Evaluation of CYFRA 21.1 as a Dedifferentiation Marker of Advanced Thyroid Cancer

Abstract

Purpose of the Study: Well-differentiated thyroid carcinomas have good prognosis, but as it de-differentiates, the survival rates go down. Early identification of such patients needs a marker which indicates the dedifferentiation process. CYFRA 21.1 has also shown to be increased in patients with 131I refractory thyroid cancer. We tested whether CYFRA 21.1 can differentiate between 131I avid and refractory tumors. Methodology: Well-differentiated thyroid cancer patients with known distant metastases were accrued and tested for stimulated and unstimulated thyroglobulin and CYFRA 21.1. All patients underwent 131I whole-body scan, 131I post therapy scan, and 18F-Fluorodeoxyglucose positron emission tomography-computed tomography. Those with even a single 131I nonavid lesion were considered 131I refractory disease. CYFRA 21.1 of both 131I avid and nonavid was compared, and CYFRA 21.1 levels against disease extent were analyzed. Results: CYFRA 21.1 levels were significantly elevated in 131I refractory group. A cutoff value of 2.07 ng/ml distinguished between 131I avid and refractory disease with high sensitivity and specificity (88% and 89.7%, respectively). However, CYFRA 21.1 levels were similar in patients when analyzed based on disease sites. Conclusion: CYFRA 21.1 can be utilized to differentiate between 131I avid and refractory diseases. Further long-term studies are required to use it as a predictive and prognostic marker.

Keywords: 131I refractory, 18F-Fluorodeoxyglucose positron emission tomography-computed tomography, dedifferentiation, thyroid cancer

Introduction

Well-differentiated thyroid carcinomas have good prognosis, but as dedifferentiation sets in, the survival rates go down. Response in such cases to conventional treatment modalities such as radiotherapy and chemotherapy is poor, and therefore, there is a need for new effective treatment modalities. With new insights into thyroid carcinogenesis evolving, newer targeted therapeutic agents are being investigated, and some treatments such as multitask inhibitors have shown better and promising results in such refractory cases.

Early identification of such patients needs a marker which indicates dedifferentiation process. The cytokeratin 19 (CK19) is an acidic protein which is highly expressed in differentiated thyroid cancer (DTC), particularly in papillary carcinoma of thyroid (PTC). The soluble fragments of CK19 (CYFRA 21.1) were found to be increased preoperatively in patients with locally aggressive DTC histotypes but not primary and metastatic classic DTC histotypes. Thus, it promises to be a potential predictive marker for the dedifferentiation of thyroid cancer. CYFRA 21.1 has also shown to be increased in patients with 131I refractory thyroid cancer. Furthermore, 18F-Fluorodeoxyglucose positron emission tomography/computed tomography (18F-FDG PET/CT) is known to be a prognostic marker of dedifferentiated thyroid cancer (deDTC) showing increased FDG uptake in such tumors. There is no study done to compare the bulk of the disease with CYFRA 21.1 levels. No study has been done in the Indian population regarding CYFRA 21.1 in thyroid cancer. We intend to test the same.

Methodology

Patients attending the thyroid clinic of the department of nuclear medicine, with known distant metastasis from histologically proven well DTC and meeting all inclusion and exclusion criteria were recruited for the study [Figure 1]. The study was approved

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by ethics committee, and informed consent was obtained from all the patients.

**Inclusion criteria**

Histologically proven DTC adult patients (>18-year-old) with known distant metastases, diagnosed either by ¹³¹I whole-body scan (WBS) done post total thyroidectomy or by biopsy in patients with metastatic presentation, were recruited for the study.

**Exclusion criteria**

Patients with high antithyroglobulin (Tg) antibody, pregnant women, and those who had any previous therapies with cytotoxic chemotherapy were excluded from the study.

Levothyroxine (LT4), if given, was withdrawn 4–6 weeks before ¹³¹I therapy, to achieve TSH level of >30 mIU/mL. In addition, all patients were advised to be of any diet and drug-containing a high amount of ¹³¹I 4 weeks before therapy.

All patients underwent ¹³¹I WBS and whole body ¹⁸F-FDG PET/CT before ¹³¹I therapy. ¹³¹I WBS was acquired 24 h after the administration of 2 mCi ¹³¹I on a single-head gamma camera (Seimens E. CAM) with a medium energy collimator at a speed of 12 cm/min on each side.

**Positron emission tomography/computed tomography image analysis**

All scans were evaluated independently by two experienced nuclear medicine physicians. PET images were looked for area of increased radiotracer uptake. Corresponding areas in CT images and fused PET/CT images were corroborated, and the extent of disease in the PET/CT scan was analyzed.

**¹³¹I therapy**

¹³¹I therapy was given according to a fixed-dose protocol, i.e., patients with lung metastasis received 150 mCi ¹³¹I (5.5 GBq) and 200 mCi (7.4GBq) with bone metastasis (with or without lung metastasis). All patients underwent ¹³¹I post therapy scan (¹³¹I PTS) 24–48 h after the therapy. The scan was acquired using a single-head gamma camera (Seimens E. CAM) with medium energy collimator at a speed of 20 cm/min on each side.

**Image analysis**

All scans were evaluated independently by two experienced nuclear medicine physicians. ¹⁸F-FDG PET images were looked for area of increased radiotracer uptake. Corresponding areas in CT images and fused ¹⁸F-FDG PET/CT images were corroborated, and the extent of disease in the PET/CT scan was analyzed. ¹⁸F-FDG PET/CT scans were compared to ¹³¹I PTS. Patients were categorized as ¹³¹I refractory disease if any additional lesion was found on PET/CT scan that was not showing any ¹³¹I avidity.

**Biochemical analysis**

Serum Tg and CYFRA 21.1 levels were measured both before (on LT4) and 4–6 weeks after LT4 withdrawal in each patient. Serum Tg was measured by immunoradiometric assay using a commercial reagent set (DynotestTg-plus; Brahms Diagnostica, Berlin, Germany), and CYFRA 21.1 was measured by ELISA using a commercial reagent kit (TM-CYFRA 21.1 ELISA Kit, Weldon Biotech India Private Limited, Delhi, India).

**Statistical analysis**

Statistical analysis was done using SPSS 11.5 (SPSS Inc., Chicago, Illinois, USA) software. Normally distributed data were expressed as mean ± standard division. The normality of Tg and CYFRA 21.1 distribution was assessed using Shapiro–Wilk test. t-test and Mann–Whitney U test were applied to compare the distribution of variance in different
results. $P < 0.05$ is considered to indicate statistical significance.

**Results**

A total of 61 patients were recruited for the study. Six patients did not turn up for CYFRA 21.1 and Tg analysis, 4 weeks after being put on thyroxine supplementation. One advance thyroid cancer patient in the $^{131}$I refractory group died of disease and was subsequently deleted from the study. Hence, the final analysis was done on 54 patients with 25 patients in $^{131}$I avid group and 29 in $^{131}$I refractory group. Patients in both the groups were matching in their baseline parameters, namely age, gender, histopathology, and stage [Table 1].

$^{18}$F-FDG PET/CT was done in all patients after thyroxine withdrawal to improve the sensitivity of the scan. Seven patients in $^{131}$I avid group showed lung only metastases as compared to 14 patients in $^{131}$I refractory group. Most of the cases in $^{131}$I avid group had micronodular metastases [Figure 2a-c], whereas $^{131}$I refractory group had mixed micro and macronodular metastatic pattern [Figure 3a-c]. Bone only metastases were seen in 15 patients in $^{131}$I avid group, whereas only one patient in $^{131}$I refractory group had bone-only metastasis. This patient had initially presented with left hip pain, which on evaluation found to have lytic lesion in the left ilium and biopsy done form the lesion showed metastatic follicular carcinoma of thyroid. Patients with both lung and bone metastases were less in $^{131}$I avid group, only three patients, whereas it was common in $^{131}$I refractory group (14 patients).

The Tg levels, both on and off thyroxine, did not differ in $^{131}$I avid and $^{131}$I refractory group. Off-thyroxine Tg levels were significantly elevated in both the groups. Serum CYFRA 21.1 was not affected by T4 therapy but was

| Table 1: Patient demographic table |
|-----------------------------------|
|                                | Iodine avid | Iodine refractory | $P$   |
| Age (year)                      | 44.8±13.88  | 51.6±14.88        | 0.736 |
| Sex (females)                   | 19          | 18                | 0.4207|
| HPE                              |             |                   |       |
| PCT                             | 10          | 15                | 0.5432|
| FCT                             | 13          | 10                | 0.2897|
| FVPCT                            | 0           | 4                 |       |
| Hurthle cell                    | 1           | 0                 |       |
| Distant metastases              |             |                   |       |
| Lung                            | 7           | 14                | 0.2209|
| Bone                            | 15          | 1                 | <0.0001|
| Lung and bone                   | 3           | 14                | 0.0108|

No significant difference was seen between $^{131}$I avid and $^{131}$I refractory groups with respect to patient age, gender distribution and histopathology. Age mentioned above is given in mean±SD. Bone only metastases were significantly more in $^{131}$I avid group. A $P$ value of $<0.05$ was considered significant. HPE: Histopathology, PCT: Papillary carcinoma of thyroid, FCT: Follicular carcinoma of thyroid, FVPCT: Follicular variant of PCT, SD: Standard deviation

![Figure 2](image1.png)

**Figure 2:** (a and b) $^{18}$F-Fluorodeoxyglucose positron emission tomography/computed tomography of a patient in iodine avid group showing suspicious nodule in the right lung posterior lobe with no significant fluorodeoxyglucose uptake. (c) $^{131}$I whole-body scan done in the same patient which shows residual thyroid tissue with bilateral lung metastases. This patient's CYFRA 21.1 level was 1.49 ng/ml and had a stimulated thyroglobulin of 640 ng/ml

![Figure 3](image2.png)

**Figure 3:** (a and b) Multiple fluorodeoxyglucose avid bilateral lung nodules noted in a 52-year-old female patient with papillary carcinoma of thyroid (Follicular variant) and its corresponding noncontrast computed tomography image. (c) $^{131}$I whole-body scan done in the same patient which shows no abnormal $^{131}$I concentration. Stimulated thyroglobulin of this patient was 654 ng/ml, and CYFRA 21.1 was 2.6 ng/ml

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significantly higher in patients with ¹³¹I refractory disease compared with patients with ¹³¹I avid disease \(P < 0.0001\); Table 2). One of the patients in ¹³¹I avid group had an abnormally high CYFRA 21.1 level (49.2 ng/ml) but was later confirmed to have carcinoma lung. Comparing CYFRA 21.1 values between the groups, even after the exclusion of this patient, showed a significant difference.

CYFRA 21.1 values were compared between the patients off each group and analyzed. Interestingly, CYFRA 21.1 values did not differ with the bulk of disease or with the site of the disease, i.e., patients having lung only or bone-only metastases, or both lung and bone metastases showed similar CYFRA 21.1 values. Results were similar in both the groups [Tables 3 and 4].

CYFRA 21.1 values of ¹³¹I avid and ¹³¹I refractory group were analyzed using the receiver operating characteristic (ROC) curve [Figure 4]. A cutoff value of 2.07 ng/ml distinguished between 131I avid and refractory disease with high sensitivity and specificity (88% and 89.7%, respectively).

**Discussion**

The management of deDTC is a therapeutic challenge. The ¹³¹I refractory and ¹⁸F-FDG PET/CT positive thyroid cancer have a poor prognosis in contrast to the well DTC.\(^2,11,12\) Conventional treatment is of marginal benefit for advanced thyroid cancers, emphasizing the importance of developing novel effective therapies. The role of tumor markers, which can predict such aggressive tumors, is thus important to direct the line of management, which can lead to better outcomes.

Higher CYFRA 21.1 levels were found in patients with primary aggressive DTC but not in conventional DTC histotypes.\(^8,9,13\) Such differences suggest that ¹³¹I refractory thyroid cancer cells are likely the source of increased serum CYFRA 21.1. Previous studies in human lung and liver cancer cell lines showed that among CK19-producing cells, only those with caspase-3 (an enzyme involved in apoptosis phenomena) expression induced high CYFRA 21.1 levels in culture supernatants.\(^14-16\) Serum caspase-3 enzyme activity is detectable in patients with metastatic ¹³¹I refractory thyroid cancer.\(^8\) The same is reflected in

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**Table 2: Comparison of thyroglobulin and cytokeratin fragment 21.1 between ¹³¹I avid and refractory groups**

| Tumor markers | ¹³¹I avid | ¹³¹I refractory | \(P\) |
|---------------|-----------|-----------------|------|
| Tg (off T4)   | 709.5 (5-4145) | 640 (82-3420) | 0.2491 |
| Tg (on T4)    | 137 (0.9-3450) | 94 (9-1324) | 0.1921 |
| CYFRA 21.1 (off T4) | 1.28 (0.75-3.3) | 2.6 (0.9-49) | <0.0001 |
| CYFRA 21.1 (on T4) | 1.47 (0.99-2.42) | 2.7 (0.88-49.2) | <0.0001 |
| CYFRA 21.1 (on T4)* | 1.47 (0.99-2.42) | 2.65 (0.88-9.4)* | <0.0001 |

On comparison between ¹³¹I avid and ¹³¹I refractory groups, no difference was seen in serum Tg levels, but a significant difference was seen in CYFRA 21.1 levels. *CYFRA 21.1 after exclusion of a patient with secondary lung malignancy having abnormally high CYFRA 21.1 levels. A \(P<0.05\) was considered statistically significant. Tg: Thyroglobulin, T4: Thyroxine, CYFRA 21.1: Cytokeratin fragment 21.1

**Table 3: Comparison of cytokeratin fragment 21.1 with ¹⁸F-fluorodeoxyglucose positron emission tomography/computed tomography-based disease extent in ¹³¹I avid group**

|       | Lungs (n=7) | Bone (n=15) | \(P\) |
|-------|-------------|-------------|------|
| CYFRA 21.1 | 1.43 (0.81-3.3) | 1.24 (0.75-2.04) | 0.1586 |
| Lungs (n=7) | 1.43 (0.81-3.3) | 1.69 (1-2.2) | 0.8333 |
| Bone (n=15) | 1.24 (0.75-2.04) | 1.69 (1-2.2) | 0.2863 |

On comparison with extent of disease on ¹⁸F-FDG PET/CT, no significant difference was noted in the extent of disease and cytokeratin fragment 21.1 levels in ¹³¹I avid group. A \(P<0.05\) was considered statistically significant. T4: Thyroxine, CYFRA 21.1: Cytokeratin fragments 21.1, ¹⁸F-FDG PET/CT: ¹⁸F-fluorodeoxyglucose positron emission tomography/computed tomography

**Table 4: Comparison of cytokeratin fragments 21.1 with ¹⁸F-fluorodeoxyglucose positron emission tomography/computed tomography-based disease extent in ¹³¹I refractory group**

|       | Lungs (n=14) | Lungs and bone (n=14) | \(P\) |
|-------|-------------|----------------------|------|
| CYFRA 21.1 | 2.5 (0.9-4.6) | 2.95 (1.48-49) | 0.2505 |

On comparison of CYFRA 21.1 with the extent of disease on ¹⁸F-FDG PET/CT, no significant difference was noted in the ¹³¹I refractory group. A \(P<0.05\) was considered statistically significant. T4: Thyroxine, CYFRA 21.1: Cytokeratin fragments 21.1, ¹⁸F-FDG PET/CT: ¹⁸F-fluorodeoxyglucose positron emission tomography/computed tomography
our studies where significantly higher levels of CYFRA 21.1 are noted in 131I refractory disease as compared to well-differentiated metastatic thyroid cancer \( P < 0.0001 \), Table 2. Aggressive thyroid tumors, i.e., tumors with high proliferation rate, which have increased rate of apoptosis and subsequent necrosis, are more likely to release CYFRA 21.1 into the serum. This is reflected in a study done by Gao et al. and Giovanella et al., in which negative tissue CK19 staining of aggressive thyroid tumors showed high levels of CK19-soluble fragments in serum due to the fast processing of CK19 molecules in such tumors.\(^{[9,10]}\) Interesting fact in this study is that even though only those patients with primary well-differentiated tumor histotypes were recruited, patients with 131I refractory metastatic disease showed increased CYFRA 21.1 levels. This is in concordance to the fact that genetically, the metastatic disease tends to have more chromosomal abnormalities than the primary, the dedifferentiation leading to increased serum CYFRA 21.1 level. One of the patients from the 131I refractory group was found out to have secondary primary carcinoma in the lung; thus, it had a very high CYFRA 21.1 level. Analysis is done after excluding the patient also showed significant difference \( (P = 0.001) \) between CYFRA 21.1 levels of 131I refractory and 131I avid groups. On doing ROC analysis, a cutoff value of 2.07 ng/ml differentiated between 131I avid and refractory diseases with high sensitivity and specificity of 88% and 89.7%, respectively.

Patients with increased CYFRA 21.1 levels had variable Tg levels. Tg levels were not significantly different between I–131 refractory and 131I avid groups [Table 2]. One possible reason could be due to the selection criteria as the disease was termed 131I refractory even if one of the lesions or an additional lesion found on 18F-FDG PET/CT was not 131I avid. Second, all the patients had well-differentiated tumors to start with, thus having differentiating properties such as Tg production and Sodium iodide symporter (NIS) expression. The genetic aberrations leading to decreased NIS expression and nonthyroglobulin secreting metastatic tumors though overlapping evolve differently as seen in thyroglobulin-elevated negative iodine scintigraphy syndrome, thus giving a different phenotypic presentation with some tumors retaining either of the differentiating properties.

In our study, PTCs with lung metastases were far more common in 131I refractory than the 131I avid group. PTCs with different mutations have distinct histopathologic appearance and biologic properties.\(^{[17]}\) Tumors associated with RET/PTC1 rearrangements are of conventional type with indolent course, whereas those with B-Rapidly Accelerated Fibrosarcoma (B-Raf), Rat Sarcoma virus (RAS), and Telomerase reverse transcriptase (TERT) mutations are associated with aggressive variants, decreased 131I avidity, distant metastases, and high recurrence rates.\(^{[18]}\) BRAF mutations are commonly seen in PTC, particularly in the solid variants, and maybe one of the reasons for having increased number of PCTs with lung metastases in 131I refractory group.

It is now a well-known fact that 18F-FDG PET/CT has the ability to locate residual or metastatic lesions in patients suspected of recurrence, with loss of ability to concentrate 131I in situations of high Tg levels or rising anti-Tg antibodies titers.\(^{[19,21]}\) In our study, 18F-FDG PET/CT was done to know the extent of disease. Lesions were called as metastatic based on the uptake and by their CT characteristics when uptake was minimal, as noted in well DTCs. Hence, all lesions, irrespective of uptake, were taken into account as all the patients were diagnosed cases of distant metastases, i.e., with lung and skeletal metastases. The FDG uptake seen in 131I-negative lesions could indicate the growth of more aggressive tumor cells in metastatic sites that have lost the activity of the NIS but that have increased expression of the glucose transporter 1 gene.\(^{[22]}\) However, analyses of CYFRA 21.1 in relation to the site of metastases did not reveal any significant difference [Tables 3 and 4]. Patients with bone-only or lung only metastases had similar CYFRA 21.1 values as compared to those who had both lung and bone metastases. This might probably indicate that CYFRA 21.1 levels are not related to the bulk of disease, but nature of the tumor per se, i.e., if all the sites are well-differentiated and 131I avid, no matter the number of lesions CYFRA 21.1 values will be low. Whether such an indication can make CYFRA 21.1 a better prognostic marker, needs to be evaluated. Quantitative analyses with standardized uptake value were not done due to the low avidity of FDG in well DTC, and presence of both iodine avid and iodine refractory lesions in 131I refractory group. Lesion-wise analyses were not done in this study due to difference in lesion wise distribution in both the groups (only one patient in 131I refractory group had solitary bone metastases, and almost all patients had mixed macro and micronodular pulmonary metastases in 131I refractory group). Comparison with exact number of lesions in a larger number of patients might provide conclusive evidence in future.

Serum Tg (Tg) is the best biomarker so far for postoperative follow-up of DTC, but it is not perfect for the following reasons: In many cases, persistent Tg cannot tell thyroid tissue remnant from residual or recurrent tumor; anti-tyroglobulin autoantibody present in many DTC patients can interfere with serum Tg measurement in immunometric assays, causing inappropriately low Tg values and lastly non stimulated Tg might be falsely low which is used in follow-up. Thus, there is a need for a novel tumor marker which overcomes the limitations of Tg and can predict poor prognosis, particularly in those who are on redifferentiation therapy, in whom Tg levels are erratic.

**Conclusion**

Serum CYFRA 21.1 levels are significantly increased in 131I refractory deDTC. The cutoff serum value of 2.07 ng/ml differentiates between well-differentiated...
and dedifferentiated metastatic thyroid cancer with high specificity and sensitivity. However, there is a need for larger prospective randomized control trial to know the prognostic implications of higher CYFRA 21.1 levels and its role in those undergoing redifferentiation therapies. Can pretherapeutic absolute level of CYFRA 21.1 or its dynamicity predict the dedifferentiation process, needs to be evaluated.

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**Conflicts of interest**
There are no conflicts of interest.

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