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A DESCRIPTIVE CROSS SECTIONAL STUDY FOR ESTIMATION OF ANTI NUCLEAR AUTOANTIBODIES IN PAEDIATRIC & ADOLESCENT PATIENTS WITH AUTOIMMUNE THYROID DISEASES IN A TERTIARY CARE HOSPITAL IN KOLKATA, WEST BENGAL

Biochemistry

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ABSTRACT

Worldwide study showed frequency of ANA positivity in children with autoimmune thyroiditis (but without any signs & symptoms of other autoimmune disease) ,was between the range of 30%-70%. This cross sectional , descriptive,non- interventional study took place in Medical College,Kolkata on 100 paediatric & adoloscent age group (5-15 yr) patients suffering from autoimmune thyroid disease(i.e. Hashimoto's Thyroidits & Graeves Disease) but without any signs & symptoms of other autoimmune disease to estimate their ANA positivity & their ANA- HEP 2 pattern for prediction of their future chance of getting other autoimmune diseases . Total 52 patients were found to be ANA positive out of the 100 patients, ANA HEP 2 indirect immunofluorescence showed most of them i.e. 57.69% had coarse/fine speckled pattern. To precisely establish the role between ANA positivity in paediatric & adoloscent age group patients with autoimmune thyroid disease and future autoimmune disease occurence, more prospective and cohort studies with larger sample size are needed, as the limitation of this study was small sample size & patient follow up was not done.

KEYWORDS

Anti nuclear antibody, Autoimmune thyroid disease, HEP 2.

INTRODUCTION : -

Autoimmune thyroid diseases (ATD) are among the most common autoimmune diseases in humans and also referred as the prototypic single-organ directed autoimmune disease [1]. Most commonly the patients suffer from chronic lymphocytic thyroiditis,also referred to as Hashimoto disease (HT) & also from Graves Disease ,these 2 diseases often have similar kind of clinical presentation [1]. In these disorders, several autoantibodies against thyroid antigens such as thyroid peroxidase antibody, thyroglobulin antibody, and anti-TSH-receptor antibody are identified. In addition, non-organ specific autoantibodies such as antinuclear antibodies (ANA) and rheumatoid factor (RF), may be present [2]. Often there is an association between autoimmune thyroid diseases & rheumatic diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and Sjogren's syndrome (SS) [3]. Therefore, finding an autoantibody such as ANA or RF in an ATD patient may warrant further investigations.

But on the other hand , ANA and RF are not specific for rheumatic diseases & causes such as some drugs , neoplasm or infection may alter these autoantibodies without any special significance [4]. When antinuclear antibodies appear in other autoimmune non-rheumatic syndromes it becomes a question of concern whether these autoantibodies are merely a serological finding or whether they are markers of an associated preclinical rheumatic disorder even before appearance of the symptoms .

According to some cohort studies done wordwide, the frequency of ANA positivity in children with autoimmune thyroiditis was between the range of 30% to 70% [5]. Possible explanation behind the high association of ANA with autoimmune thyroiditis are enhanced apoptosis of thyroid follicular cells thus exposing nuclear antigens to elicit development of ANA, and B cell hyperactivity with production of multiple autoantibodies [6,7].

From our clinical experience & also from clinical studies done worlwide, we have noticed an association between ANA positivity and thyroid antibody positivity in patients without a known autoimmune disease; however, this phenomenon has not been extensively studied in the pediatric population of eastern India. Therefore, this study was designed to estimate the incidence of ANA positivity & to study ANA pattern in thyroid antibody positive (Anti-Thyroid Peroxidase [ATPO] antibodies positive) children without symptoms suggestive of SLE or yet to develop symptoms.

MATERIALS & METHODS :-

This cross-sectional,observational,non-interventional study was done in the Dept. Of Biochemistry,Medical College & Hospital,Kolkata between January 2019 – April 2019. It included 100 paediatric & adoloscent age group (5-15 yr) patients with Autoimmune Thyroid Disease (80 patients suffering from Hashimoto's thyroiditis and 20 patients suffering from Graeve's Disease). The sampling technique comprised all paediatric& adoloscent patients suffering from autoimmune thyroid disease visiting the Endocrinology Outpatient Clinic from Medical College & Hospital,Kolkata which is a tertiary hospital. They came for regular consultation during the period mentioned and they were included according to inclusion/exclusion criteria, appointment order and willingness to participate in the study. Patients with a previous diagnosis of rheumatic disorders were excluded. Patients taking drugs such as – penicillin , streptomucin ,tetracyclins ,INH ,sulphonamides ,phenytoin ,hydantoin, procainamide ,practolol ,reserpine ,hydralazine ,chlorpromazine ,penicillamine & allopurinol were excluded as these drugs are known causative factors for drug induced Lupus. [8] . Hashimoto's Thyroiditis was defined based on the presence of thyroid autoantibody (Anti TPO Ab) more than 2 times the upper normal value and thyroid ultrasound evaluation showing reduced echogenicity compatible with thyroiditis, with or without hypothyroidism. Graeve's Disease was defined by clinical symptoms and laboratorial tests (TSH and free T4) of hyperthyroidism. Treatment for thyroid disease was required in 95.6 % of these patients.

The enrolled patients had to undergo a complete physical examination and the clinical and laboratory data were collected in a standardized form, which included their demographics, any significant past medical history ,comorbidities, and previous and recent treatments. All children and their parents were interviewed according to a questionnaire seeking signs and symptoms related to rheumatic diseases in children. The questionnaire included the following signs and symptoms like: joint pain, joint swelling, back pain, morning stiffness, asthenia, Raynaud's phenomenon, xerostomia, xerophthalmia, pleuritis, and pericarditis. Children with signs and/or symptoms suggestive of immune rheumatic diseases were excluded from the study & were later examined by a rheumatologist.

TSH, free T4 and anti TPOAb were measured by chemiluminescence assays; normal TSH value varied between 0.55 and 4.78 mIU/L and free thyroxine between 0.8- 1.76 ng/dL & anti TPO Ab upto 35 IU/ml. [9]

Ten mL of venous blood were drawn, aliquoted, and preserved at –20 ° C until ANA and RF tests were done. All the samples were screened for ANA by indirect immunofluorescence on hep-2 cells, using a commercially available kit ANA hep-2 (EUROIMMUN Medizinische Labordiagnostika AG.Germany), as recommended by the manufacturer, a titer of 1: 100 or higher was considered to indicate ANA positivity. Immunofluorscence intensity ranged from + to ++++.The fluorescence patterns were interpreted as fine speckled, coarse speckled, homogeneous, peripheral, centromeric, nucleolar, and cytoplasmic patterns. Samples with positive ANA tests were assessed by ELISA for antibodies against extractible nuclear antigens (ENA; SS-A/ Ro, SS-B/La, Sm, RNP, Jo-1,Ro-52, CENP-B, PCNA,

dsDNA, nucleosomes, histone, rib.P.prot, AMA M2 and Scl-70), using individual ENA kit (EUROIMMUN Medizinische Labordiagnostika AG.Germany) for detection and confirmation of the test. The cut-off level was set at > 1:100, as recommended by the manufacturer. [10]

Statistical calculations were performed using SPSS for Windows Version 17 (SPSS, Chicago, IL, USA). Data were expressed as mean and standard deviation for continuous data. values < 0.05 were considered statistically significant.

RESULTS :-

Total 100 paediatric & adoloscent age group (5-15 yr) patients with Autoimmune Thyroid Disease (80 Hashimoto's thyroiditis and 20 Graeve's Disease) participated in this study.

Number of female patients were 76 & male patients were 24.

Table -1 – Depicting Mean Values Of Tsh,ft4 & Anti Tpo Ab In Hashimoto's Thyroiditis

TSH	FT4	ANTI TPO Ab
10.45 +- 0.98 mIU/ml	0.5+-0.24 ng/dl	80 .5 +- 2.5 IU/ml

TABLE -2 – Depicting mean values of TSH & FT4 in Graeve's Disease

TSH	FT4
0.35 +- 0.09 mIU/ml	2.5+-0.29 ng/dl

ANA positivity was found in 52/100 children (52%), anti-dsDNA antibodies were found in 15/100 (15 %) .

- ENA-specific autoantibodies were determined in following number of patients –

A) anti-RNP/Sm positive – 15,
B) anti-Jo-1 positive – 3 ,
C) anti SS-A/ Ro positive – 12 ,
D) anti PCNA positive – 4,
E) anti SSB positive – 2 ,
F) anti Rib P positive – 3,
G) anti nucleosome positive – 3,
H) anti histone positive – 7 ,
I) anti AMA M2 positive – 3 .

The ANA pattern was coarse/fine speckled in 30/52 (57.69%), homogeneous in 14/52 (26.92%), nucleolar in 4/52 (7.69 %) & cytoplasmic in 4/52(7.69 %). (Table 3) (Fig. 1 to 5).

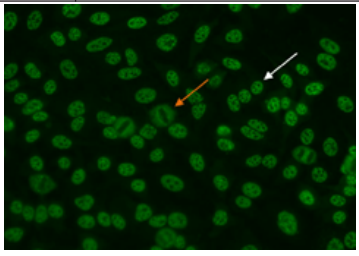
The IIFA intensity was +++ in 18/52 (34.61%), ++ in 22/52 (42.31%), and + in 12/52 (23.07%) cases.

No significant differences were found between the ANA-positive and ANA-negative groups with respect to age, sex, L-thyroxine treatment, TSH & TPOAb levels.

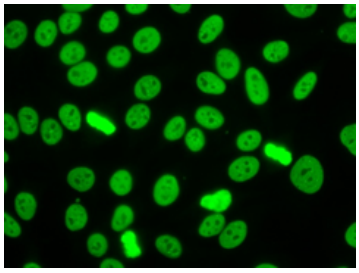
Children with persistent joint pain were referred to a rheumatologist (FC). Children with Raynaud's phenomenon underwent capillaroscopy. No evidence of SLE, RA, or any other systemic autoimmune disease was found.

TABLE 3

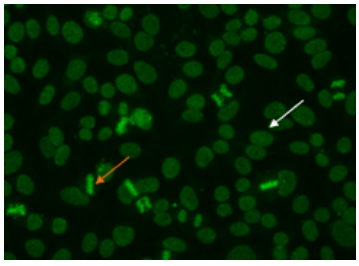
	ANA Pattern- total number of paediatric patients (52)
coarse/fine speckled	30 (57.69%)
homogeneous	14 (26.92%)
nucleolar	4 (7.69 %)
cytoplasmic	4 (7.69 %)



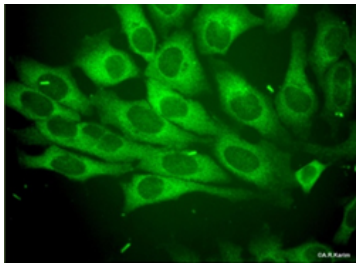
Coarse Speckled pattern (Fig. 1)



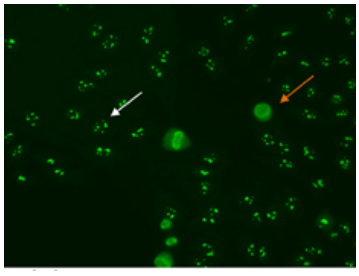
Homogenous pattern(Fig. 2)



Fine Speckled pattern(Fig. 3)



Cytoplasmic Pattern(Fig. 4)



Nucleolar pattern(Fig. 5)

DISCUSSION :-

Autoimmune diseases are complex disorders caused by a combination of genetic susceptibility and environmental factors that may disrupt the immune system by attacking self organs. These disruptions often becomes the cause for future development of autoimmune diseases making patients with one autoimmune disease vulnerable for other autoimmune diseases[11-12].

AITD is one of the commonest autoimmune disease and it is frequently associated with other autoimmune disorders which are organ and non-organ-specific [13-15]. In adult patients suffering from AITD a variable ANA prevalence up to 45% has been reported [16,17]. ANA is associated with different autoimmune disorders (i.e., SLE, progressive systemic sclerosis, Sjogren's syndrome, juvenile idiopathic arthritis, mixed connective-tissue disease, autoimmune hepatitis and primary autoimmune cholangitis). It is also associated with infections (18,19). Most important role of ANA is that it can be detected in over ninety percent of patients with SLE, an autoimmune disease, which is multifactorial involving genetic and environmental factors, characterized by a wide range of autoantibodies and clinical manifestations [19, 20].

ANA can be also found in healthy people [18]. A recent study done by M Satoh et al which is a cross-sectional analysis done on 4754 individuals older than 12 years , showed a prevalence of ANA of 13.8%

[21]. M. O. Esteves Hil´ario et al showed similar prevalence of ANA positivity ,i.e. 12.6% in healthy children, with higher titres found between 5 and 10 years of age [22]. Y. Inamo et al (1997) investigated ANA prevalence in children with AITD, they found an incidence of ANA positivity significantly higher in patients with untreated Graeves disease (71%) than in Hashimotos thyroiditis (33%),(using a cutoff of 1 : 40 for ANA IIFA on HEp-2 cells) [23]. They also suggested that the ANA positivity may be a predictive factor for poor response to antithyroid drugs in Graeves Disease. In our study, Hashimotos thyroiditis represented 80% of total enrolled patients versus 36% in the previous study, and we included only 20 children affected with Graeves Disease.

According to our study there was no significant difference in ANA - positive and ANA-negative group with respect to age, sex, LT4 treatment, TSH & TPOAb levels.

As per our study, 12 out of 100 patients were anti SS-A/ Ro positive, this finding was supported by Tektonidou et al. [24] found a prevalence of 10% of anti-Ro/SS-A positivity, and Elnady et al. [25],with a positivity of 14.8% in their sample. But on the other hand, Morita et al. [26] did not find anti-Ro/SS-A in their 50 patients with autoimmune thyroid disease. The finding of anti-Ro/SS-A positivity in a disease with female predominance is very important, as this autoantibody may cross the placenta barrier in pregnant women and cause neonatal lupus with a risk of congenital heart block, independently of the diagnosis of a background disease [27]. Most mothers of babies born with neonatal lupus do not have SLE or another autoimmune disease, just the autoantibody [28]. Neonatal lupus accounts for 90–95% of cases of congenital heart block occurring in utero or in the neonatal period [27,28]. As this is a serious situation that may be prevented by glucocorticoid administration to the mother [27], the purpose of this study was to propose ANA screening (and further search for anti-Ro/SS-A) in ATD patients who in the future will become pregnant, to avoid this complication. In our study, all anti-Ro-positive patients were females.

- **Table depicting future chance of getting autoimmune disease –[29]**

anti-RNP/Sm positive	95 % prevalence of Mixed Connective Tissue Disease
anti-Jo-1 positive	25% - 35% prevalence of myositis
anti SS-A/ Ro positive	40%- 95 % prevalence of SLE, 95% - 100 % of neonatal lupus erythematosus
anti PCNA positive	3% prevalence of SLE
anti SSB positive	40%- 95 % prevalence of SLE, 75 % of neonatal lupus erythematosus
anti Rib P positive	10% prevalence of SLE
anti nucleosome positive	40% - 70% prevalence of SLE
anti histone positive	95% - 100 % prevalence of drug induced SLE
anti AMA M2 positive	Upto 96 % prevalence of primary billiary cholangitis

According to different researches done worldwide the probable explanation behind autoimmune disease is that a defect in the mechanisms involved in the engulfment of dead cell with inappropriate clearance of self-nucleic acids can cause autoimmune diseases. A deficiency of clearance of apoptotic cells is considered one of the causes of SLE and may be due to autoimmunity itself [30-31]. The activation of the innate and acquired immune response inside the body can be induced by infection, inflammation, or tissue injury, & these factors may have an impact on the development of autoimmunity in the thyroid [32,33].

In our study fine or coarse speckled pattern was the most commonly found pattern & this finding was supported by similar finding by Nisihara R et al (2018) [34].

This high prevalence of ANA in paediatric & adoloscent AITD may be the cause behind the involvement of self nucleic acids in the development of AITD.

So to conclude, after analysis of a limited number of cases, our study demonstrated ANA positivity in over 50% of children & adoloscents with autoimmune thyroid diseases and this ANA positivity at the time of the study was not related to overt immune-rheumatic diseases.

The purpose of the study was that the finding of ANA positivity in children and adolescents affected by AITD needs a careful reevaluation: it can be seen as a manifestation of "activated autoimmunity" without clinical relevance at the time of the study, because the positivity of ANA and other nonorgan- specific autoantibodies can appear in blood even many years before the onset of systemic autoimmune diseases [35,36].

To precisely establish the role between ANA positivity in paediatric& adoloscent age group patients with autoimmune thyroid disease and future autoimmune disease occurence, more prospective and cohort studies with larger sample size are needed. As the limitation of this study was small sample size and as it was a cross sectional study patients were not followed up.

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