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RESEARCH ARTICLE

PROSPECTIVE STUDY TO COMPARE RESULTS OF THYROID FUNCTION TESTS OF FLUORESCENT IMMUNOASSAY(FIA) AND ENZYME LINKED RADIOIMMUNOASSAY(ELISA) WITH GOLD STANDARD CHEMILUMINISCENCE TEST(CLIA) TEST IN A TERTIARY CARE HOSPITAL

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Key words:-
CLIA (Chemiluminiscence), ELISA (Enzyme Linked Immunosorbant Assay, FIA (Fluroimmunoassay), TFT (Thyroid Function Test)

Abstract

Background: Thyroid function test is widely used parameter to diagnose thyroid illness. Since introduction of radioimmunoassay method, several improvements are made in this technique. so it is important to define best sensitive assay with diagnostic accuracy to perform TFT in small laboratories . Therefore we performed analytical evaluation of results of serum total T3, total T4, TSH level by ELISA , FIA with gold standard CLIA method.

Material & Methods: In this study total of 40 participants were enrolled. Subjects were chosen randomly either attending OPD & IPD . Serum total T3, total T4, TSH levels were measured in subjects by all three method FIA, ELISA and CLIA. Results of FIA and ELISA test were compared with gold standard CLIA test in terms of sensitivity, specificity, positive predictive value and negative predictive value & diagnostic accuracy.

Results: Sensitivity of serum total T3, totalT4, TSH by ELISA is 75% FIA 88%, specificity of serum total T3,T4, TSH by ELISA is 67% and FIA 75%, PPV of all three parameter by ELISA is 60% & FIA 70%, NPV by ELISA is 80% & FIA is 90%

Conclusion: Results of serum total T3,serum total T4 and serum TSH by FIA method is comparable with results of CLIA method in term of sensitivity, specificity, positive predictive value and negative predictive value & can be used as reliable &efficient diagnostic test for measurement of serum Total T3, serum Total T4, serum TSH in small laboratories.

Introduction:-

Thyroid disease is one of the most common endocrine disorders (1). The laboratory diagnosis and monitoring of thyroid diseases are based on thyroid function test measurement of serum TSH along with serum total T4 and total

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T3 (2). Therefore, Thyroid function test are widely used for diagnosis of thyroid illness & follow up by clinicians(2). In subclinical hypothyroid stages analytical sensitivity of serum TSH is important where serum total T4 and T3 levels are in normal range(3). With rapid advancement and automation in laboratory ,there are many methodology developed to estimate TFT. As third generation Chemiluminiscence assay is now a days considered as gold standard for estimating serum thyroid hormone level due to its high diagnostic accuracy but it is expensive, requires trained personnel and good laboratory set up(2). ELISA is a popular analytic method widely used in many laboratories for measurement of thyroid hormones showing disadvantage of laborious technique and high turnaround time(4) Fluroimmunoaasay (FIA) can be used as Point of care technique(POCT) is rapid and cost effective which is needed in small laboratories. (5) . Due to availability of different method & lack of comparative data patients may go over and under treatment may have to bear brunt of discrepant results produced by different technique. so it is important to define the best sensitive assay with diagnostic accuracy where gold standard method is not available, therefore we performed analytical evaluation of ELISA and FIA method with gold standard CLIA method

Material & Methods:-

Cross sectional study was conducted between October 2023 to November 2023 at GMC Akola after obtaining ethical committee clearance no 435/2023. 40 participants between 20 to 60yrs old who are on fasting chosen randomly either attending OPD & IPD. Subjects with diabetes mellitus, hypertension , any chronic illness & pregnant women were excluded from study. With all aspectic precaution fasting blood sample was collected and serum was separated & Serum total T3, total T4, TSH levels were estimated in patients by all three method ELISA (biotek EPOCH2) , FIA (fincare III plus) and CLIA(ABBOTT Architect I 1000sr, Germany). Results of FIA and ELISA test were measured and data was obtained &compared with gold standard CLIA test in terms of sensitivity, specificity, positive predictive value and negative predictive value and diagnostic accuracy.

Result:-

In this study out of 40 subjects 8 were between age 20 to 35yrs, 22subjects between age 36yrs to 50yrs & 10 were above age 50yrs . Out of which 27 were females and 13 were males (table no 1).

We found that out of 40 subjects 16 were with disease subjects & 24 were without disease subjects by CLIA method (table no2).

We compared ELISA method with CLIA method and found that 12 were true positive , 16 were true negative and 8 were false positive & 4 were false negative subjects (tale no 2).

We compared FIA method with CLIA method and found that 14 were true positive , 18 were true negative and 6were false positive & 2 were false negative subjects(table no 3).

After data analysis,sensitivity of serum T3,T4 TSH by ELISA is 75% FIA 88%, specificity of T3,T4, TSH by ELISA is 67% and FIA 75%, PPV of all three parameter by ELISA is 60% & FIA 70%, NPV by ELISA is 80% & FIA is 90% & diagnostic accuracy of ELISA is 70% & FIA 80%.(graph no 1)

AGE	Number
(20 to 35yrs)	8(Male 1, Female 7)
(35 t0 50 yrs)	22(Male 8, Female 14)
(>50yrs)	10(Male 4, Female 6)

Table no 1:- Age and sex wise distribution of subjects.

	CLIA diseased 16(n=40)	CLIA (without disease)24 (n=40)
ELISA(positive)	12(n=40)	8(n=40)
ELISA(negative)	4(n=40)	16(n=24)

Table no 2:- Comparision of results of ELISA with gold standard method CLIA results.

	CLIA diseased 16(n=40)	CLIA (without disease)24 (n=40)
FIA(positive)	14 (n=40)	6 (n=40)
FIA(negative)	2 (n=40)	18 (n=24)

Table no 3:- comparision of results of FIA with gold standard method CLIA results

Graph no1: Comparision of results of TFT by ELISA and FIA method with CLIA method

Discussion:-

As it is known,the most trustworthy single marker for ruling out primary thyroid dysfunction is the measurement of serum TSH levels [1]. The American Thyroid Association's most recent recommendation explains use & importance of the TSH measurement as a primary test to diagnose hyperthyroidism as well as hyperthyroidism [6,7,8,9]. In our study, thyroid illness is more common in females as compared to males which is consistent with naz etal and shah etal , Paczkowska findlings(6,7,8) In ELISA, antigens from the test specimen adjoined to a surface are treated with specific antibody; and enzyme substrate is added as a final step. End point reaction is color change in the substrate producing a detectable signal. It is having high error while pipetting,washing showing high false positive and high false negative cases (10)

FIA is direct solid sandwich immunodetection method, where sample is added to the sample well of the test, the fluorescence-labeled detector antibody on the membrane separately binds to antigen in blood specimen & mixture migrates on the nitrocellulose matrix of test strip by capillary action, the complexes of detector antibody are separately captured by antibody that has been immobilized on test strip Signal intensity of fluorescence of detector antibody reflects amount of antigen captured having low pipetting, washing error and less turnaround time(11).Our study indicate comparable results in term of sensitivity, specificity, PPV, NPV and diagnostic accuracy in measurement of TFT by FIA tchnique than ELISA for diagnosing diseased thyroid illness when compare with CLIA method(12)

Conclusion:-

FIA technique is rapid immunometric diagnostic point of care assay test obtained within 20 mins and with 80% of diagnostic accuracy, requires less amount of sample so can be used as reliable diagnostic test for estimation of serum Total T3, serum Total T4, serum TSH as compared to ELISA technique for laboratories where gold standard CLIA technique is not available

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