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SALIVARY FLUORIDE LEVEL, WATER FLUORIDE LEVEL AND CORRELATION WITH DENTAL CARIES IN 20-60 YRS AGE GROUP

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ABSTRACT

Aims: Previous studies have indicated that there may be a relationship between salivary fluoride concentrations and dental caries. Aim & Objective: Our study focuses on quantitative evaluation of salivary fluoride level of a random group of patients and measurement of the water fluoride levels of their respective locality and find out their possible correlation with the prevalence of dental caries. Materials and Methods: 40 random patients of 20-60 years age group attending the outdoor of the department of Conservative Dentistry & Endodontics and residents of a locality in a particular place called Chhatna, Bankura were selected for the study. Their saliva samples were collected and drinking water from all tube wells and bore wells in the residential area of patients were collected. Salivary fluoride and water fluoride levels were estimated using an ion-specific electrode. For each patient, dentition status score was assigned for each tooth (Oral Health Survey; Basic Methods; WHO 1997) and the DMFT score was calculated from it for each patient. Results: A negative correlation (i.e. less caries DMFT score for increasing in salivary fluride level) was found between salivary fluride and caries DMFT score in both N24 Parganas and Chhatna, Bankura. The mean DMFT score of N24 parganas was significantly higher (p<.05) than that of Bankura. A negative correlation was found between water fluoride level and dental caries in 30-40 and 50-60 age group in study population of Bankura i.e. decrease in DMFT score with increase in water fluoride level and in 40-50 yrs age group in N24 parganas.

Conclusion: With increase in salivary fluoride level, there is a decrease in caries and with increase in water fluoride level, there is a decrease in caries.

KEYWORDS: Dental caries; salivary fluoride; water fluoride; correlation; N24 Parganas; Chhatna; tubewells; borewells; ion specific electrode; age group; DMFT score

INTRODUCTION

Dental scientists living and working during the last 50 to 60 years have contributed to our understanding that dental caries is a chronic, dietomicrobial, site-specific disease caused by shifts from protective factors favouring tooth remineralization to destructive factors leading to demineralization.^[1] Of the infectious diseases that affect humans, dental caries may be the most prevalent, according to a 1996 bulletin from the Centers for Disease Control and Prevention. We now know that caries results from complex interactions among the tooth structure, the dental biofilm, and dietary, salivary and genetic influences.^[1] This view of the disease was the basis for the proposal of many preventive philosophies still in use nowadays, based on the treatment of the disease itself, and not of the sequelae of the disease (the cavities). Although the concepts brought up by pioneer studies on the multiple factors involved in caries disease changed the paradigm of caries and moved Dentistry to a higher level of quality in patient care, in order to understand how fluoride could interfere with the caries disease, we must leave the prominent "Keyes diagram" behind and move forward to a deeper understanding of the biological and social factors involved in the disease.^[2] Using a broader definition to

 Table 1: Distribution of Salivary fluoride level (mean± s.d.) and correlation between salivary fluoride and Caries

 DMFT score for different age group of 24-Pargans (N)

Age Group (in years)	Salivary fluride (Mean± s.d.) (in ppm)	Caries DMFT score (Mean± s.d.)	Correlation (Between Salivary fluride and Caries DMFT score) (r)	p-value
20-30	0.147(0.16)	5.00(1.73)	-0.13	0.839
30-40	0.249(0.32)	2.00(2.35)	-0.61	0.279
40-50	0.111(0.07)	4.20(2.59)	-0.02	0.971
50-60	0.184(0.25)	7.00(5.34)	-0.46	0.440

 Table 2: Distribution of Salivary fluride level (mean± s.d.) and correlation between salivary fluride and Caries

 DMFT score for different age group of Bankura

Age Group (in years)	Salivary fluride (Mean± s.d.) (in ppm)	Caries DMFT score (Mean± s.d.)	Correlation (Between Salivary fluride and Caries DMFT score) (r)	p-value
20-30	0.058(0.02)	0.80(1.79)	-0.48	0.412
30-40	0.066(0.01)	2.20(2.17)	0.29	0.642
40-50	0.055(0.02)	2.20(3.35)	-0.17	0.789
50-60	0.050(0.01)	4.00(1.22)	-0.12	0.846

 Table 3: Overall distribution of Salivary fluride level (mean± s.d.) and correlation between salivary fluride and

 Caries DMFT score of the two districts

District	Salivary fluride (Mean± s.d.) (in ppm)	Caries DMFT score (Mean± s.d.)	Correlation (Between Salivary fluride and Caries DMFT score) (r)	p-value
24-Pargans	0.173(0.21)	4.550(3.55)	-0.37	0.112
Bankura	0.057(0.02)	2.300(2.39)	-0.21	0.370

 Table 4: Overall distribution of Salivary fluride level (mean± s.d.) and correlation between salivary fluride and

 Caries DMFT score (combined value of the two districts)

Overall	Salivary fluride (Mean± s.d.) (in ppm)	Caries DMFT score (Mean± s.d.)	Correlation (Between Salivary fluride and Caries DMFT score) (r)	p-value
	0.115 (0.16)	3.425 (3.19)	-0.14	0.389

understand the disease.^[2,3] dental caries can only occur if a necessary factor is present: biofilm accumulation on the teeth. But the presence of biofilm is not sufficient for the disease to develop; fermentable carbohydrates must also be present so that acid can be produced in the restricted environment of the biofilm, inducing mineral loss from the underlying tooth structure. The exposure to sugar can thus be considered a determinant factor in dental caries disease, especially if it occurs at a high frequency (e.g. more than 6 times/day⁴). If the sugar is sucrose, bacteria in the biofilm are able to not only produce acids, but also synthesize extracellular polysaccharides - sugar polymers that enhance the biofilm cariogenicity by changing its diffusion and adherence properties.^[2] Other determinant factors in the development of the disease are saliva and fluoride. Both have significant,

positive effects on the reduction of mineral loss, by either clearing out the fermentable substrates and acids or buffering the latter, in the case of saliva, or else enhancing mineral precipitation back on teeth, in the case of fluoride. Fluoride can only exert its effect if it is free, soluble in the aqueous oral environment (biofilm fluid or saliva).^[3] As such, fluoride will physicochemically induce mineral precipitation on the tooth structure in the form of fluorapatite; this can happen while demineralization is occurring within the biofilm milieu (an effect called reduction of demineralization), or after acids have been cleared from the biofilm or the biofilm itself was removed (the so-called enhancement of remineralization).^[4,5] Thus, fluoride deposited on the tooth mineral must be regarded as a consequence of reduced mineral loss occurring in the presence of fluoride, and not





Fig. 7

the goal of its preventive action. Such concepts as "fluoride strengthening teeth", "increasing the resistance of teeth to acids" and "reducing the acid produced by bacteria", although theoretically reasonable, are no longer accepted as clinically relevant to the reduction of caries associated with fluoride use.^[6] In other words, fluoride is not able to affect biofilm accumulation (necessary factor) and the production of acids from its exposure to sugars (determinant factor), but will chemically reduce the mineral loss induced by the combination of these two factors, through the



Fig. 8

precipitation of a fluoridated mineral on teeth. Fluoride available in the ionic form in the oral cavity is able to counterbalance the mineral losses caused by acid production in the biofilm, by inducing the precipitation of the less soluble mineral phase fluorapatite in the tooth structure. Perhaps the great effect of fluoride to control caries is based on the concentration needed for it to exert its effect: when fluoride is present at concentrations as low as 1 μ M (approximately 0.02 ppm F), the oral fluids (saliva, plaque fluid) are supersaturated with respect to the mineral

Salivary & water fluoride level

phase fluorapatite. Thus, even when available at very low concentrations in the mouth, fluoride can induce the precipitation of minerals on teeth. This effect, occurring every day, results in delayed mineral loss and prolongs the time needed for caries lesions to be clinically observed - or even maintains the mineral loss at subclinical stages for the whole life of an individual.^[2] However, the limitations of the anticaries effect of fluoride need to be considered. Fluoride does not interfere with the factors responsible for the disease, namely biofilm accumulation and sugar use. The antibacterial effect of fluoride in the concentrations remaining in the oral cavity, predominantly below 10 ppm, cannot affect bacterial metabolism.^[6] Also, once a great mineral loss has occurred, and the clinical signs are already visible (e.g. a white spot lesion), fluoride is not able to replenish the porous area inside the enamel with minerals, but will help impair the process, resulting in the arrestment of the caries lesion progression. The white spot will eventually have a shiny surface, as a result of surface polishing and remineralization in the presence of fluoride, but the white aspect, from the porous areas underneath, will partially remain.^[4] Fluoride in drinking water prevents dental caries but excessive ingestion during the period of tooth mineralization causes dental fluorosis.^[7] It was known that individuals living in fluoridated area have a lower incidence of dental caries than those living in a non-fluoridated area.^[8] Now it is agreed that fluoride available in the oral cavity in the topical form plays a significant role in caries reduction. Hence the focus of the fluoride usage is on topical fluorides. But water fluoridation is also considered as one of the major public health initiative, which reduces the health related disparities among various communities. Hence water fluoridation is also an effective tool in reducing dental caries as a part of mass strategy. Fluoride concentration in whole saliva has been related to the efficacy of caries prevention. Salivary fluoride levels vary from 0.01-0.10 mg/L depending on the water fluoride level and the fluoride usage and diet of the individual. The baseline salivary fluoride level in some studies have ranged from .02ppm to 1.93 ppm.^[9] Lagerlof Oliveby^[10] stated that saliva influences and caries attack mainly by its rate of flow and by its fluoride content. This group also reported that the

salivary fluoride level was not influenced by the flow rate or by diurnal variation. Dawes et al., quote the normal concentration of fluoride in saliva as being about 1 mµmol/L (0.019mg/L), and also confirmed that salivary fluoride levels were independent of flow rates, and that higher concentrations of fluoride in saliva led to the formation of calcium fluoride which had a longer clearance time.^[11] Many researchers now believe that continuous low concentrations of fluoride in saliva, particularly at the plaque/saliva/enamel interface are necessary for caries prevention [ten Cate, 1997; Featherstone, 1999].^[12,13] Leverett et al., showed that caries-free subjects had higher salivary fluoride than high caries subjects.^[14] Shields et al., showed that subjects with no caries experience, from both fluoridated and nonfluoridated communities, had salivary fluoride levels of 0.04 mg/L or greater, whereas high caries subjects from both fluoridated and nonfluoridated communities had salivary fluoride levels of 0.02 mg/L or less.^[15] Bruun and Thylstrup studied the salivary fluoride levels of 13 year old Danish children residing in high (2.31mg/L) and low (0.36 mg/L) water fluoride areas and their caries status.^[16] They reported that the children residing in the higher water fluoride area had higher salivary fluoride levels (0.047 mg/L) compared to the lower water area (0.026 mg/L) and correspondingly lower DMFS scores (7.0 versus 15.2). Duggal et al., also showed a consistent inverse relationship between salivary fluoride concentration and dental caries in 272 children living in rural areas in North India.^[17] So in the present context, studies investigating the effect of salivary and water fluoride level on the caries prevalence have become the need of the hour. Our study focuses on quantitative evaluation of salivary fluoride level of a random group of 40 patients of 20-60 years age group and measurement of the water fluoride levels of their respective locality and find out their possible correlation with the prevalence of dental caries.

MATERIALS AND METHODS

The present study was conducted in the department of Conservative Dentistry & Endodontics, Guru Nanak Institute of Dental Science & Research, 157/F Nilgunj Road, Kolkata - 700114. 40 random patients of 20-60 years age group attending the outdoor of the dept of Conservative Dentistry & Endodontics and

residents of a locality in a particular place called Chhatna, Bankura were selected for the study. The majority of patients attending the outdoor were found to be residents of areas in and around Sodepur, mostly of north 24 Parganas. Patients of age group 20-60 yrs were randomly selected for the study. A history sheet containing duly filled details pertaining to the study was obtained from each patient. The sample size necessary for the study was calculated on the basis of prevalence of dental caries in adults. **Inclusion Criteria**

- 1. Random patients of 20-60 years age group were selected for the study.
- 2. Patients irrespective of any medical condition were selected for the study.
- 3. Both male and female patients were selected for the study.
- 4. Only patients who are residents of North 24 Parganas and Chhatna, Bankura were selected for the study.
- 5. Drinking water from all borewells and tubewells in the respective residential areas of patients were selected for the study.

Exclusion Criteria

- 1. Patients below 20 years of age and above 60 years were not selected for the study.
- 2. Residents of districts other than North 24 Parganas and other places of Bankura apart from Chhatna were not selected for the study.
- 3. Drinking water from sources other than tubewells and borewells in the respective residential areas of patients and from other districts than those selected for the study were not included in the study.
- 4. Teeth missing due to reasons other than that of caries are not scored.
- 5. Teeth having restorations due to aesthetic purposes or reasons other than caries like laminates, crowns are not scored

Intraoral Examination

Intraoral examination was carried out in a dental chair by a mouth mirror, a standard WHO probe and adequate illumination (as described in Oral Health Survey; Basic Methods; WHO 1997) and caries status of each patient was assigned using the WHO index (Oral Health Survey; Basic Methods; WHO 1997) for the dentition status and treatment need. The treatment need score was not assigned. For each patient, dentition status score was assigned for each tooth (Oral Health Survey; Basic Methods; WHO 1997) and the DMFT score was calculated from it for each patient. The examination was carried out by two observers separately in the same day to avoid the chances of bias.

SALIVA COLLECTION METHOD:

All the saliva samples were collected between 9 am - 12 pm to minimise diurnal variations associated with saliva sampling. The patients were asked to come at least two hours after the last meal and at least 1 hour after brushing. Before the extraction of saliva the subjects rinsed their mouth twice with tap water and waited for 10 mins before commencing collection to remove food debris and other non salivary elements that could interfere with measurements. The patients were asked to swallow the saliva in their mouth for 5-10 secs before commencing collection. In order to obtain mechanically stimulated saliva, the participants were asked to chew onto a piece of sterile paraffin wax and expectorate every 30 seconds for a total of 5 minutes into a sterile container. Approximately 2 m.l. of saliva was collected. The samples were immediately sent to the laboratory for investigations.

SALIVARY FLUORIDE ESTIMATION

The salivary samples were treated with TISAB II (total ionic strength adjustment buffer) in a 1:1 ratio to provide a constant background ionic strength, decomplexes fluoride ions and adjusts the solution pH (preparation of TISAB II: place 500ml of distilled water in a 1 liter beaker. Add 57 ml of glacial acetic acid and 58g of reagent grade sodium chloride to the beaker. Place the beaker in a water bath for cooling. Immerse a calibrated pH electrode into the solution and slowly add 5 M NaoH until the pH is between 5.0 and 5.5. cool the solution to room temperature and pour it into a 1 liter volumetric flask and dilute to flask mark with distilled water. All reagents should be pure to keep the fluoride level low in the buffer.) The fluoride estimation was then carried out in an ion specific electrode ISE ORION Thermoscientific (Model No-M-9609BNWP, Sr No. OX1-13627) coupled to an ion analyzer (Orion-4 Star)by direct calibration method

WATER FLUORIDE ESTIMATION

The sources of drinking water were noted from the details filled out by patients in questionnaire form. Drinking water from all tubewells and borewells in the residential area of patients were collected. The water samples were treated with TISAB-I in a 1:1 ratio and estimated for fluoride levels in a_ion specific electrode ISE ORION Thermoscientific (Model No-M-9609BNWP, Sr No. OX1-13627) coupled to an ion analyzer (Orion-4 Star)by direct calibration method. The mean fluoride level of drinking water sources in N24 parganas was found to be .193ppm and in Chhatna, Bankura was found to be 1.7ppm.

RESULTS

Statistical Analysis was performed with the help of Epi Info (TM) 3.5.3. EPI INFO is a trademark of the Centers for Disease Control Prevention (CDC). Descriptive statistical and analysis was performed to calculate the means with corresponding standard deviations (s.d.). One Way Analysis of variance Also (ANOVA) followed by Tukey's Test was performed with the help of Critical Difference (CD) or Least Significant Difference (LSD) at 5% and 1% level of significance to compare the mean values. $p \le 0.05$ was taken to be statistically significant.

Distribution of Salivary fluoride level (mean± s.d.) and correlation between salivary fluoride and Caries DMFT score for different age group of 24-Pargans (N): Mean water fluoride level -0.193±0.19 ppm and mean salivary fluoride level - 0.173±0.21. t-test showed that there was no significant difference in mean water fluoride level and mean salivary fluoride level (p>0.05). ANOVA test showed that there was no significant difference in salivary fluride level of different age groups ($F_{3,16}=0.25$;p>0.05). However, mean salivary fluride level for the age group 30-40 year was higher than other age group. Also for this group highest negative correlation (i.e. less caries DMFT score for increasing in salivary fluride level) was found between salivary fluride and caries DMFT score but not statistically significant(p> .05). ANOVA test showed that there was no significant difference in caries DMFT score of different age groups (F_{3.16}=0.33;p>0.0). However, mean caries DMFT score for the age group 50-60 year was higher than other age group (Table 1, Fig. 1 & Fig. 2).

Distribution of Salivary fluride level (mean \pm s.d.) and correlation between salivary fluride and Caries DMFT score for different age group of Bankura: Mean water fluride level - 1.7 \pm 0.34 ppm and mean salivary fluride level - 0.057 ± 0.02 . t-test showed that mean salivary fluride level was significantly lower than mean water fluride level (p<0.05). ANOVA test showed that there was no significant difference in salivary fluride level of different age groups $(F_{3,16}=1.63;p>0.05).$ However, mean salivary fluride level for the age group 30-40 year was higher than other age group. Also for this group highest positive correlation (i.e.More caries DMFT score for increasing in salivary fluoride level) was found between salivary fluoride and caries DMFT score but not statistically significant (p>.05). ANOVA test showed that there was no significant difference in caries DMFT score of different age groups (F_{3.16}=0.84;p>0.0). However, mean caries DMFT score for the age group 50-60 year was higher than other age group (Table 2, Fig. 3 & Fig. 4).

Overall distribution of Salivary fluride level (mean± s.d.) and correlation between salivary fluride and Caries DMFT score of the two districts: In overall, t-test showed that mean salivary fluride level and mean caries DMFT score were significantly higher in N24-Parganas that that of Bankura (p<0.05). Also higher negative correlation (i.e. less caries DMFT score for increasing in salivary fluride level) was found between salivary fluride and caries DMFT score 24-Pargans but not statistically for significant(p>.05) (Table 3, Fig. 5 & Fig. 6).

Overall distribution of Salivary fluoride level (mean \pm s.d.) and correlation between salivary fluoride and Caries DMFT score (combined value of the two districts) Mean water fluoride level - 0.947 \pm 0.26 ppm. Combined data of the two districts showed that mean salivary fluoride level was significantly lower than that of mean water fluoride level (p<0.05). Also negative correlation was observed between salivary fluoride and caries DMFT score but not statistically significant(p>.05) (Table 4).

Correlations for N24-Parganas: For the age group 50-60 years highest negative correlation (i.e. less salivary fluoride level for increasing in water fluoride level) was found between salivary fluoride and water fluoride level. For the age group 40-50 years highest negative correlation (i.e. less caries DMFT score for increasing in water fluoride level) was found between water fluoride level and caries DMFT score and it was

statistically significant (p<.05) (Fig. 7 & Fig. 8). *Correlations for Bankura:* For the age group 20-30 years highest negative correlation (i.e. less salivary fluoride level for increase in water fluride level) was found between salivary fluoride and water fluoride level but not statistically significant (p>.05). For the age group 30-40 years and 50-60 yrs negative correlation (i.e. less caries DMFT score for increase in water fluride level) was found between water fluoride level and caries DMFT score but not statistically significant (p>.05) (Fig. 9 & Fig. 10).

DISCUSSION

The intent of the present study was to estimate the relative effect of water fluoride on salivary fluoride, dental caries, and effect of salivary fluoride on dental caries prevalence among people of two different districts having varying levels of fluoride in drinking water. Several districts in West Bengal have been identified with high fluoride level in groundwater. Chhatna, Bankura have been identified as one of the areas in Bankura with high fluoride level ranging from <.5 ppm to a maximum of 4.25 ppm with at least 11 sites recording groundwater fluoride level of >1.5 ppm(Chakrabarti, 2012).^[18] The mean salivary fluoride level for the people of N24 Parganas was found to be significantly higher than that of Chhatna, Bankura as seen from results of this study, inspite of the fact that the water fluoride level of Chhatna, Bankura was higher (1.7 ppm, which is higher than the optimum water fluoride .7-1.2 ppm) than that of N24 Parganas (.193 ppm). This can be attributed to the difference in brushing habits, use of fluoridated tooth pastes, food habits and socioeconomic status of the residents of the two districts. The mean DMFT score of N24 parganas was significantly higher (p<.05) than that of Bankura which can be attributed to the fact that the water fluoride level of Chhatna, Bankura was higher than that of N 24 parganas. In both N24 parganas and Bankura there was a high negative correlation found between salivary fluoride level and DMFT scores which means that with increasing salivary fluoride levels there is a decrease in dental caries score which confirms the cariostatic and caries-protective effects of fluoride. The mean salivary fluoride level of participants of both the districts was found to be higher in 30-40 age group which can be attributed

to food habits, brushing habits, use of fluoridated tooth paste and socio-economic status and the availability of dental health care facilities,dental awareness etc. The mean DMFT score was higher in the 50-60 yrs age group which can be attributed to longer duration of exposure of tooth to food and irritants in the oral cavity, lack of meticulous oral hygiene maintenance owing to age-related loss of manual dexterity, food habits, etc. Previous studies^[19] on excretion of fluoride in human saliva have revealed that the concentration of fluoride in whole saliva mirrored the fluoride concentration in plasma, but at a lower level. Variations in salivary flow rate (0.34 +/- 0.15 ml/min for unstimulated and 1.06 +/- 0.28 ml/min for stimulated) did not affect the salivary fluoride concentration. In our study, a negative correlation was obtained between salivary fluoride level and water fluoride level, i.e. with increase in water fluoride level, a decrease in salivary fluoride level was seen contrary to previous studies by Bruun and Thylstrup,^[16] and Ingram and Morgan,^[20] Bhargava *et al.*,^[21] where a positive correlation was obtained. But all these studies used water fluoride concentrations of 1, 4 and even >4 ppm whereas in our study design only 1.7 ppm and .193 ppm water fluoride concentrations are used and moreover the status of artificial fluoridation of water sources of the studied population in our study are not known. Further studies on a larger population need to be conducted to confirm the results. Regarding correlation of water fluoride and dental caries, negative correlation was found in 30-40 and 50-60 age group in study population of Bankura i.e., decrease in DMFT score with increase in water fluoride level and in 40-50 yrs age group in N24 parganas. Overall negative correlation was found between salivary fluoride level and dental caries in both the districts i.e., with increase in salivary fluoride level there is decrease in DMFT score but not statistically significant. Moreover the mean salivary fluoride concentration was found to be .05 ppm for people of Chhatna, Bankura and .1 ppm for N24 Parganas in accordance with the findings that the salivary fluoride concentration of 0.03 ppm or higher is sufficient for anticaries effect.

CONCLUSION

Within the limitations of the study it can be concluded that:

- The mean salivary fluoride level of participants of both the districts N24 parganas and Chhatna, Bankura was found to be higher in 30-40 age group though not statistically significant (p>.05).
- 2) The mean salivary fluoride level and mean caries DMFT score of N24 parganas was significantly higher (p<.05) than that of Bankura. The mean DMFT score was higher in the 50-60 yrs age group in both the districts.
- 3) In N24 parganas and Bankura there was a high negative correlation found between salivary fluoride level and DMFT scores which means that with increasing salivary fluoride levels there is a decrease in dental caries score, with N24 Parganas having a higher negative correlation.
- 4) The mean DMFT score of participants of N 24 parganas was higher with a lower mean water fluoride level of .193 ppm whereas the mean DMFT score of Chhatna, Bankura was lower with a higher mean water fluoride level of 1.7 ppm.
- 5) There was no significant difference in mean water fluoride level and mean salivary fluoride level (p>0.05) in N24 parganas whereas mean salivary fluride level was significantly lower than mean water fluride level (p<0.05) for Chhatna, Bankura.
- 6) A statistically not significant negative correlation was found between water fluoride level and dental caries in 30-40 and 50-60 age group in study population of Bankura i.e. decrease in DMFT score with increase in water fluoride level and in 40-50 yrs age group in N24 parganas(statistically significant).

Further studies need to be conducted on a larger population for more conclusive results..

CONFLICT OF INTEREST & SOURCE OF FUNDING

The author declares that there is no source of funding and there is no conflict of interest among all authors.

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