staining in the tooth increases with use.
Severe effects (5)	Teeth can be stained or discoloured. Teeth show definite mottling, hypo calcification, and hypoplasia. Increase rate of wear, erosion, and pitting of teeth with use.

Krook *et al.,* (1983), worked on a new scale and identified some of the defects in the enamel of cattle that had been overlooked by the Scaling criteria given by the National Academy of Sciences[77]. He defined five principle defects and gave an ordinal scale of 1-5 as follows:

Score 1: Hypercementosis with tooth ankylosis, cementum necrosis, and cyst formation.
Score 2: Permanent incisor teeth to show delayed eruption.
Score 3: Necrosis in alveolar bone with the bone and gingiva recession.
Score 4: Permanent teeth can be visualized erupted in an oblique fashion. In addition, hypoplasia of teeth accompanied by diastema.
Score 5: Increased progression of the lesions in teeth along with tooth loss.

In 2002, Swarup and Dwivedi gave scoring criteria for examining dental fluorosis in cattle[45]. This criterion was based on a numerical scale of 0-5 that recognized cattle dental fluorosis from the range of normal to severe Table 7. Although different authors have used different indexes for their studies, studies with their outcomes have been compiled in Table 8.

Table 7: Scoring and classification of the dental lesion by Swarup and Dwivedi (2002)

Score (Type)	Description
0 (Normal)	Translucent, glossy, and white enamel. The normal shape of teeth is observed.
1 (Questionable effect)	Tooth appearance has deviated from normal translucent enamel; the cause of which is not known. Mottling is not observed, but unilateral and bilateral cavities may be present; flecks observed
2 (Slight effects)	The shape of teeth is normal but little mottling of enamel and some discoloration may be observed
3 (Mild effects)	Moderate mottling and chalky enamel are observed. Discoloration and slight abrasion are seen.
4 (Moderate effects)	Definite mottling, hypo calcification along with hypoplasia, and discoloration are present. The colour of the enamel maybe cream. Pitting of enamel is observed, teeth abrasion.
5 (Excessive effects)	Definite mottling, hypo calcification, may have enamel pitting, discoloration or cream-colored, excessive teeth abrasion.

Table 8: Dental fluorosis Studies in humans and cattle

References	Country	Fluoride (ppm)	Indexes	Age group	Fluorosis Examination	Remark
			Human Studies			
78	Brazil	0.06-1.98	The European Academy of Pediatric Dentistry Criteria, TF index, WHO index	6 to 12 years)	610(age group-Clinical	Molar-incisor hypomineralisation was likely to be associated with dental fluorosis
79	India	>1.5	Dean's Index	1299 (age group-12 to 18 years)	Clinical	The overall prevalence of dental fluorosis was 21%
80	Malaysia	0.5-0.7	Dean's index	1155 (age group-9 to 12 years)	Photographic	Fluorosis prevalence was lower among younger children
81	India	1.5-4.5	Dean's Index, TSIF, ICMR index	300 (12-15 years)	Photographic and visual	ICMR index is easy to use and better as compared to others
82	England	Water fluoridation-1ppm and non-fluoridated areas	TF index	580 (18- 52 years)	Digital photography	Aesthetic impacts of fluoride seem to diminish with age
83	Nigeria	0.07-2.13	TF index and modified DDE index	322 (8 years old)	Clinical	Drinking water fluoride was a positive predictor of dental fluorosis
84	Georgia	0.08-0.4 (fluoride deficit regions)	TF Index	570 (1-6 years)	Descriptive analysis	Indoor coal-burning that led to the exposure of fluoride during pregnancy was a reason for dental fluorosis risk in children
85	US	2.5-5.1	TF Index	308 (15 years old)	Clinical	Self-perception of dental fluorosis affects adolescents
86	Iran	1.2-1.4	Dean's Index	100 (15-18 years)	Clinical, Quality of life was evaluated through a questionnaire	Dental fluorosis is associated with a decrease in Quality of Life.
87	India	1.5 4.5	Dean's Index	30 extracted teeth (patient's age–15 to 40 years)	Ground sections of teeth analyzed under light micrograph	Dental fluorosis features are comparable to those under specialized microscopes
88	Brazil	0.06-1.14	DDE Index	566 (5-year-old children)	Clinical	Enamel defects
89	Sweden	<0.1	DDE Index	796 (Children- 11, 15, and 19 years)	Clinical	Incisors (upper central) and permanent first premolars were more affected
90	Poland	0.3-0.9	DDE Index	2,522 (11 to 15 years) and 3,122 (5 to 8 years)	Clinical The prevalence of DDE in populations was low	
			Cattle Studies			
91	Kenya	1-30	Dean's Index	242	Clinical	Most animals were affected by fluoride at the early stages of their growth
92	India	5-6.2	Shupe et al.,	197960 (20 buffaloes, 20 cattle, 20 goats)	Clinical	Buffaloes were more severely affected as compared to other animals.
93	India	1.37	Shupe et al., 1979	270	Clinical	Interaction between fluoride and other minerals affects the severity of fluorosis.
94	India	4-4.75	Swarup and Dwivedi, 2002	492	Clinical	Reduced plasma calcium levels and elevated alkaline phosphate activity

Future Perspective in Dental Lesion Characterization

Dental fluorosis indexes are most commonly and widely used for the assessment of dental fluorosis. Direct clinical methods are the traditional techniques that have been used ever since. With the advancements of technologies, imaging technologies came into existence for assessing dental fluorosis. Imaging techniques include conventional images and digital photographs[95]. Photography for assessing dental fluorosis can be advantageous to remove bias and better reliability. High-quality photographs can be remotely sensed and analyzed by several examiners[96], but such analysis requires trained examiners, Image processing techniques, and skills. Since all current dental fluorosis indexes recommend using ordinal scales thus, the scores can be considered only subjective points and a range of change[69]. To develop a continuous scale Vieira *et al.,* (2005) developed a Visual Analog Scale (VAS). T-F index was used in the development of VAS for dental fluorosis. A 100 mm scale was graded based upon the best and worst tooth surface. A total of five photographs were used as an indicator of the scale Fig. 2. The advantages of using such scales lie in the fact that this is a continuous scale and simple to use. In addition, the analysis is more robust and has meaningful parameters[97]. The main reason for disapproval of the VAS is that it does not give specified criteria for its scale points that can be susceptible to examiner bias. Even though images of fluorosis with varying degrees of dental fluorosis were utilized as indications, the assessment was still regarded as subjective, making examiner training and calibration problematic. More research and usage of the score in epidemiological surveys using images or other appropriate techniques are needed to validate this indicator.

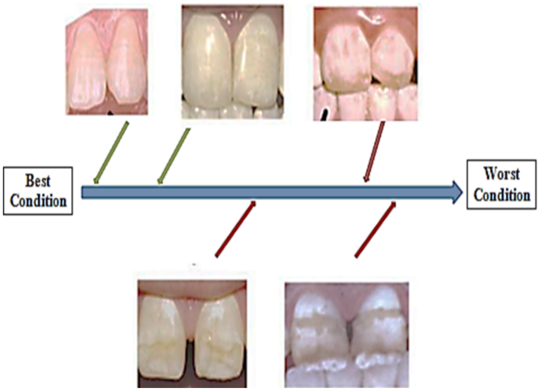

Fig. 2. Visual Analog Scale for Dental Fluorosis with visual indicators[97]

In 2006, Pretty and colleagues recommended the use of Quantitative Light Fluorescence[98]. Their study had the objective to use the fluorotic system and analyze its association with the TF index. The principle of QLF is that it compares differences in fluorescence between sound enamel and 'unsound enamel' (loss of fluorescence intensity in areas of enamel hypomineralisation). The use of computer software was recommended for assessing the images. Guerra *et al.,* (2015) worked on the Developmental Defects of Enamel (DDE) index[99]. They used spectrophotometric evaluation for examining the defects. 39 teeth that represented DDE defects on the labial surface were collected for this study. These samples were then analyzed using a spectroshade evaluation. The study found this method to be more reliable and concludable. Results obtained were free from bias and the data obtained were correct[99]. Such methods can be used for analyzing dental fluorosis studies.

CONCLUSION

1. All the dental Fluorosis Indexes reviewed in this paper have been used extensively and find a place in the literature. However, it should also be considered that none is without limitations.
2. Employing new diagnosis techniques can be used to overcome certain limitations and prevent biases.
3. New techniques proposed by different researchers can be employed for *In vivo* diagnosis of teeth and scoring; however, pilot studies need to ensure the reliability of such techniques.
4. Apart from aesthetic appearance and histological conditions, other optical properties, chemical, and spectroscopic analysis can be employed as an alternative to clinical diagnosis.
5. Extensive research and detailed analysis are needed for improvement and advancement in this direction.

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Conflict of interest

Authors have no relevant financial or non-financial interest to declare.

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