Pacific Journal of Medical and Health Sciences
ISSN: 2456-7450 | Vol.4, No.2, 2022, pp-01-16

PJMHS
PACIFIC JOURNAL OF MEDICAL AND HEALTH SCIENCES

Research Paper

Comparative Study of TSH Quantification in Two Similar Novel Microfluidic Technology Hormone Analyzers

Ankit Koirala[1], Nitesh Kumar Sah[1]*

[1]Assistant Professor

[1]Shree Medical and Technical College, Bharatpur, Nepal
[1]*Faculty of Agriculture, Far West University, Nepal

*Corresponding author Email: niteshkumarsah5@gmail.com

ABSTRACT

Prevalence of the endocrine and metabolic disorders is common globally, and thyroid disorders constitute a major problem. Serum TSH (thyroid stimulating hormone) level determines the functional status of thyroid gland. Therefore, estimation of thyroid stimulating hormone (TSH) levels is a basic attempt to diagnose and manage the treatment of thyroid disorders. Recently, analytical technologies for biological fluids has been developed to replace the traditional analytical methods that rely on large sophisticated instruments that require more turn over time, energy, skilled manpower and are less sensitive and cost high. In contrast, immunoassays are popular because of its inexpensive and convenient nature. Immunoassay employs antibody to identify and neutralize variety of exogenous substances to diagnose any diseases and disorder. But their reaction principles and detection procedures limit their sensitivity. Microfluidic technology miniaturizes many lab procedures through the application of small volumes of fluid and flow. Microfluidic immunoassays have been studied broadly and explored as a promising tool for the estimation of TSH. These techniques are concerned with the miniaturization of conventional assay techniques and have gained advantage over the sample volume, reagent volume, contamination, turnover time including cost. The combination of both the immunoassays and microfluidic techniques are explored as a promising platform for automated, sensitive and multiplexed point-of-care diagnostics in developing areas. In the present study, we compared serum TSH level using similar microfluidic technology hormone analyzer. In the present study, we observed that estimation of serum TSH levels can be done rapidly and at at reasonable price. We also observed that this method is sustainable, effective, efficient, portable, eco-friendly, requires less men power, energy saving, error free, cost relevant, timeliness and appropriate for use in health clinics and in rural area.

KEYWORDS: Microfluidic, TSH, POCT

INTRODUCTION

Thyroid gland is a ductless and largest endocrine gland that develops approximately the 24th day of gestation period under the influence of fibroblast growth factor signaling pathways and is endodermal origin[1]. It is highly vascular organ with many follicular cells, situated just below the larynx and anterior to the trachea. The follicular cells are made to store the thyroid hormones. The weight of thyroid gland varies from author to author though it's normal, weigh ranges from 2-3 grams in neonates and 18-16 grams in adults[2].

Anatomy of Thyroid Gland

In 1656, Thomas Wharton is credited for the discovery of exact location for the structure of the thyroid gland along

with its primary function in human beings. According to him, thyroid gland provides size and beauty to the neck as this gland heat thyroid cartilage because thyroid cartilage is normally cold, lubricate the neck[3].

Thyroid gland is located at the front and sides of the neck; it is conical shaped bilobed structure in which both the lobes are linked with middle line by the isthmus. The apex part of each lobe is directed upward and lateral ward whereas the base is directed downward at the level of fifth or sixth tracheal ring. Each lobe measures about 5 cm. in length; about 3 cm in width, and about 2 cm in thickness. It is lightly weighty in female than male. Its convex shaped superficial surface is covered with skin, superficial and deep fascia, sternocleidomastoideus, superior belly of the omohyoideus, Sternohyoideus and sternothyreoideus, and beneath by the pretracheal layer of the deep fascia. The medial surface is shaped over thyroid and cricoid cartilage, trachea, constrictor pharynges, inferior and posterior part of the Cricothyreoideus, the esophagus, the superior and inferior thyroid arteries, and the recurrent nerves.

The thin anterior border inclines obliquely from above downward toward the middle line of the neck, while the posterior thick border overlaps the common carotid artery, and, as a rule, the parathyroid. An anastomotic branch uniting the two superior thyroid arteries runs across its anterior boarder while inferior thyroid veins runs at its lower border. Frequently, the upper part of the isthmus give rise to a conical shape structure called as pyramidal lobe ascends as far as hyoid bone. Fibrous band known as levator glandulae thyreoideæ is found attached above the hyoid bone and below to the isthmus. Sometimes accessory glands are present in the vicinity of lateral lobe or above the isthamus[4].

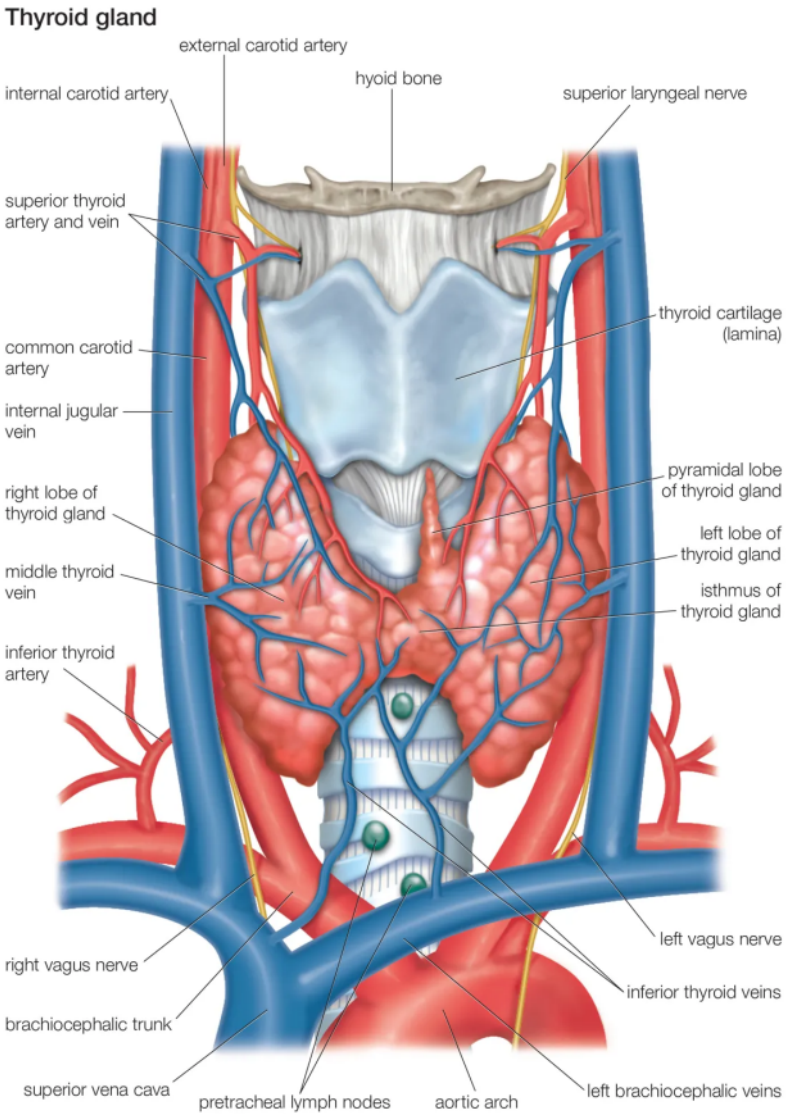

Figure 1: Anatomy of Thyroid Gland [5]

Secretions of Thyroid Gland

Thyroid gland mainly secretes two types of hormones namely, tri-iodothyronine (T3), and Thyroxin (T4). Organification of iodine is the first step of the synthesis of thyroid hormones in which iodine after absorption, forms iodide. Thus formed iodide is then condensed with tyrosine residue that resides along the thyroglobulin to form either a mono-iodinated tyrosine or di-iodinated tyrosine. This newly formed iodothyroglobulin constitutes most important constituent of colloidal material in the follicle of thyroid unit. The next step after the organification is a coupling reaction to which the first step is linked closely. Coupling reaction is nothing but it is coupling of iodotyrosine molecules together where two di-iodotyrosine molecules bind together to produce thyroxin (T4).Tri-iodothyronine (T3) is produced outside of the thyroid gland from coupling of a di-iodotyrosine and a mono-iodotyrosine. Deionization of thyroxin (T4) produces tri-iodothyronine (T3) which is biologically more active than T4. Iodine is the key component for the production of thyroid hormones whereas thyroperoxidase (TPO) is the primary enzymes that facilitate the synthesis of T3 and T4. The release of T3 and T4 in the circulatory system is controlled by HPT (hypothalamic-pituitary-thyroid) axis through its negative feedback mechanism. As T3 and T4 can't move freely in the blood stream therefore they binds with thyroxin binding globulin (TBG). Various metabolic process, energy consumption, growth, differentiation and development of tissue, turnover of hormones, synthesis of vitamins, and synthesis of many other substrates are regulated by T3 and T4. Therefore, to maintain the normal level of thyroid hormone, it is essential to supply adequate amount of iodine. Intake of iodine less than its recommended dose (about 150 gram per day) is mainly associated with goiter where as high iodine levels is associated with inhibition of thyroglobulin proteolysis, iodide oxidation and organification[6,7,8].

Hypothalamus secretes thyrotropin releasing hormone (TRH) when it senses decreased serum level of T3 and T4. Thyrotropin releasing hormone stimulates the anterior pituitary gland to secrete thyroid-stimulating hormone (TSH). Increased concentration of T3 in serum T3 inhibits secretion of TRH and TSH7. According to the American Association of Clinical Endocrinology, the normal level of TSH in serum ranges from 0.3 to 3.0 mIU/L. Whereas National Academy of Clinical Biochemistry accept the value from 0.4 to2.5 mIU/L. However, widely accepted value is that the upper limit of normal level should exceed more than 4.12 mIU/L[9,10,11].

Physiological Impact of Thyroid Hormones

Thyroid hormones, mainly T3 binds with two different receptorsh TR-α1 and hTR-β1to synthesize T3-receptor complex in the tissue. DNA binds with the T3-receptor complex and brings change in the expression of enzyme those encoding genes which have control over metabolism at cellular level.

Thyroid hormones involves in the regulation of growth and development of bones, CNS, lipid metabolism in adipose tissue, intestinal absorption of carbohydrate and degradation of muscle protein, formation of 2,3-diphosphoglycerate (DPG)

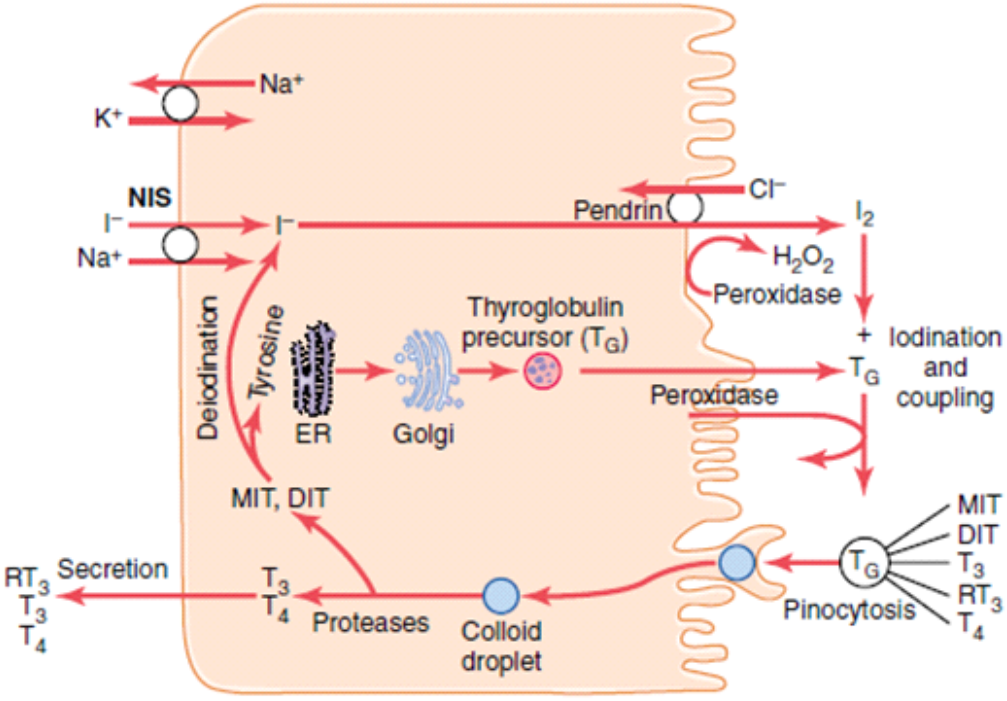

Figure 2: Synthesis and Secretion of T3 (tri-iodothyronine) and T4 (thyroxine)[5]

for oxygen dissociation from haemoglobin. Along with this almost all cell of the body is targeted by thyroid hormones, especially, metabolically active tissues which require large amount of oxygen. Hence thyroid hormone has wise effect on physiologic process which is briefly discussed as follows:

Metabolism: Thyroid hormones increases basal metabolic rate (BMR) by stimulating many metabolic processes in actively dividing tissues. Stimulation of various metabolic processes due to the action of thyroid hormones accounts for excess heat production in the body those results from increased rate of ATP hydrolysis and oxygen consumption.

In lipid metabolism: The concentration of fatty acids increases in the plasma when there is increased level of thyroid hormone. Because increased level of thyroid hormones stimulates fat mobilization in plasma. Also, thyroid hormones enhance fatty acid oxidation in many tissues. Concentration of cholesterol and triglycerides in plasma are inversely proportional to concentration of thyroid hormones; - increased blood cholesterol concentration is a diagnostic indication for hypothyroidism.

In carbohydrate metabolism: Thyroid hormones stimulate the insulin dependent entry of glucose in to the cell to produce free glucose.

In growth and development: Thyroid hormones enhance normal growth and development process of children and young animals. Adequate level of TH is essential for the normal development of foetal and neonatal brain.

In cardiovascular system: Heart rate, cardiac contractility and cardiac output are increased by thyroid hormones. They are required for vasodilatation to increase blood flow to many organs of the body.

In central nervous system: Altered mental state is associated with the altered concentration of thyroid hormones. Decreased concentration of thyroid hormone brings about mentally sluggish, while increased concentration causes anxiety and nervousness.

In reproductive system: Normal serum levels of thyroid hormones determine the normal functioning of reproductive system. Hypothyroidism is most commonly associated with infertility.

Regulation of Thyroid Hormones

Pituitary gland secretes thyroid stimulating hormone (TSH) under the influence of thyrotropin - releasing hormone (TRH) from the hypothalamus, controls the secretion of thyroid hormones from thyroid gland. TSH allows thyroid hormone production and secretion from the thyroid gland by a complex mechanism of positive and negative regulation[12,13].

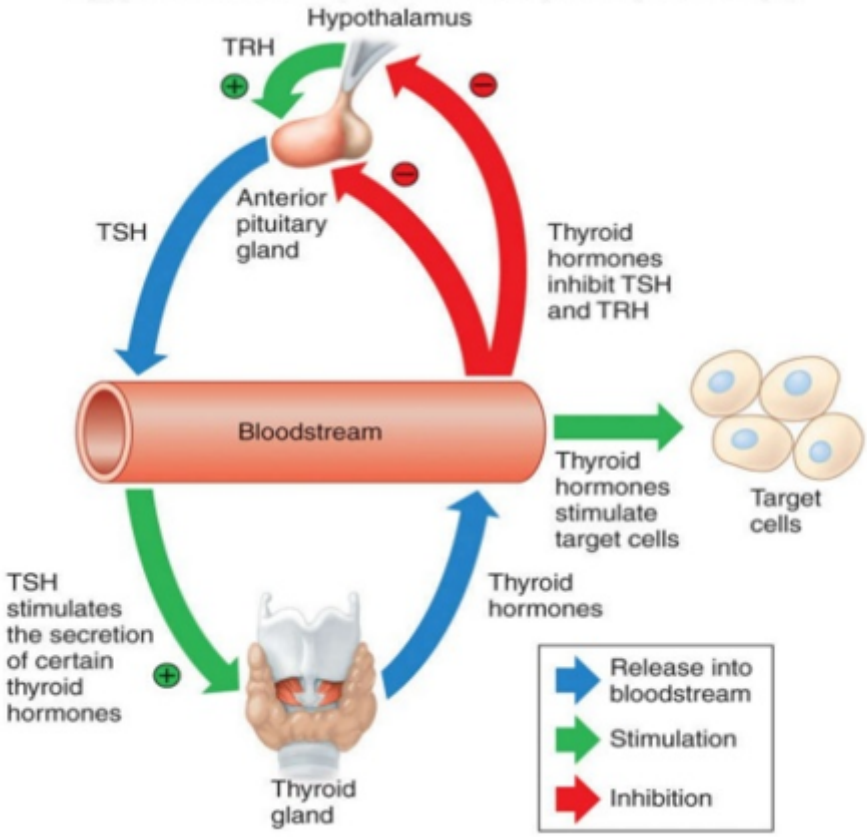

Figure 3: Regulation of Thyroid Secretion[5]

Endocrine and metabolic disorders emerge as common medical problems in worldwide due to iodine deficiency. Iodine deficiency is considered as significant cause of thyroid disorder that sets off the growth of thyroid gland which ultimately results in goiter, thyroid nodule formation and, hypothyroidism if not treated. Because the biosynthesis of Thyroid hormone requires the presence of iodide including thyroid peroxidase, supply of hydrogen peroxide, thyroglobulin- thyroid colloid protein identified by Adolf Oswald, proteolytic enzymes and iodotyrosine deiodinase[14,15].

T3 and T4 are the secretions thyroid gland are essential in regulation of the heart rate, blood pressure, body temperature, growth & development and the rate of metabolic process to generate energy in the body. The production of the Thyroid hormones is regulated by negative feedback mechanism[16,17].

Disorders of Thyroid Gland

Goiter has always been the most common diseases of interest. In Western medicine, thyroid gland was thought to lubricate the trachea. Goiter is the fullness or swelling of neck those results from thyroid enlargement was referred to by the Greeks, including Galen as hernia or swelling of the windpipe. According to Hippocrates and Gaius Plinius Secundus of Pliny, drinking of snow-water is the sole cause of goiter. Initially goiter was believed to be an enlargement of larynx but it was not considered as an enlargement of the thyroid gland[18].

In 1786 thyrotoxicosis was first described by Caleb Parry which was reported in 1825. Exophthalmic goiter was first described by Robert James Graves and the disease was named after him[19]. Fine needle aspiration introduced by Martin and Ellis in 1930s is widely accepted as gold standard technique for the evaluation of thyroid nodules[20]. Theodore Kocher, "Father of thyroid surgery" is credited for the advances in thyroid surgery. He recognized the need to preserve the parathyroid and reported more than 5000 successful thyroidectomies by 1912[21]. With this, thyroid extracts have been accepted as a part of current medical practice. With the demonstration of auto antibodies in Hashimoto's disease; Roitt, Doniach, Campbell and Hudson bring advances in thyroidology in 1956[22]. Albucasis removed a large goiter under opium sedation for the first time in the 10th century, Guy de Chaliac, the French surgeon reported goiter as a hereditary disease and recommended surgical treatment for this[24].

Thyroid diseases are the most common medical problem of immense interest associated with poor nutritious habits, iodine deficiency and lack of awareness. The bio physiological basis of the disease was not understood until 19th century but the discovery of structure and function of thyroid gland accredited modern scientists to cope up with reasonable explanations of the thyroid diseases. The disorder can either be harmless goiter which doesn't need any treatment or it can be life threatening cancer. The abnormal production of thyroid hormones results in two conditions namely hyperthyroidism (production of too much thyroid hormone) and hypothyroidism (production of insufficient thyroid hormone).They may present with localized or generalized enlargement of the gland (Goiter). Therefore, TSH evaluation is significant to diagnose thyroid disorders.

Table 1: Normal ranges of TSH

CONDITION	TSH (µIU/ml)
Cord (>37 week)	2.3 – 13.2
Birth – 4 days	1.0 – 39.0
2 – 20 week	1.7 – 9.1
21 week – 20 years	0.7 – 64
21 – 54 years	0.4 – 4.2
55 – 87 years	0.5 – 8.9
Pregnancy	
1st trimester	0.3 – 4.5
2nd trimester	0.5 – 4.6
3rd trimester	0.8 – 5.2

Table 2: Normal ranges of T3

CONDITIONS	T3 (ng/dL)
Cord (>37 week)	5 – 141
1 – 3 days	100- 700
1 – 11 month	105 – 245
1 – 5 years	105 – 269
6 – 10 years	94 – 241
11 – 15 years	82 – 213
16 – 20 years	80 – 210
20 – 25 years	70 – 204
50 – 90 years	40 – 181
Pregnancy	
1st trimester	81 – 190
2nd & 3rd trimester	100 – 260

Table 3: Normal ranges of T4

CONDITIONS	T4 (µg / dL)
Cord	7.4 – 13.1
1 – 3 days	11.8 – 22.6
1 – 2 wk	9.9 – 16.6
1 – 4 month	7.2 – 14.4
4 – 12 month	7.8 – 16.5
1 – 5 years	7.3 – 15.0
5 – 10 years	6.4 – 13.3
10 – 15 years	5.6 – 11.7
Adult 15 – 60 years	
Male	4.6- 10.5
Female	5.5 – 11.1
>60 years	5 – 10.7

Table 4: Normal ranges of FT3

CONDITIONS	FREE T3(pg/dL)
Cord	15 – 391
Child & Adult	210 – 440
Pregnancy	200 – 380

Hyperthyroid Disorders

It is a condition where thyroid gland produce increased level of thyroid hormone. It occurs due to increased production of T3 and T4. It is characterized by weight loss, anxiety, excess sweating, tremulousness, diarrhea, palpitations, muscular weakness, heat intolerance, sleeping disorders, etc. Common cause of hypothyroidism includes Grave's disease, toxic adenoma, and thyroid cancer.

Table 5: Normal ranges of FT4

CONDITIONS	FREE T4(/dL)
New born(1 – 4 days)	2.2 – 5.3
Children (2 wk – 20 yrs)	1.8 – 2.0
Adults	1.8 – 2.7
Pregnancy	
1st trimester	0.7 – 2.0
2nd& 3rd trimester	0.5 – 1.6

Hypothyroid Disorders

In this condition, thyroid gland does not produce much more thyroid hormone, as result of which metabolic and physiochemical process of the body are disturbed. It is caused by inflammation of thyroid gland, autoimmune diseases such as Hashimoto's thyroiditis, complete removal of the thyroid gland and this condition is characterized by fatigue, weight gain, cold intolerance, constipation, dry and thinning of hair, irregular menstrual cycle and problems related to pregnancy, depression, memory problems, a slowed heart rate, etc[23].

Thyroid diseases are the most common medical problem of immense interest associated with poor nutritious habits, iodine deficiency and lack of awareness. The bio physiological basis of the disease was not understood until 19th century but the discovery of thyroid gland and its functions accredited modern scientists to cope up with reasonable explanations of the thyroid gland diseases. The disorder can either be harmless goiter which doesn't need any treatment or it can be life threatening cancer. Therefore, serum estimation of TSH along with T3, T4, FT3 and FT4 in thyroid disorder is significant to evaluate and monitor the status of thyroid gland either it is functioning normal or not[25,26]. The National Academy of Clinical Biochemistry (NACB) has recommended that the functional sensitivity of TSH assay should be less or equal to 0.02 mIU/L so that one can distinguish a non-thyroid illness with primary hyperthyroidism. This is mainly important in patients hospitalized with non-thyroid illness[27].

Various methods have been developed for the estimation of TSH which includes isotopes method and non-isotopes method. Isotopes methods are radio-immuno assay (RIA), immuno-radio-Metric Assay (IRMA), competitive protein binding (CPB), and radio- receptor assay (RRA) where as non-isotope method includes enzyme immune assay (EIA), enzyme linked immunosorbent assay (ELISA), microparticle enzyme Immuno Assay (MEIA), fluoro immuno assay (FIA)[41-43]. RIA was the first generation assay methodology with limited

functional sensitivity (1.0mIU/L) used between 1965 and 1985 whereas IMA methodology with improved functional sensitivity is the second generation assay method developed in mid-80's[28-30]. With the invention of the third generation assay method, the results are more precise, rapid and better with respect to analytical, operational and clinical outcomes[31]. Functional sensitivities for three generations of TSH assays measure the precision. For each subsequent generation of TSH assays, the functional sensitivity limit assay shifts to lower concentration by one order of magnitude.

The functional sensitivity limit of first-generation assays (1 to 2μIU/mL) occurs at approximately the middle of the euthyroid range for TSH concentrations. The first generation assays cannot distinguish between normal and suppressed TSH levels. In contrast, second-generation assays permit quantization of TSH in the low, normal and subnormal range, down whereas third-generation assays extend the range another tenfold. In addition, third generation also have far superior precision in the subnormal TSH range[31].

With the rapid advance in bio-technology, microfluidic technology emerges as outstanding invention of 21st-century for the quantification of thyroid hormones that are cost effective, time saving, energy saving, use less consumables and rapid detections. Microfluidic technology is a sub-discipline of fluid mechanics that uses small volume of fluid to be on the micro - and nanometer scale to study any disorder and the effect of treatment[32,33].

With the increase in population and the condition of the people being isolated very much far from testing cares, recent technologies advances have developed another laboratory-medicine discipline technology that applies same principal as microfluidics is POCT (point-of-care testing). Unlike other tests, POCT shortens the time for making clinical decision about additional testing or therapy, the test results are rapidly available as delays are no longer because of transport and sample preparations[34].

POCT device uses a small chip containing a microfluidic channel in which is TSH, T3 and T4 gels are trapped and tagged with fluorescence compound. A small volume of serum sample (65ul) is pushed into the microfluidic channel inside the analyzer. When the serum sample reaches the gel, the TSH molecule in the serum sample gets attached to their respective antibody (antigen-antibody reaction). The unbound antigen (TSH molecule in the serum) are washed with a buffer and a fluorescence labeled anti human antibody then comes and binds to the complex which then emits fluorescence. The intensity of fluorescence is detected by a fluorescence diode which gives the quantity of TSH present in the serum. The test principle is similar to existing chemiluminescence methods, with microfluidic technology. POCT are generally functions outside the clinical laboratories for the direct evaluation of a number of laboratories parameters[35]. Some POCTs for thyroid stimulating hormone (TSH) measurement have been developed and proposed[36-40] suggesting that the need to compare these tools to standard laboratory approaches. In the present study, firstly, we aimed to comparative study of TSH quantification in two similar microfluidic technology hormone analyzer, and secondly, to validate a bed side (POCT) instrument for estimation of TSH.

Novel microfluidic technology is an outcome of advances in biotechnology. It is a new means to manage the treatment of various diseases including thyroid disorders. Microfluidics is used for processing small (10 to 1018 mictro liters) amounts of fluids, using channels with dimensions of a ten to several hundred microns. It uses small size of channels and laminar flow of fluids in micro channels and offers fundamentally new capabilities in control of concentrations of molecules in space and time[31].

Using similar principal of microfluidics, a POCT device is used for the assay of thyroid hormones. It uses a small chip that contains a microfluidic channel in which is TSH, T3 and T4 gels are trapped and tagged with fluorescence compound and about 65ul volume of serum sample is passed into the microfluidic channel for antigen antibody reaction where the TSH molecule of serum sample (antigen) bind to their respective antibody. The unbound TSH molecule in the serum (antigen) are washed off with a buffer solution and a fluorescence labeled anti human antibody then binds with the complex which then emits fluorescence. The intensity of fluorescence is detected by a fluorescence diode which measures the quantity of TSH present in the serum. For competitive assays, such as T3 and T4, the serum antigen bind with their corresponding antibody present in the gel that are already tagged with fluorescence. This results reduction of fluorescence and the amount of reduction represents of the amount of T3, T4 in the serum sample.

The advantage of this method being used for the estimation of thyroid hormones is that this instrument can be setup in a doctor's cabin and results can be obtained within around 30-40 minutes. Therefore, the purpose behind developing of this device is to provide cost effective and rapid healthcare solution to the patients in rural areas.

MATERIALS AND METHODS

Patients who were attending at Padmashree Diagnostic Centre, Vijayanagar Main Road, Bengaluru, for regular health check and were accessing TSH hormones were recruited for this study. After completely explaining the study to the patients, a written informed consent was obtained from patients. A total of twenty eight patients were recruited for the present study. Following criteria was used to include or exclude the subjects for the study:

Selection criteria	Patient's detail
Inclusion Criteria	
Age in Years	Any aged patient
Gender	Male and Female
Healthy volunteers	Yes
Diseased state of the thyroid	Yes
Patient on/ not on Drugs	Yes
Exclusion Criteria	
Patient who don't have TSH estimation	No
Patient from whom a written consent form was not obtained	No

Specimen Handling and Analysis

The blood specimens collected from the patients was centrifuged at 1800 x g for 15 minutes to obtain the cellular components and the cell free serum. Thus obtained serum sample was processed for the analysis of routine biochemical parameters sought by the treating clinicians. Remaining specimens were aliquoted, labeled and stored at – 20ºC for further analysis. Aliquots of specimens, once thawed were used for the analysis on the same day. Aliquots of specimens were not subjected for repeated freezing and thawing to avoid pre-analytical errors.

METHODS

Patient Selection Method

A total of 28 patients with regular medical follow up were recruited for this study. Patient details like body mass index, education, smoking, alcohol intake, dietary habits and family history was considered before selecting the patients.

Sample Size: One Group of 28 Subjects

Sampling method: Random Sampling

Analytical Methods

Serum TSH level was estimated by using a novel point of care instrument which is based on microfluidic technology (lateral flow immunofluorescence method).

Sources of Data

Available literature information from recent publications was updated during the course of study. The study design was standardized /modified depending upon the situation before applying the same for the sample analysis. Information with respect to study outcome was procured from the patient medical records and clinical expertise opinion was sought before relating the study outcome.

Statistical Analysis

The obtained data was expressed as Mean ± SD. The two tailed student's' test was employed to assess the differences between the groups comparing with available reference interval from available literature. A repeated measure of ANOVA was carried out to evaluate the significance between 1st and 6th visit. In order to test between the two treatment regimens, the repeated measure of ANOVA was computed following by Pearson's correlation.

Assay Protocols

Microfluidics Technology Test Principle

Method

Anti TSH antibody bound to the gel was allowed to react with the serum TSH followed by washing of unbound antigen using washing buffer. Then anti human antibody tagged with fluorophore was added which binds with antigen-antibody complex. The amount of fluorescence was read by the fluorescence reader which is directly proportional to amount of TSH present in patient's sample.

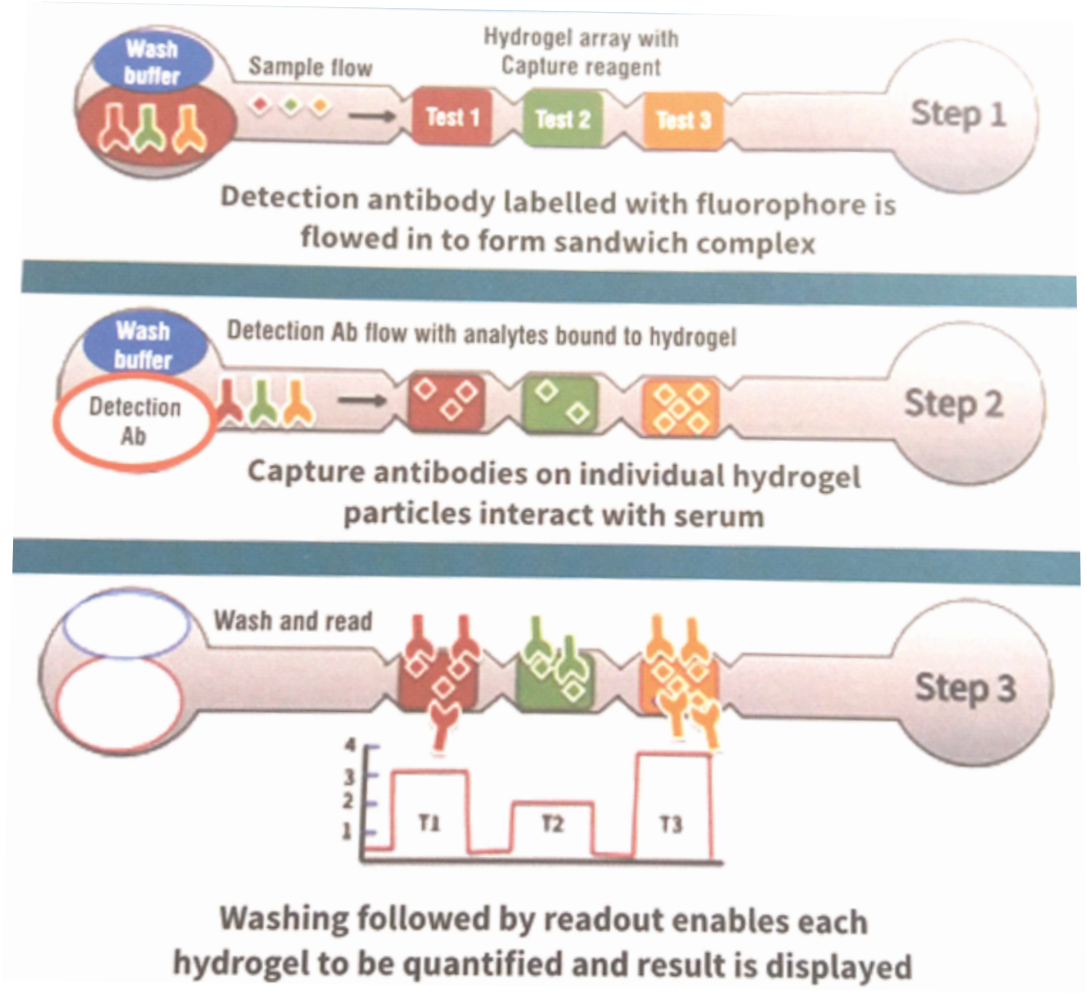

Figure 4: Microfluidics Principle

Figure 5: Cartridge

RESULTS AND DISCUSSION

Thyroid diseases have always been a disease of interest in all over the world due to its widespread prevalence in the increasing population. Estimation of TSH plays significant role in the management of the treatment of thyroid disorders such as hyperthyroidism, hypothyroidism, etc. Therefore, estimating TSH has become necessary test to be done for all patients before it results to a major health problem. But huge population of the world is living in the rural area where sophisticated medical laboratories set up are unavailable to diagnose various health problems including thyroid disorder. POCT address health care problem in order to provide cost effective, rapid and quality diagnosis and treatment to any health problems for all the people, particularly those who are living in rural area who cannot afford for sophisticated medical laboratories. POCT device is based on microfluidic technology that balances cost and clinical adequacy for the diagnosis and treatment of different disease and health related disorders. Various studies have been carried out to compare POCT with sophisticated laboratory based method for its effectiveness, impact, efficiency, and sustainability. The present study is carried out between two similar novel microfluidic technologies for TSH quantification to validate the two novel POCT devices and check any variation in the test values and also the correlation between the two similar instruments when the same samples are being tested at the same time.

The patients were recruited for this study at Padmashree Diagnostics, Department of Clinical Biochemistry, Vijayanagar, Bangalore.

The study initiation started after obtaining the written informed consent. The study population consisted of a group of 28 samples collected from different patients who visit the clinicians comes for regular health checkup for TSH (n=28).

Methodological Investigations

TSH estimation was done in two similar POCT instrument based on Microfluidic Technology

The details of assay protocols of microfluidics are mentioned as follows:

Specification of Microfluidics (ACIX)

Instrument type	Portable immunoassay analyser
Throughput per run	Up to4 parameters per run
Measuring time	<30 min using fertility markers
Sampling material	Plasma , serum
Measuring principle	Fluorescence
Type of assay	Fluorescence immunoassay on hydrogel
Sample volume	20µl
Wavelength	635 nm
Data storage	Patient data : 1000
Calibration data	250
QC data	250
Dimensions	290(w) x 300(h) mm
Weight	5 kg
Electrical requirements	100 – 240 Vac
Power consumption	Max 12W
Battery backup	Up to 4 hours
Printer	Integrated
PC	Integrated through USB
Interface	Rs-232C/USB
Calibration	Factory calibration, 2-point calibration every weeks
Monitor/keyboard	LCD Touch screen

Table 6: Mean and SD of TSH values by both methods

Methods	N	Minimum	Maximum	Mean	SD	Unpaired t-test	Test retest reliability
Instrument-I	28	0.30	72.84	12.10	± 21.27	t =.0.222^{NS}, p=0.825	r=0.994, p=0.000
Instrument-II	28	0.30	85.90	13.42	± 23.02		

The data above represents the two similar novel instrument which is based on microfluidic technology in which 28 samples for TSH estimation have being tested which gives results ranging from 0.30 to 72.84 mIU/L, mean 12.10 and SD 21.27 on one instrument and test values ranging from 0.30 to 85.90, mean 13.42 and SD 23.02 in the other instrument respectively.

Note: S denotes significant ($p < 0.05$) and NS denotes Not significant ($p > 0.05$)

The values in the unpaired t-test t=0.222 suggest that the differences were not significant which means both the instruments were giving nearly same results whereas the test reliability with r = 0.994 and p=0.000 suggests the results coincides and correlating.

Statistically both the instruments gave nearly same values, mean and SD.

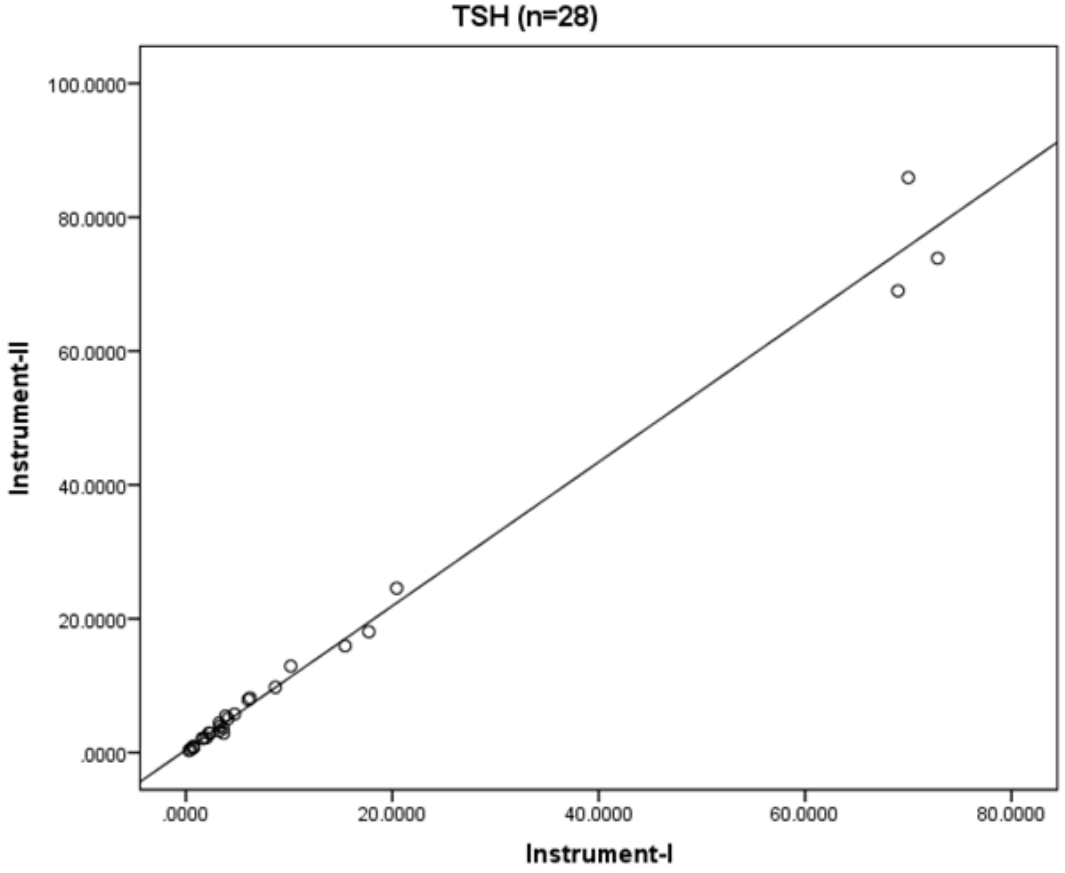

Figure 6: The line graph represents the serum TSH Values between the two instrument expressed as Mean ± SD. Student's 't' test: *: $p < 0.05$;**: $p < 0.01$: ***: $p < 0.001$

Table 7: Mean and SD of TSH values by both methods TSH (0.5-5.5)

Methods	N	Minimum	Maximum	Mean	SD	Unpaired t-test	Test retest reliability
Instrument-I	16	1	5	2.69	±1.25	T=0.354^{NS}, p=0.872	r=0.920, p=0.000
Instrument-II	16	1	6	3.06	±1.52		

The above data represents the two instruments in which patient's samples were tested. 16 patient samples out of a total 28 patient's TSH values came between 0.5 - 5.5 where the least detected value in both the instruments was 1 and the highest value obtained was 5 and 6 in instrument 1 and instrument II, mean and SD 2.69,1.25 and 3.06,1.52 for the two instruments of microfluidics respectively.

The values in the unpaired t-test t=0.354 suggest that the differences were not significant which means both the instruments were giving nearly same results whereas the test reliability with r=0.920 and p=0.000 suggests the results coincides and correlating.

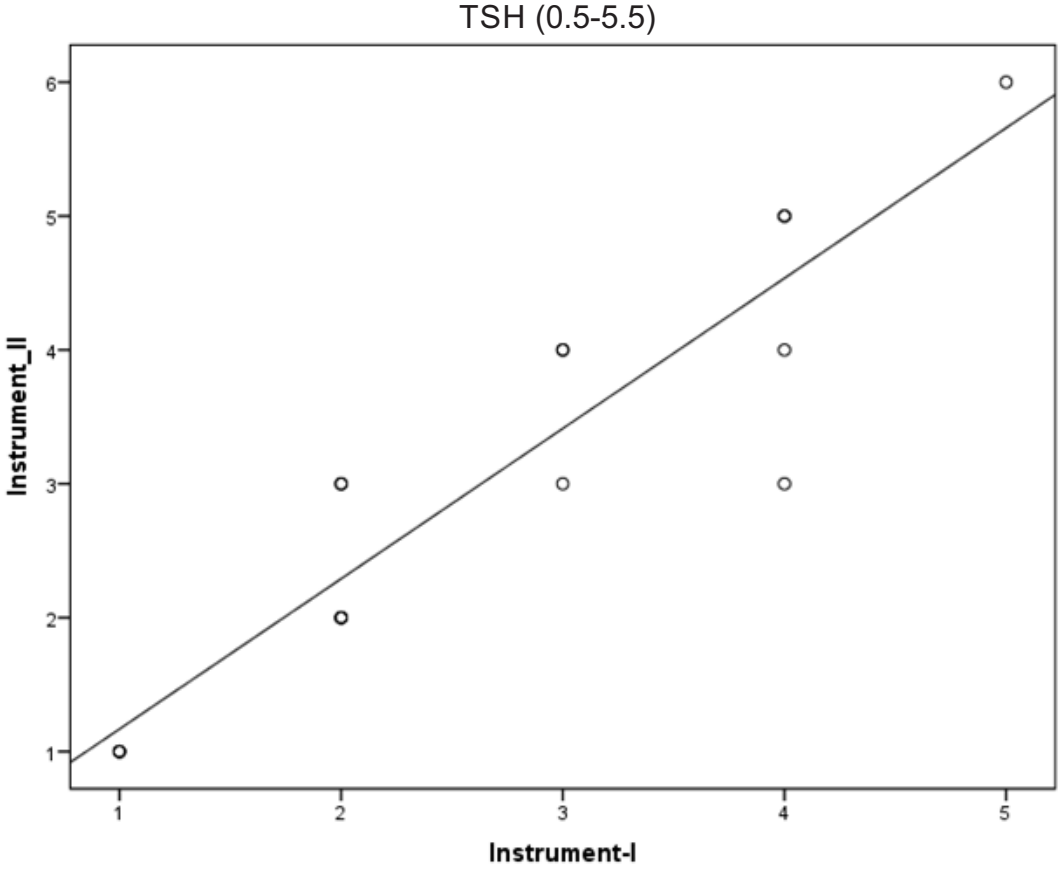

Figure 7: The line graph represents the TSH values which were tested in the two Microfluidic instruments and the values were expressed as Mean ± SD. Student's 't' test: *: p < 0.05;**: p < 0.01: ***: p < 0.001

Table 8: Mean and SD of TSH values by both methods (TSH >5.5)

Methods	N	Minimum	Maximum	Mean	SD	Unpaired t-test	Test retest reliability
Instrument-1	10	6.04	72.84	29.65	±28.67	$t=0.040^{NS}$, p=0.969	r=0.990, p=0.000
Instrument-2	10	7.91	85.90	32.60	±30.80		

The above data represents the two instruments in which patient's samples were tested. 10 patient samples out of a total 28 patient's TSH values came above 5.5 where the highest detected value were 72.84 and 85.90 in the two instruments, mean and SD 29.65,28.67 and 32.60 and 30.80 for the two instruments of microfluidics respectively.

The values in the unpaired t-test t=0.040 suggest that the differences were not significant which means both the instruments were giving nearly same results whereas the test reliability with r=0.990 and p=0.000 suggests the results coincides and correlating however serum TSH values above 40µIU/ml were not correlative and unpredictable.

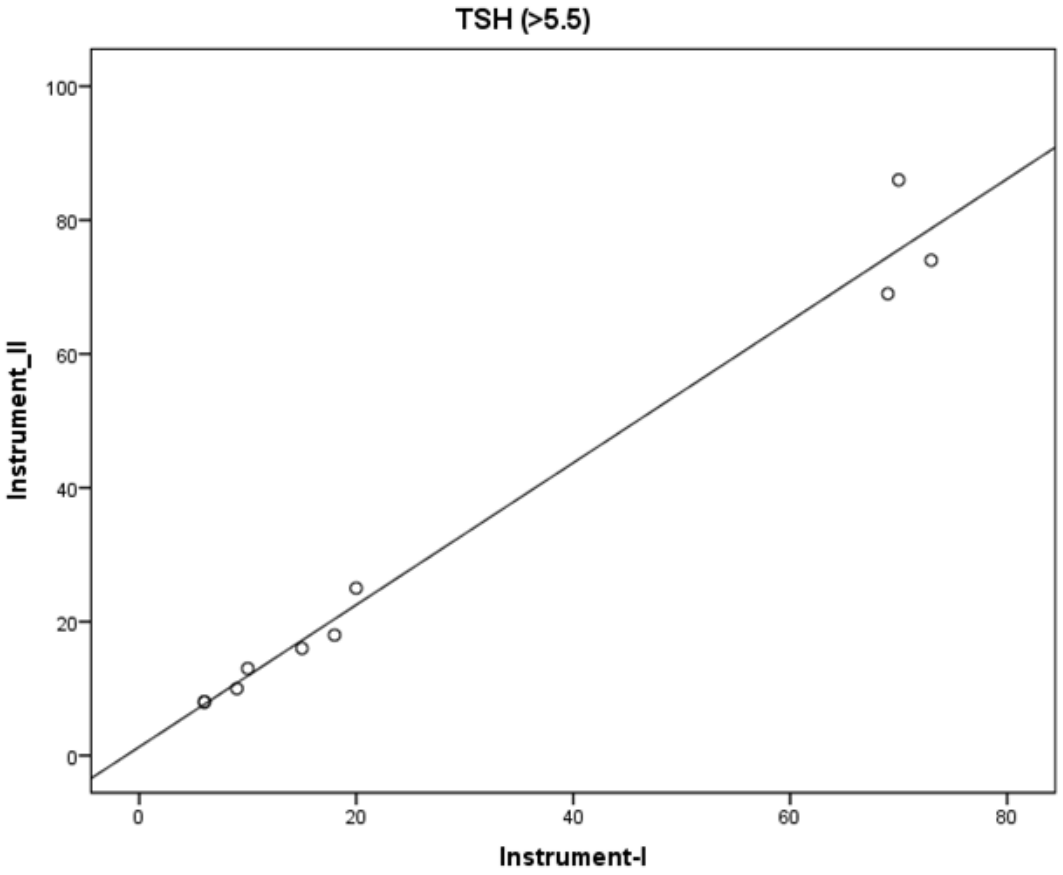

Figure 8: The line graph represents the TSH values >5.5 measured in the two MICROFLUICICS instrument. Values expressed as Mean ± SD. Student's 't' test: *: $p < 0.05$;**: $p < 0.01$: ***: $p < 0.001$

SUMMARY AND CONCLUSION

With the increase in population, the prevalence of thyroid disorders is worldwide and estimation of TSH becomes a common test to be done for every patient for the management of treatment of thyroid disorders. The availability of sophisticated medical labs are very much limited to certain areas particularly in the developing countries where patient in the rural areas deprived of getting access to such central labs. Also, for the people who are living in cities with well facilitated laboratories set up who provide TSH estimation facilities sometimes becomes unaffordable e due to high test cost. Because of this, majority of the patients with thyroid disorders left untreated. In these cases this microfluidic technology based hormone analyzer ACIX can be a good alternative to meet the demand of clinicians and patients of socio-economically backgrounds in terms of cost, time and accuracy for a treatment/diagnosis of thyroid disorder.

In this study serum TSH estimation have been done with two novel microfluidic technologies. In this study, linearity proves that the two novel instrument based on microfluidic technology is comparable, the values falling within normal range i.e. 0.5-5.5 μIU/ml is excellently comparable between the two microfluidic instruments but the results above 40μIU/ml shows higher variability and the test values are unpredictable between the two Microfluidic instruments. This novel microfluidic instruments can be used as a POCT device and can easily diagnose a case of hyper and Hypo thyroidism despite high values showing variability with reference method as the TSH level above 5.5 is accurately measured by the ACIX and any value above which is classified as hypothyroid status.

Figure 9: ACIX Immunoanalyser

CONCLUSION

We conclude our study that the new instrument based on Immunofluorescence technique using microfluidics are comparable and so can be used as an alternative method for diagnosis/treatment of thyroid disorders. This instrument can be very beneficial as its size, light weight makes it portable and so it can be set up anywhere particularly in rural areas and provide health care solutions to patients and clinicians with a service quality, availability, cost, relevance and timeliness.

CONFLICT OF INTEREST: None

FINANCIAL SUPPORT: None

REFERENCES

1.Moore KL, Persaud TV, Torchia MG. The developing human-e-book: clinically oriented embryology. Elsevier sci.; 2018 Dec 23.

2.Kirsten D. The thyroid gland: physiology and pathophysiology. Neonatal Network. 2000; 19(8):11-26.

3.Rolleston HD. The endocrine organs in health and disease: With an historical review. Oxford University Press, H. Milford; 1936.

4.Gray H. Anatomy of the human body. Lea & Febiger; 1878

5.Khonsary SA. Guyton and Hall: textbook of medical physiology. Surg. Neurol. Int. 2017; 8.

6.Dumont JE, Corvilain B, Maenhaut C. The phylogeny, ontogeny, anatomy, and metabolic regulation of the thyroid. The Thyroid and its Diseases. 2002.

7.Wier FA, Farley CL. Clinical controversies in screening women for thyroid disorders during pregnancy. J Midwifery Women's Health. 2006; 51(3):152-8.

8.Kayodeoo. Thyroid and iodine status in pregnancy at a tertiary hospital in Lagos Nigeria. Faculty of internal medicine. 2013.

9.Baskin HJ, Cobin RH, Duick DS, Gharib H,et al. American Association of Clinical Endocrinologists medical guidelines for clinical practice for the evaluation and treatment of hyperthyroidism and hypothyroidism. Endocrine practice. 2002; 8(6):457-69.

10.Wier FA, Farley CL. Clinical controversies in screening women for thyroid disorders during pregnancy. J Midwifery Women's Health. 2006; 51(3):152-8.

11.Spencer CA. Assay of thyroid hormones and related substances. Endotext [Internet]. 2017 Feb 20.

12.Dohan O, De la Vieja A, Paroder V, Riedel C et al. The sodium/iodide symporter (NIS): characterization, regulation, and medical significance. Endocrine reviews. 2003; 24(1):48-77.

13.Wondisford FE, Steinfelder HJ, Nations M, Radovick S. AP-1 antagonizes thyroid hormone receptor action on the thyrotropin beta-subunit gene. J. Biol. Chem. 1993; 268(4):2749-54.

14.Weart EL. The Conquest of Goiter. N. Am. Rev. 1929; 228(3):359-66.

15.Citterio CE, Rivolta CM, Targovnik HM. Structure and genetic variants of thyroglobulin: Pathophysiological implications. Mol. Cell. Endocrinol. 2021; 528:111227.

16.Shahid MA, Ashraf MA, Sharma S. Physiology, thyroid hormone. InStatPearls [Internet] 2022 May 8. StatPearls Publishing.

17.Saeed BA, Bhat U, Sathyamurthy B. Screening for the level of specific biochemical markers in thyroid disorders.

18.Niazi AK, Kalra S, Irfan A, Islam A. Thyroidology over the ages. Indian J Endocrinol Metab. 2011; 15(l2):S121.

19.Sawin CT. Theories of causation of graves'disease: A Historical Perspective. Endocrinol. Metab. Clin. 1998; 27(1):63-72.

20.Yu XM, Patel PN, Chen H, Sippel RS. False-negative fine-needle aspiration of thyroid nodules cannot be attributed to sampling error alone. Am. J. Surg. 2012; 203(3):331-4.

21.Hannan SA. The magnificent seven: a history of modern thyroid surgery. Int J Surg. 2006; 4(3):187-91.

22.Roitt IM, Doniach D, Campbell PN, Hudson RV. Auto-antibodies in Hashimoto's disease (lymphadenoid goitre). The Lancet. 1956; 268(6947):820-1.

23.Triggiani V, Tafaro E, Giagulli VA, Sabbà C et al. Role of iodine, selenium and other micronutrients in thyroid function and disorders. Endocrine, Metabolic & Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune, Endocrine & Metabolic Disorders). 2009; 9(3):277-94.

24.Leoutsakos V. A short history of the thyroid gland. HORMONES-ATHENS-. 2004; 3:268-71.

25.Castro MR, Gharib H. Thyroid disorders. Evidence-Based Endocrinology. 2007:34-8.

26.Roberts RF, La'ulu SL, Roberts WL. Performance characteristics of seven automated thyroxine and T-uptake methods. Clinicachimicaacta. 2007; 377(1-2):248-55.

27.Rawlins ML, Roberts WL. Performance characteristics of six third-generation assays for thyroid-stimulating hormone. Clin. Chem. 2004;50(12):2338-44.

28.Matyjaszek-Matuszek B, Pyzik A, Nowakowski A, Jarosz MJ. Diagnostic methods of TSH in thyroid screening tests. Agric Environ Med.. 2013; 20(4).

29.Kaur MA, Gupta S, Kaur V, Chopra B, Singh K. Comparison of measurement of serum TSH by two 3rd generation techniques. Int. J. Bioassays. 2014; 3:3040-3.

30.Rabiee M, Ghasemnia NN, Rabiee N, Bagherzadeh M. Microfluidic devices and drug delivery systems. In Biomedical Applications of Microfluidic Devices. Academic Press. 2021 Jan 1 (pp. 153-186).

31.Dong J, Ueda H. ELISA-type assays of trace biomarkers using microfluidic methods. Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. 2017; 9(5):e1457.

32.Luppa PB, Müller C, Schlichtiger A, Schlebusch H. Point-of-care testing (POCT): Current techniques and future perspectives. TrAC, Trends Anal. Chem. 2011; 30(6):887-98.

33.Di Cerbo A, Quagliano N, Napolitano A, Pezzuto F, Iannitti T, Di Cerbo A. Comparison between an emerging point-of-care tool for TSH evaluation and a centralized laboratory-based method in a cohort of patients from Southern Italy. Diagnostics. 2021; 11(9):1590.

34.Park HS, Yoo J, Lee H, Choi AR, Ryu J, Park KH, Oh EJ. Analytical Evaluation of Portable and Simple FREND Fluorescent Immunoassay for Rapid Quantification of Thyroid-Stimulating Hormone and Free Thyroxine. Clin. Lab. 2016; 62(12):2455-60.

35.Jung W, Han J, Kai J, Lim JY, Sul D, Ahn CH. An innovative sample-to-answer polymer lab-on-a-chip with on-chip reservoirs for the POCT of thyroid stimulating hormone (TSH). Lab Chip. 2013; 13(23):4653-62.

36.Znoyko SL, Orlov AV, Bragina VA, Nikitin MP, Nikitin PI. Nanomagnetic lateral flow assay for high-precision quantification of diagnostically relevant concentrations of serum TSH. Talanta. 2020; 216:120961.

37.Yazawa Y, Oonishi T, Watanabe K, Shiratori A, System-on-fluidics immunoassay device integrating wireless radio-frequency-identification sensor chips. J. Biosci. Bioeng.. 2014; 118(3):344-9.

38.Ylikotila J, Välimaa L, Vehniäinen M, Takalo H, Lövgren T, Pettersson K. A sensitive TSH assay in spot-coated microwells utilizing recombinant antibody fragments. J. Immunol. Methods2005; 306(1-2):104-14.