

CLINICAL STUDY OF RADIO ACTIVE IODINE UPTAKE IN DIFFERENT GOITRE CASES IN CO-RELLATION WITH NORMAL INDIVIDUALS

Yogananda Reddy Indla^{*1}, Shakeela Dasari², Ragam Ravi Sunder¹, Purru Ravi Kumar¹,
Rajani Santha Kumari³

^{*1}Department of Physiology ,RIMS Ongole,Andhra Pradesh, India-523001

²Department of Physiology, Andhra Medical College, Vishakapatnam

³Department of Physiology ,MIMS Ghanpur Medical Mandal Hyderabad, A.P, India

***Corresponding Author:** yoga4udotcom@yahoo.co.in

This article is available online at www.ssijournals.com

ABSTRACT

Aim: to measure the radioactive iodine uptake in different variants of goiter
Materials & Methods: subjects with normal levels of T₃, T₄ & TSH and no goiter (control group) and examination group which is divided in to three sub groups eu-thyroid goiter, hypo-thyroid goiter and hyper-thyroid goitre cases. Each group is having 10 subjects. All the subjects are females aged 35 yrs±5 and no one is receiving any drugs since 6 weeks just before the beginning of the study. Radioactive iodine uptake is measured by using the bellow formula

$$\% \text{ uptake} = \frac{\text{CPM in thyroid} - \text{CPM of background activity}}{\text{CPM standard}} \times \frac{\text{CPM standard}}{\text{CPM dose administered}} \times 100$$

Results and Observations: At 2 hrs: in hypo-thyroid cases RAIU is elevated, in hyper-thyroid subjects RAIU uptake is increased and in eu-thyroid subjects the uptake is decreased. At 24 hrs: in hypo-thyroid cases RAIU is decreased, in hyper-thyroid subjects RAIU uptake is increased and in eu-thyroid subjects the uptake is decreased in comparison with the control group.

Conclusion: Radioactive iodine uptake is increased at 2 hrs and decreased at 24 hrs and 48 hrs in hypo-thyroid subjects, P value is significant at 24 and 48 hrs only.

Keywords: Goitre, RAIU, RIAK-5/5a

1. INTRODUCTION

Thyroid gland is a centre of attraction for the biological research. Goitre is the enlargement of thyroid gland. Goitre could be with normal T₃, T₄ and TSH levels in the blood (eu-thyroid), could be with increased T₃, T₄ levels with normal or decreased TSH levels⁵ (hyper-thyroid) and it could be with decreased T₃, T₄ levels with increased TSH levels (hypo-thyroid). There are so many tests to study the functioning of thyroid gland like f MRI, PET scan etc., but radio active uptake of iodine studies along with blood T₃, T₄ assay is considered to be the most sensitive and more reliable than the other tests. In the clinical study we are correlating the uptake of radioactive iodine in eu-thyroid, hypo-thyroid and hyper-thyroid goitre cases with normal individuals.

1.1 Aims & Objectives: To study the radioactive iodine uptake in different variants of goiter in comparison with normal individuals.

2. MATERIAL & METHODS

All the subjects including examination group and control group are female's only. Average

age of the subjects is 30yrs±5. Normal subjects without goitre and with normal plasma T₃, T₄ and TSH levels are as control group. We have selected 30 cases of goitre as examination group and divided the subjects in to three groups one group is eu-thyroid, second group is hypo-thyroid and the third group is hyper-thyroid and each group is consisting of 10 subjects. We have classified the subjects in to eu-thyroid, hypo-thyroid and hyper-thyroid basing on plasma T₃, T₄ and TSH levels⁴. The subjects were not taking any thyroid medications since 6 weeks just before the test³. Blood sample was collected from the subjects in to the test tube during the early hours of the day before breakfast at 7-8 am. Collected blood sample is allowed to clot at room temperature then centrifuged and serum is collected and stored at 2-8 degree centigrade temperature for assay on the same day.

2.1 Radio immune assay (RIA) of T₃ T₄ and TSH: in our study the kits used for RIA of T₃ and T₄ are RIAK – 4/4 A and RIAK – 5/5, respectively⁷. For TSH estimation Immuno-Radio Metric Assay (IRMA)¹ Kit for Human thyroid stimulating hormone – IRMAK -9 is used. These kits were collected from radio

pharmaceuticals operations, board of radiation and isotope technology of navi Mumbai.

2.2 Reagents of RIAK – 4/4 A:

1. T₃ Standard (lyophilized)
2. Antisera complex (lyophilized)
3. ¹²⁵I- T₃ (lyophilized)
4. T₃ free human serum (lyophilized)
5. Control serum A and B (lyophilized)
6. Concentrated assay buffer (solution)
7. Polyethylene glycol (PEG) dry powder

Reconstitution of Reagents: the reagents of the T₃ assay kit are reconstituted as follows:

1. Assay Buffer: 100 ml of double distilled water is added to the contents of concentrated buffer vial. This reconstituted assay buffer contains 0.1% Gelatine in 0.14 M THAM (Tris-Hydroxymethyl Amino Methane) buffer, PH adjusted to 8.6.

2. T₃ standard: 5 ml of assay buffer is added to the T₃ standard and gently mixed. The reconstituted standard is 2.4 ng/ ml and called standard A. Other standards are reconstituted as follows:

| S.N. | Standard | B | C | D | E |
|------|-----------------------|-----|-----|-----|------|
| 1 | Assay buffer in ml | 0.5 | 1.5 | 3.5 | 7.5 |
| 2 | Standard A in ml | 0.5 | 0.5 | 0.5 | 0.5 |
| 3 | Concentration (ng/ml) | 1.2 | 0.6 | 0.3 | 0.15 |

3. T₃ Antisera complex: 10 ml of buffer is added to T₃ anti sera complex.

4. ¹²⁵I – T₃: 10 ml of buffer is added and gently mixed.

5. T₃ free serum: 2 ml of doubled distilled water is added.

6. Control serum A&B: 0.5 ml of doubled distilled water & mixed gently.

7. Polyethylene glycol (PEG): PEG powder is transferred to a 125 ml reagent bottle containing 100 ml of 1% (W/V) NaCl. The powder is mixed well till dissolution is obtained. All the reconstituted reagents are stored at 5⁰ C, thus stored reagents are stable up to one week.

T₃ ASSAY FLOW CHART

Volumes in micro-liters

| Tube No. | Buffer | Free Serum | Std/ sample | Antiserum Complex | ¹²⁵ I – T ₃ |
|----------|--------|------------|-------------|-------------------|-----------------------------------|
| 1,2 | 400 | 50 | - | - | 100 |
| 3,4 | 300 | 50 | - | 100 | 100 |
| 5,6 | 200 | 50 | 100 (E) | 100 | 100 |
| 7,8 | 200 | 50 | 100 (D) | 100 | 100 |
| 9,10 | 200 | 50 | 100 (C) | 100 | 100 |
| 11,12 | 200 | 50 | 100 (B) | 100 | 100 |
| 13,14 | 200 | 50 | 100 (A) | 100 | 100 |
| 15,16 | 300 | - | 50 (S1) | 100 | 100 |
| 17,18 | 300 | - | 50 (S2) | 100 | 100 |
| T1,T2 | - | - | - | - | 100 |

Tubes are mixed gently. Later incubated at 37⁰ C for 45 min or at room temperature for 3hrs. 1 ml of PEG Solution is added to all tubes except T1& T2, Tubes are mixed and centrifuged at 2250 RPM for 20 min. Supernatant is discarded and carefully removed the last traces of the supernatant without touching the precipitate.

| Content | Tube No | CPM | Corrected Avg CTS | B/Bo |
|----------|---------|----------------|-------------------|-------|
| Total | T1, T2 | 37790 37933 | 37511 | |
| Blank | 1,2 | 1612 1697 | 1405 | |
| Zero Std | 3,4 | 17248 17199 | 15569 | 100.0 |
| Std E | 5,6 | 15354 15162 | 13604 | 87.4 |
| Std D | 7,8 | 12978 13167 | 11418 | 73.3 |
| Std C | 9,10 | 9953 9892 | 8268 | 53.1 |
| Std B | 11,12 | 7107 6873 | 5595 | 35.9 |
| Std A | 13,14 | 4986 5047 | 3362 | 21.3 |
| Sample 1 | 15,16 | 11367 11616 | 9837 | 63.2 |
| Sample 2 | 17,18 | 7114 7110 | 5507 | 35.4 |

2.3 CALCULATION OF RESULTS

Average counts per minute (CPM) are recorded for all duplicate tubes. Then corrected for background. Average counts of tubes 1&2 are called blank counts.

% Blank = Blank counts/Total counts X 100

1. Blank counts per minute are subtracted from the average of all the remaining duplicates. These are called average counts.
2. Zero binding is calculated as follows:
% Blank /B/o = corrected Average counts of 3&4/corrected Total counts X 100
3. % Blank /B/o = corrected Average counts of Std/ Sample3&4/ corrected average counts of zero Std X 100.
4. Standard curve is plotted as given below:
Corrected average counts against concentration of T₃ (ng/ml) on a linear graph.
5. Sample value is read from the standard curve, T₃ Concentration of the sample can be expressed as ng/ml by multiplying the value by 2.

2.4 R I A of T₄

Reagents of RIAK – 5/5 A;

1. T₄ Standard (lyophilized)
 2. T₄ Antisera complex (lyophilized)
 3. ¹²⁵I- T₄ (lyophilized)
 4. T₄ free human serum (lyophilized)
 5. Control serum A and B (lyophilized)
 6. Concentrated assay buffer (solution)
 7. Polyethylene glycol (PEG) dry powder
- Reconstitution of Reagents; the reagents of the T₄ assay kit are reconstituted as follows:

1. Assay Buffer: 100 ml of double distilled water is added to the contents of concentrated buffer vial. This reconstituted assay buffer contains 0.1% Gelatine in 0.14 MTHAM (Tris- Hydroxy methyl Amino Methane) buffer, PH adjusted to 8.6.
2. T₄ Standard: 4 ml of assay buffer is added to the T₄ standard and gently mixed. The reconstituted standard is 20 ng/ ml and called standard A. Other standards are reconstituted as follows:

| S.No | Standard | B | C | D |
|------|-----------------------|-----|-----|-----|
| 1 | Assay buffer in ml | 0.5 | 1.5 | 3.5 |
| 2 | Standard A in ml | 0.5 | 0.5 | 0.5 |
| 3 | Concentration (ng/ml) | 10 | 5 | 2.5 |

3. T₄ Antisera complex: 10 ml of buffer is added to T₄ anti sera complex.
4. ¹²⁵I – T₄: 10 ml of buffer is added and gently mixed

5. T₄ free serum: 5 ml of doubled distilled water is added

6. Control serum A&B: 0.5 ml of doubled distilled water is added

7. Polyethylene glycol (PEG): PEG powder is transferred to a 125 ml reagent bottle containing 90 ml of 1% (W/V) NaCl. The powder is mixed well till dissolution is obtained. All the reconstituted reagents are stored at 5⁰ C, thus stored reagents are stable up to one week.

T₄ ASSAY FLOW CHART

| Tube No. | Buffer | Free Serum | Std/ sample | Anti serum Complex | 125I – T ₃ |
|---------------|--------|------------|-------------|--------------------|-----------------------|
| 1,2 (NSB) | 200 | 100 | - | - | 100 |
| 3,4 (ZER O) | 100 | 100 | - | 100 | 100 |
| 5,6 | - | 100 | 100 (D) | 100 | 100 |
| 7,8 | - | 100 | 100 (C) | 100 | 100 |
| 9,10 | - | 100 | 100 (B) | 100 | 100 |
| 11,12 | - | 100 | 100 (A) | 100 | 100 |
| 13,14 | 100 | | 100 (S1) | 100 | 100 |
| 15,16 | 100 | - | 50 (S2) | 100 | 100 |
| T1,T2 (Total) | - | - | - | - | 100 |

Tubes are mixed gently. later incubated at 37⁰ C for 30 min or at room temperature for 75 min. 1 ml of PEG Solution is added to all tubes except T1& T2, Tubes are mixed and centrifuged at 250 RPM for 20 min. Supernatant is discarded and carefully removed the last traces of the supernatant without touching the precipitate

| Content | Tube No | CPM | Corrected Average cts | %B/B ₀ |
|----------|---------|----------------|-----------------------|-------------------|
| Total | T1, T2 | 37790 37933 | 37511 | |
| Blank | 1,2 | 1612 1697 | 1405 | |
| Zero Std | 3,4 | 17248 17199 | 15569 | 100 |
| Std D | 5,6 | 12978 13167 | 11418 | 67.7 |
| Std C | 7,8 | 9953 9892 | 8268 | 47.9 |
| Std B | 9,10 | 7107 6873 | 5595 | 32.2 |
| Std A | 11,12 | 4986 5047 | 3362 | 18.3 |
| Sample 1 | 13,14 | 11367 11616 | 9837 | 44 |
| Sample 2 | 15,16 | 7114 7110 | 5507 | 28 |

2.5 CALCULATION OF RESULTS

Average counts per minute (CPM) are recorded for all duplicate tubes. Then corrected for background. Average counts of tubes 1&2 are called blank counts.

$$\% \text{ Blank} = \text{Blank counts} / \text{Total counts} \times 100$$

1. Blank counts per minute are subtracted from the average of all the remaining duplicates. These are called average counts.
2. Zero binding is calculated as follows:
 $\% \text{ Blank} / \text{B/o} = \text{corrected Average counts of 3\&4} / \text{corrected Total counts} \times 100$
3. $\% \text{ Blank} / \text{B/o} = \text{corrected Average counts of Std/ Sample 3\&4} / \text{corrected average counts of zero Std} \times 100$.
4. Standard curve is plotted as given below: Corrected average counts against concentration of T3 (ng/ml) on a linear graph.
5. Sample value is read from the standard curve, T3 Concentration of the sample can be expressed as ng/ml by multiplying the value by 2.

2.6 IMMUNORADIO-METRIC ASSAY (IRMA) KIT FOR HUMAN THYROID STIMULATING HORMONE

TSH estimation is done as follows:

Kit contents:

| REAGENT | QUANTITY |
|--|----------------------|
| 1.h TSH monoclonal antibody coated tubes | 100 |
| 2.Anti h TSH -125 | 11 ml |
| 3.h TSH standards in animal serum 0-100 μ U/ml | 8 vials |
| 4.Wash diluents | 1 vial (50 ml) |
| 5.Controls | 2 vial (lyophilized) |

Both anti h TSH and h TSH standards are used directly. Other reagents are reconstituted as follows

2.7 EQUIPMENT REQUIRED:

1. Test tube rack
2. Laboratory vortex mixture
3. Pipettes that can accurately and precisely deliver the required volumes
4. Gamma counter calibration for Iodine ¹²⁵

2.8 Reconstitution:

Wash diluents: it is diluted with 950 ml of double distilled water

Controls: diluted with 1 ml of double distilled water and allowed to stand for 30 minutes .later

gently mixed by keeping the tube in vortex mixture.

Assay procedure:

1. All standard samples, controls, coated tubes and anti h TSH -125 are brought to room temperature
2. Required number of anti h TSH coated tubes are serially numbered and placed in the test tube racks
3. 200 μ l of each standard , control or sample are pipetted in to its respective tubes
4. 100 μ l of anti h TSH – 125 added in to all tubes .then they are mixed and incubated for 2 hours with gentle shaking (350 rpm) at room temperature
5. All tubes are washed by the following procedure:
 - (a) 2 ml of diluted solution is added to all tubes
 - (b) Test tube rack is gently shaken for 5 seconds
 - (c) Solution from each of the tube is emptied
 - (d) Washing is repeated again
 - (e) Counting of the empty tubes for one minute is done in a gamma counter calibrated for Iodine ¹²⁵

ASSAY PROTOCOL

| Tube no | Std sample (μ l) | Anti –h TSH | Description (μ IU/ml) |
|---------|-----------------------|-------------|----------------------------|
| 1,2 | 200 | 100 | 0 (A) |
| 3,4 | 200 | 100 | 0.15 (B) |
| 5,6 | 200 | 100 | 0.5 (C) |
| 7,8 | 200 | 100 | 1.5 (D) |
| 9,10 | 200 | 100 | 5.0 (E) |
| 11,12 | 200 | 100 | 15.0 (F) |
| 13,14 | 200 | 100 | 50 (G) |
| 15,16 | 200 | 100 | 100 (H) |
| 17,18 | 200 | 100 | Control-A |
| 19,20 | 200 | 100 | Control-B |
| 21,22 | 200 | 100 | Sample-1 |
| 23,24 | 200 | 100 | Sample-2 |

2.9 CALCULATIONS:

- (a) Average of the background corrected counts of all duplicates are recorded
- (b) Later average counts are plotted against standard concentration and standard curve is established
- (c) By using standard curve the h TSH concentrations of each sample are determined

2.10 RADI ACTIVE IODINE

RAIU is done as follows:

Instrumentation: the probe used for uptake measurement is specialized scintillation detector that consists of 5 cm sodium Iodide crystal and a small collimator angled sufficiently to visualize the entire gland easily from a thyroid to crystal distance of 35 cm. Even though the collimator is small extra thyroidal neck activity is always detected. There is a plastic phantom which stimulates the shape and diameter of the neck. It is used for measurement of a known standard of radio iodine capsule.

Subject preparation:

1. No synthetic thyroxin therapy with inn 6 weeks
2. No intravenous iodinated contrast study with in 4 to 6 weeks
3. No anti- thyroid drugs since 1 week
4. Light breakfast on the first day of study
5. Protenacious food is restricted for 8 hours
6. Entire procedure and the time duration of 48 hours for the complete study are explained

2.11 Technique

1. Counts per minute (CPM) are recorded from the subjects dose (Iodine Capsule) and standard
2. The tracer radio iodine ^{131}I of 25 μCi cap is administered and after 2 hours neck counts are obtained by using scintillation detector
3. 2 minutes neck counts are recorded and average CPM is taken
4. Then neck background activity is recorded by keeping lead blocker over the thyroid area
5. Then counts are recorded at 24 hrs and 48 hrs
6. Percent uptake is calculated by using the formula

% uptake =

$$\frac{\text{CPM in thyroid} - \text{CPM of background activity}}{\text{X CPM standard}} \times 100$$

CPM standard CPM dose administered

3. RESULTS & OBSERVATIONS:

At 2 hours: in hypo-thyroid subjects RAIU is elevated though non -significant indicating either iodine deficiency or Grave's thyroiditis². In thyroiditis⁸ trapping function is normal or increased and organification is impaired, in iodine deficiency RAIU is increased .The uptake is increased in hyper-thyroid subjects

suggesting hyper functioning of gland. In eu-thyroid cases the uptake is decreased and it could be because of decompensated dys hormonogenesis or thyroiditis. At 24 hours: in hypo-thyroid cases RAIU at 24 hours is decreased, in hyper-thyroid subjects RAIU uptake is increased and in eu-thyroid subjects the uptake is decreased. At 48 hrs: RAIU uptake is reduced in hypo-thyroid individuals, in hyper-thyroid subjects the uptake is increased but not significantly and in eu-thyroid subjects the uptake is decreased⁶.

Table – 1 Values of Radio Active Iodine Uptake After 2 Hours In Euthyroid, Hypothyroid And Hyperthyroid

| Group | Mean | P value |
|--------------|------|---------|
| Control | 12.3 | - |
| euthyroid | 8.3 | < 0.01 |
| hypothyroid | 13.5 | > 0.5 |
| hyperthyroid | 29.7 | < 0.001 |

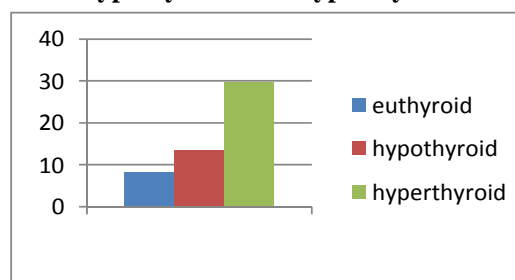
Table – 2 Values of radioactive iodine uptake after 24 hours in euthyroid, hypothyroid and hyperthyroid

| Group | Mean | P value |
|--------------|------|---------|
| Control | 30.8 | - |
| euthyroid | 20.8 | < 0.01 |
| hypothyroid | 21.1 | < 0.01 |
| hyperthyroid | 46.4 | < 0.001 |

Table - 3 Values of radioactive iodine uptake after 48 hours in euthyroid, hypothyroid and hyperthyroid

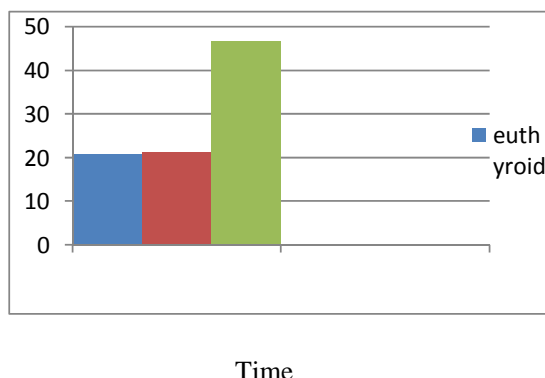
| Group | Mean | P value |
|--------------|------|---------|
| Control | 31.8 | - |
| euthyroid | 21.3 | < 0.001 |
| hypothyroid | 20.8 | < 0.001 |
| hyperthyroid | 45.3 | > 0.05 |

Graph – 1 Mean Values of Radioactive Iodine Uptake After 2 Hours In Euthyroid, Hypothyroid And Hyperthyroid

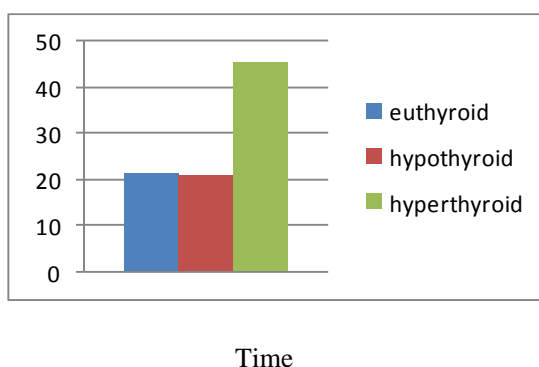


Time

Graph – 2 Mean values of radioactive iodine uptake after 24 hours in euthyroid, hypothyroid and hyperthyroid



Graph-3 Mean Values of Radioactive Iodine Uptake After 48 Hours In Euthyroid, Hypothyroid And Hyperthyroid



CONCLUSION

1. Radioactive iodine uptake is reduced at 2 hrs, 24 hrs and 48 hrs in eu-thyroid subjects P value in all three cases is significant
2. Radioactive iodine uptake is increased at 2 hrs and decreased at 24 hrs and 48 hrs in hypo-thyroid subjects P value is significant at 24 and 48 hrs only
3. Radioactive iodine uptake is reduced at 2 hrs, 24 hrs and 48 hrs in hyper-thyroid subjects P value is significant in all three cases.

REFERENCESS

1. Comparative evaluation of serum thyroxine , free thyroxine and thyrotropin determinations in screening of thyroid function, Nuutila P , Irjala K, Viikari J, Prinssi VP, Kaihola HL.,Ann Clin Res. 1998; 20 (3); 158-63.

2. Copper DS: Antithyroid drugs. N.Engl.J.Med.1984;311:1353
3. Degroot LJ, Larsen PR, Refetoff S,et all; The Thyroid and its diseases. New York, John Wiley & Sons, 1984
4. Larsen PR: Thyroid –Pituitary interaction: Feedback regulation of thyrotropin secretion by thyroid hormones. N eng J Med 1982;306:23
5. Review of Medical Physiology William F.Ganong, 19th edition 1999, pp: 303-317.
6. Samson Wright's Applied Physiology, Curi A.Keele, Eric Neil, and Norman Joels, 13th ed., 2000, pp: 537-545.
7. The practical use of thyroid function tests, Feldman JM., Am Fam physician.sep; 16 (3); 159-65.
8. Thyroiditis, Crile. G., Jr .Ann.Surg 127,640