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Original Research Article

Prevalence of hypothyroidism and its association with anti-thyroid peroxidase antibody among adult sea food consuming population attending a tertiary health care centre in Kerala

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Abstract

Background: There is less data on the prevalence of hypothyroidism in adult population of India. Currently with iodine supplementation and availability of iodine rich nutritious food, the iodine deficiency etiology in hypothyroidism is no more applicable. So it is useful to estimate thyroid auto-antibodies to thyroid peroxidase in these people to exclude autoimmune thyroiditis.

Objectives: This study was undertaken to know the prevalence of hypothyroidism and its association with Anti-TPO antibody among adult sea food consuming population.

Materials and Methods: A hospital-based cross-sectional study involving 300 patients was conducted in the Department of General surgery, Dr. SMCSI Medical College, Karakonam, Trivandrum during December 2012 to January 2014. Patient's data collected, the relevant laboratory test (serum FT3, FT4 and Thyroid Stimulating Hormone [TSH]) done. Thyroid abnormalities were diagnosed on the basis of these laboratory results. Anti TPO antibody was estimated in those patients with hypothyroidism (High TSH) using Calbiotech Thyroid Peroxidase (TPO) IgG ELISA Kit.

Results: Totally 30.4% of subjects had thyroid dysfunction, prevalence of hypothyroidism and subclinical hypothyroidism was 11.7 % and 15% respectively. Anti TPO antibody was positive in 71.4 % hypothyroid patients and 68.9 % subclinical hypothyroid patients. TSH had a significant positive correlation ($r = 0.324$, $p=0.003$) with anti TPO. There was a significant relation between hypothyroidism and Anti TPO Antibody suggesting thyroid autoimmunity as a risk factor for hypothyroidism.

Conclusion: Significant relation between hypothyroidism and Anti TPO Antibody was found, suggesting thyroid autoimmunity as a risk factor for hypothyroidism. Autoimmune thyroiditis can be considered as one of the etiological factors for hypothyroidism in our population. But Iodine status or autoimmunity do not explain all thyroid dysfunction .Hence further studies are required to address these problems.

Keywords: Hypothyroidism; Anti TPO Antibody; seafood; autoimmune thyroiditis

1. Introduction

Hypothyroidism is commonly seen during outpatient practice, and the improvements in assay & increased awareness has led to the evaluation of more number of patients. Though iodine supplementation is associated with large scale benefits, concerns have been raised regarding the side effects related to varying levels of iodine intake [1][2]. Literature says that iodine intake up to 1 mg/day is tolerated by normal adults [3]. But, reports suggest that continued exposure to iodine may result in clinical conditions like goiter, thyroid dysfunction (both hypo and hyper-thyroidism), and thyroid autoimmunity [4].

Currently with iodine supplementation and availability of iodine rich nutritious food (milk, fish etc.), the iodine deficiency etiology in hypothyroidism is no more applicable. The reason for higher prevalence of hypothyroidism in the coastal areas is unknown. People living in the coastal areas are using iodine rich water so it cannot be due to iodine deficiency. So it is useful to estimate thyroid auto-antibodies to thyroid peroxidase in these people to exclude autoimmune thyroiditis[5]. Hypothyroidism is mostly caused by Hashimoto's thyroiditis in iodine-replete individuals or by a lack of the thyroid gland or a deficiency of hormones from either the

hypothalamus or the pituitary gland [6]. The exact etiology of this disease is not clear though it is widely believed that genetic factors can predispose and environmental factors can trigger autoimmunity. Repletion of iodine in iodine depleted areas and chronic excess iodine intake exposure is known to trigger thyroid autoimmunity [7]. Moreover radiation and exposure to many environmental chemicals such as persistent organic pollutants [8] also were associated with development of thyroid autoimmunity.

In a population-based study done in Cochin on 971 adults, the prevalence of hypothyroidism was 3.9% [9]. The prevalence of subclinical hypothyroidism was also high, the value being 9.4%. In women, the prevalence was higher, at 11.4%, when compared with men, in whom the prevalence was 6.2%. The prevalence of subclinical hypothyroidism increased with age. 53% of subjects with subclinical hypothyroidism were positive for anti-TPO antibodies.

There is less data on thyroid dysfunction, antibody status and magnitude of thyroid disorders from Kerala. A proper study regarding the various aspects of thyroid problem such as pattern of thyroid disorders and autoimmunity in adults would be helpful to understand this health problem better.

1.1 Objectives: 1) To know the prevalence of hypothyroidism among adult seafood consuming population attending the surgical outpatient department. 2) To detect Anti-TPO antibody and its association with hypothyroidism in the population.

1.2 Hypothesis:

- Null Hypothesis-There is no association between Anti-TPO antibodies & hypothyroidism.
- Alternate Hypothesis-There is an association with hypothyroidism & presence of Anti-TPO antibodies

1.3 Review of Literature

1.3.1 Role of Iodine in the induction of Thyroid Autoimmunity

Human studies regarding the effect of iodine administration in subjects with endemic goiter showed development of thyroid autoantibodies in approximately 8-20% of the patients, depending on the dose, as well as an increased intrathyroidal lymphocytic infiltration in a number of patients[10][11]. Nevertheless, these findings were transient and after discontinuation of iodine, antibody titers and lymphocytic infiltration decreased significantly. The mechanisms by which excessive iodine is related to the development of thyroid autoimmunity are as yet unknown but several hypotheses have been put forward.

Intake of large iodine quantities results in its increased incorporation in the Tg molecule, this highly iodinated molecule characterized by alterations in its stereochemical conformation can change its properties, leading to loss of antigenic epitopes and creation of novel, iodine containing ones. New antigenic determinants may be created by tyrosine iodination at critical points within the Tg molecule [12][13]. When presented to T and/or B lymphocytes, these new determinants exhibit an increased affinity for the T cell receptor or the MHC-presenting molecule on antigen-presenting cells (APCs). This may consequently enhance the Tg presentation by APCs and lead to specific T lymphocyte activation, thereby initiating the autoimmune process. Excessive iodination of Tg can thus heighten its immunogenic potential compared with Tg containing fewer iodine atoms.

Another suggested mechanism is direct iodine toxicity to thyrocytes, possibly through induction of oxidative stress. TPO rapidly oxidizes excessive amounts of iodine in the hyperplasticthyrocytes and generates oxidative intermediates of iodine. These oxidative elements are highly reactive and able to bind to proteins, nucleic acids and membrane lipids, forming iodo compounds which damage thyroid cell and mitochondrial membrane integrity. Oxidative stress caused by the generation of free radicals can also lead to thyroid cell necrosis, while autoantigens may be released. Excessive iodine intake is also related to the induction of thyrocyteapoptosis and the development of thyroid autoimmunity [14-16].

1.3.2 Prevalence of thyroid disorders –Indian Scenario

According to a projection from various studies on thyroid disease, it has been estimated that about 42 million people in India suffer from thyroid diseases [4]. Despite becoming iodine sufficient the total goiter prevalence amongst school age children in India remains 17.9%, more than the recommended 5%which is thought to be due to the presence of unidentified goitrogens such as thiocyanate, environmental pollutants etc[4]. Regarding the pattern of thyroid functional disorders in the post iodization phase, the results from nationwide surveys among school children have clearly showed that subclinical hypothyroidism was the commonest disorder (4.9%) whereas overt hypothyroidism(0.79%), and hyperthyroidism (1.0%) were much less common[4].

Rebeca Abraham *et al*, 2009conducted a cross sectional study in Puducherry where of the total 505 women examined 15.8% had thyroid dysfunction. Of these 11.5% were hypothyroid (9.5% sub-clinical) and 1.8% hyperthyroid (1.2% clinical). 19% of women over 60 years had elevated TSH above 4.5 μIU/ml, suggesting that Hypothyroidism particularly

sub-clinical hypothyroidism is predominantly present amongst women in this iodine sufficient region [17].

In a recent population-based study done in Cochin on 971 adult subjects, the prevalence of hypothyroidism and subclinical hypothyroidism was 3.9% and 9.4% respectively [9]. In women, the prevalence was higher, at 11.4%, when compared to men with 6.2% prevalence. The study also noted the increased prevalence of subclinical hypothyroidism old age. About 53% of subjects with subclinical hypothyroidism were positive for anti-TPO antibodies. In another study in the same population, using cluster sampling strategy, urinary iodine status was studied in 954 subjects from the same population sampled, and the median value was 211 µg/l suggesting that this population was iodine sufficient.

Many studies have been conducted in Kerala to determine the prevalence of goiter and IDD. In 1976, Kochupillai *et al* undertook a study in coastal areas in Kerala to examine goiter characteristics, and reported that the prevalence of thyroid nodules was 13% in the population surveyed [18]**.** Surveys conducted in 14 districts in Kerala between 1989 and 1994 demonstrated goiter prevalence ranging from 4.7% to 27.3% [19]. These studies adequately demonstrated that though iodine levels are sufficient in most parts of Kerala, probably some iodine deficient pockets exists and prevalence of goiter in child population is still higher than expected for the level of iodination.

As most of these studies have been conducted among school children there is less data on thyroid dysfunction, antibody status and magnitude of thyroid disorders from Kerala. A proper study designed to address the various aspects of thyroid problem such as pattern of thyroid disorders and autoimmunity in adults would be really helpful to understand this health problem better.

2. Materials & Methodology

- **Study Area** – Department of surgery, Dr. S.M.C.S.I Medical College, Karakonam.
- **Study Period** - 2 yrs
- **Study Population** –All adult patients coming to surgery OPD with symptoms & signs of thyroid disease.
- **Study Subjects**-All adult patients diagnosed with symptoms and signs of hypothyroidism & confirmed with lab findings.
- **Study Design** –cross-sectional study

Sample Size -300 (Sah), Calculated using the formula-4PQ/L2

Taking P=25.7% (note from review of literature)

Q = 100-P

L = Relative precision (20% of previous prevalence)

- **Exclusion Criteria**- patients who are unwilling to participate in the study.

2.1 Methodology

A predesigned and pretested questionnaire with demographic details, nutritional history, clinical examination & laboratory investigations were used for data collection. All adults were subjected to blood sampling for estimation of thyroid function status (free T4, free T3 and TSH) after overnight fasting. FT3, FT4 and TSH were analyzed by electrochemiluminescence assay (Vitros ECi analyzer) after separating the serum from blood samples using a centrifuge. Fasting blood samples were collected by venipuncture technique and for separation of serum, the blood is centrifuged at 2000 rpm for 10 minutes. The separated serum is used to estimate serum TSH, FT3, FT4 and TPO antibodies.

Normal range for FT4, FT3 and TSH were 0.8-2 ng/dl,1.4-4.2 pg/ml and 0.4-4.2mIU/L respectively. The presence of either subclinical or overt, hypo- or hyperthyroidism was used to define thyroid dysfunction. Anti TPO antibody was estimated in patients with hypothyroidism (High TSH) using Calbiotech Thyroid Peroxidase (TPO) IgG ELISA Kit.

2.2 Definitions and Normal Values

Euthyroid- normal FT4 level (0.8-2 ng/dl) and normal TSH level (0.4-4.2mIU/L).

Hypothyroidism – Subjects with serum FT4 < 0.8ng/dl and TSH > 4.2mIU/L were categorized as hypothyroid.

Subclinical hypothyroidism - those with normal serum FT4 and TSH > 4.2mIU/L were classified as having subclinical hypothyroidism.

Hyperthyroidism - Subjects with serum FT4 >2ng/dl and TSH<0.4 mIU/L were considered as having hyperthyroidism.

Subclinical Hyperthyroidism - Subjects with normal serum FT4 and TSH<0.4 mIU/L were considered as having subclinical hyperthyroidism.

Interpretation of Anti TPO antibody

- <50IU/L-Negative
- >50IU/L-Positive

Principle of the Test

Microwells with bound highly purified human thyroid peroxidase (TPO). Antibodies against this antigen, if present in diluted serum or plasma, bind to the respective antigen. Washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human IgG immunologically detects the bound patient antibodies forming a conjugate / antibody / antigen complex. Washing of the microwells removes unbound conjugate. An enzyme

substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end – product. The intensity of this yellow color is measured photo metrically at 450 nm. The amount of color is directly proportional to the concentration of IgG antibodies present in the original sample.

2.3 Data collection method

One to one interview method.

2.3 Data entry

Data was entered in Microsoft Excel

2.4 Data analysis

Data analyzed using Statistical package for the social sciences (SPSS) version 17.0. Correlations of continuous variables calculated using Pearson's correlation coefficient and statistical test of significance of the associations with categorical variables done by Chi-Square test. A p-value of 0.05 or less was taken to indicate a significant difference.

3. Observations and Results

This is a cross sectional study conducted among adult seafood consuming population in rural Kerala to find out the prevalence of hypothyroidism and its association with Anti-TPO antibody. In this study, 300 adult patients who were suspected to have thyroid disease were evaluated in detail with clinical examination and laboratory investigations.

In this study 91 patients were found to have abnormal thyroid function tests.

Table 1: Age distribution of cases

Age	Count	Percent
<30	121	40.3
31 – 40	85	28.3
41 – 0	56	18.7
51 – 60	25	8.3
>60	13	4.3
Mean ± SD	35.9 ± 13.1	

Large number of study subjects belongs to 18-30 years age group. Mean age was 35.9 ± 13.1 years.

Table 2: Gender distribution of cases

Sex	Count	Percent
Male	44	14.7
Female	256	85.3

The ratio of female to male is around 5.8:1 in our study

Table 3: Pattern of thyroid dysfunction among the study population (N=300)

Interpretation	Count	Percent
Hypothyroidism	35	11.7
Subclinical Hypothyroidism	45	15.0
Hyperthyroidism	6	2.0
Subclinical Hyperthyroidism	5	1.7
Euthyroid	209	69.7

Totally 30.4 % of subjects have thyroid dysfunction; subclinical state was seen in more number of cases than overt hypothyroidism.

Table 4: Pattern of thyroid dysfunction among the study population based on gender (N=300)

	Gender		
	Male	Female	Total
Hypothyroidism	8	27	35
	22.9%	77.1%	100.0%
Subclinical hypothyroidism	5	40	45
	11.1%	88.9%	100.0%
Hyperthyroidism	2	4	6
	33.3%	66.7%	100.0%
Subclinical hyperthyroidism	0	5	5
	0%	100.0%	100.0%
Normal TFT	29	180	209
	13.9%	86.1%	100.0%

In the present study prevalence of thyroid dysfunction was more among females than males. The common symptoms at the time of presentation were hair loss and fatigue.

Figure 1: Graph showing mode of presentation

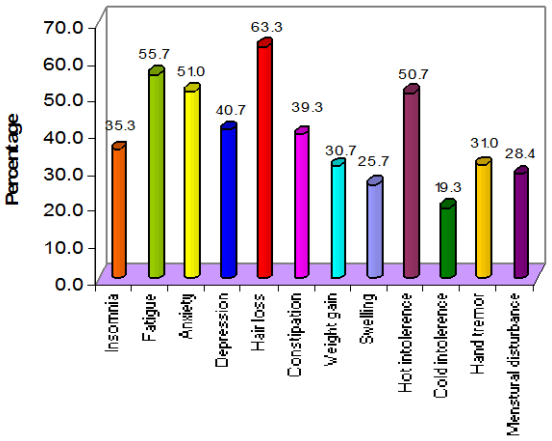

Table 5: Age distribution among hypothyroid patients

Age	Count	Percent
<30	17	48.6
31 - 40	12	34.3
41 - 50	5	14.3
51 - 60	1	2.9
Mean ± SD	31.8 ± 7.8	

Large number of study subjects belongs to 18-30 years age group. Mean age was 31.8 ± 7.8 years.

Table 6: Age distribution among Subclinical hypothyroid patients

Age	Count	Percent
<30	7	15.6
31 - 40	21	46.7
41 - 50	13	28.9
51 - 60	4	8.9
Mean ± SD	37.9 ± 8.2	

Most of study subjects belong to 31-40 years age group. Mean age was 37.9±8.2 years.

Table 7: Anti TPO Antibody positivity among hypothyroid and subclinical hypothyroid patients

Anti TPO	Hypothyroid	Subclinical hypothyroid
Positive	71.4%	68.9%
Negative	28.6%	31.1 %

Subjects with TPO positive subclinical hypothyroid cases are more likely to progress into frank hypothyroid state.

Table 8: Descriptive statistics of Anti TPO antibody among hypothyroid and subclinical hypothyroid patients

	Hypothyroid	Subclinical Hypothyroid
Mean	98.2	80.3
SD	55.0	38.9
Median	114.5	86.5
Minimum	8.0	13.0
Maximum	186.0	143.0

Fig 2: Box plot of Anti TPO antibody among hypothyroid and subclinical hypothyroid patients

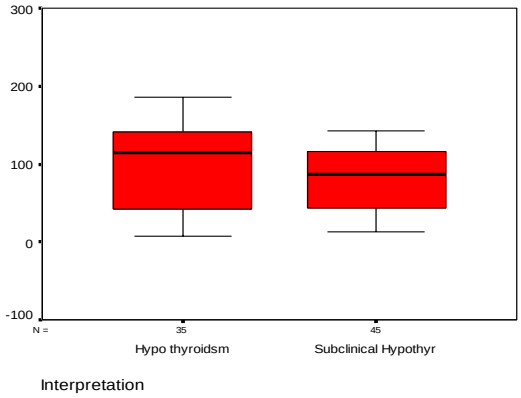

Table 9: Age distribution of cases with positive Anti TPO Antibody

Age	Count	Percent
<30	16	28.6
31 - 40	23	41.1
41 - 50	14	25.0
51 - 60	3	5.4
Mean ± SD	35.4 ± 8.4	

Most cases with Anti TPO antibody positivity were in the 31-40 age groups. The average age of the patient in the age study being 35.4 years.

Table 10: Gender distribution of cases with positive Anti TPO Antibody

Sex	Count	Percentage (%)
Male	8	14.3
Female	48	85.7

Table 11: Correlation between Anti TPO antibody and TSH

	r	P
Hypothyroidism	0.324	0.003

Linear correlation was tested between Anti TPO antibody and TSH using Pearson's' correlations. TSH showed a significant positive correlation (r = 0.324, p=0.003) with anti TPO.There is a significant relation between hypothyroidism and Anti TPO Antibody.

4. Discussion

This is a study conducted on hypothyroidism, in rural Kerala, with respect to thyroid disorders (hypothyroidism). Study demonstrates that hypothyroidism, mainly subclinical hypothyroidism, was alarmingly high in this region with female vulnerability. The prevalence of hypothyroidism and subclinical hypothyroidism was 11.7 and 15 % respectively. This is much higher than the reported prevalence of hypothyroidism in other iodine-sufficient populations [21][22]. This may be due to the effect of iodine status improvement occurred during the last 2-3 decades of salt iodization. Tunbridge *et al* [22] were the first to provide a reliable estimate about the prevalence of hypothyroidism in the general adult population. They found out that 10.3% and 0.3% were suffering from subclinical and over hypothyroidism, respectively. Another study from Tehran, Iran, on individual's ≥20 years demonstrated that 0.35% of individuals were overt and 2.2% were subclinical hypothyroid [23]. A study from five coastal areas of Japan, which has iodine rich seaweed (kelp), showed that the prevalence of hypothyroidism was 0 – 9.7% [24]. Another study from northern Japan, where iodine intakes is high, revealed that 0.7% of men and 3.1% of women were overt hypothyroid [25]. The prevalence ofelevated TSH among Colorado men and women was 9.5%[26]. NHANES III found the prevalence of subclinical hypothyroidism with a TSH>4.5m IU/L in the U.S. adult population to be 4.3% [27]. In a population-based study done in Cochin the overall prevalence of hypothyroidism was found to be 3.9%. Prevalence of subclinical hypothyroidism was also high in this study, the value being 9.4% [9].

Although all age group presented with a high prevalence of hypothyroidism, higher number of subjects was observed between age groups of 18-30 years of age. This shows that thyroid disease should be considered during routine evaluation of this susceptible group and should be followed by appropriate detection and treatment. From our study the prevalence of hypothyroidism is highest (48.6%) in the age group of 18-30 years while prevalence of subclinical hypothyroidism is highest (46.7%) in 31-40 years. Our study revealed that females are more vulnerable to hypothyroidism & subclinical hypothyroidism.

Saha *et al* reported that hypothyroidism was more prevalent (40.5 %) in the age group of 36-45 years with obvious female preponderance [20].

Another study quoted age preponderance of 34-years and above [28] while Makkah *et al* exhibited similar age group predominance of 40 ± 12 years on the prevalence of thyroid disorders [29]. The prevalence and pattern of hypothyroidism depend on ethnic, geographic and environmental factors including iodine status [30].

The total prevalence of goiter was 27% (Grade 1- 16.3%, Grade 2 -10.7 %) and was significantly higher in females than males. Grade I and II goiters were present in 6.8% and 4.5% of men and 18% and 11.7% of women respectively. In 1976, Kochupillai *et al* undertook a study in coastal areas in Kerala and reported that the prevalence of thyroid nodules was 13% in the population surveyed [18]**.** Surveys conducted in 14 districts in Kerala between 1989 and 1994 demonstrated goiter prevalence ranging from 4.7% to 27.3% [19].

Another finding that requires further elaboration is the high prevalence of positive Anti TPOAb in hypothyroid individuals, supporting the results of other studies [31]. In our study positive anti TPO antibody was seen in 71.4% of hypothyroid subjects and in 68.9% of subclinical hypothyroid suggesting thyroid autoimmunity as the etiology of these thyroid dysfunction.

Chronic thyroid dysfunction i.e. hyperthyroidism (Grave's disease) and hypothyroidism (Hashimoto's Thyroiditis) occur secondary to the actions of antibodies [32]. Anti-TPO antibodies are the most prevalent and is present in 80-90% of Hashimoto's Thyroiditis. Anti-TPO antibodies are cytotoxic, and they damage the thyroid cell by complement activation and antibody — dependent cell cytotoxicity [33]. Detection of these antibodies insignificant titers helps in establishing the etiology of hypothyroidism [34]. The antibody titers vary among individuals depending on the activity of the underlying auto immune activity. During the active phase of chronic lymphocyticthyroiditis, a high titer can be detected in the serum. Conversely, around 10-15% patients may have a negative antibody screening result in spite of the presence of an autoimmune process in the thyroid. Theinitiation of autoimmunity may be predisposed by genetic factors[35] advancing age, environmental factors[36] suchas stress[37], infections[38], trauma, smoking[39], femalesex indicating hormonal influences[40], and iodine[41] play a key role in the manifestation of autoimmune thyroiditis.

Vaseghani *et al* concluded from their study that anti-TPO antibody titer correspond to TSH titers[42]. In our study TSH had a significant positive correlation (r = 0.324, p=0.003) with anti TPO. There is a significant correlation between hypothyroidism and Anti TPO Antibody. Studies done including one in Greece showed that all patients with sub-clinical hypothyroidism had positive anti-thyroid antibodies[43][44], while some studies did not show significant difference[45]. A significant correlation between TSH or T4 concentration and elevated anti-TPO antibody was demonstrated by Ghoraishian *et al*[46].

In our study, a majority of hypothyroid subjects had an elevated TPO antibody titer (twice the upper limit of normal) establishing an autoimmune etiology. Among those, who were negative for anti-TPO antibodies, there is a possibility of some of them would still have a cytological evidence of autoimmune destruction. The high antibody titers in some of them reflect the active autoimmune process going on in the thyroid gland.

In suspected thyroid disorders, assessment begins with the evaluation of the thyroid hormone levels. In case of hypothyroidism, the presence of anti-TPO antibodies provides an etiological diagnosis. Anti-TPO antibodies, however, can be negative in 10-15% of individuals, which occurs when the autoimmune activity has subsided. In persons suspected to have other autoimmune diseases, anti-TPO antibodies can be used as a surrogate marker for the presence of autoimmunity.

5. Conclusion

Anti-TPO antibody estimation is a very useful test for establishing the etiological diagnosis of autoimmune thyroid diseases in our population, hence autoimmune thyroiditis can be considered as one of the etiological factors for hypothyroidism in our population. But Iodine status or autoimmunity do not explain all thyroid dysfunction. Other than these two factors there are genetic and environmental factors which can affect thyroid hormone levels and autoimmunity. Possible role of environmental pollutants such as perchlorate, pesticides, triclosan, tobacco etc. need to be evaluated. But estimation of these factors are expensive and beyond the scope of this study. Hence to address these issues further large studies should be designed.

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