

Photodiagnosis of the Serum Samples of Differentiated Thyroid Carcinoma Patients: A Pilot Study

Sudha D. Kamath
KMC International Center,
Manipal University, Manipal.

Srujan K
Department of Nuclear
Medicine, KMC, Manipal
University, Manipal.

Nandini Pandit
Department of Nuclear
Medicine, KMC, Manipal
University, Manipal.

ABSTRACT

Tg is a specific and sensitive marker for the presence of functioning thyroid tissue, and its measurement is fundamental in the follow-up of patients affected by differentiated thyroid carcinomas (DTCs). Serum antibodies against thyroglobulin (TgAbs) are common in patients with DTC and can interfere in thyroglobulin (Tg) assays. Unfortunately, serum Tg measurement becomes useless in approximately 10–20% of DTC cases who are positive for anti-Tg antibodies that interfere with the Tg measurement. To overcome this problem, in the present study, we have used absorption spectroscopy technique for the quantification of Tg and TgAb.

Key words: Thyroglobulin, thyroglobulin antibody, absorption, immunoglobulin antibody G (IgG).

1. INTRODUCTION

The serum thyroglobulin estimation is primarily used as a tumor marker to evaluate the effectiveness of treatment for thyroid cancer and to monitor for recurrence [1]. Thyroglobulin test can be performed, along with a TSH test, prior to thyroid cancer treatment to determine whether the cancer is producing thyroglobulin. If it is, then the test can be performed at regular intervals after treatment to monitor for cancer recurrence [1,2]. Following therapy, the patients with differentiated follicular – cell derived thyroid cancer will produce no/trivial amount of thyroglobulin which can be revealed by serum thyroglobulin level. Reappearance of circulating thyroglobulin after total thyroid ablation is pathognomonic for the presence of tumor, indicating the need for additional treatment. But, since the thyroglobulin antibodies (TgAb) interfere with the results of thyroglobulin test, false positive and negative results are common with a person with measurable anti-Tg autoantibody levels ≥ 22 IU/mL. The presence of these antibodies lessens or eliminates the usefulness of the thyroglobulin test as a tumor marker. Therefore, in anti-Tg-positive patients, serum Tg measurements should either not be used in thyroid cancer follow-up or be interpreted with extreme caution. The serum contains major proteins like albumin and globulins. Globulin is derived from lymphocytes and they are responsible for the production of antibodies and other immune substances which play an important role in the various clinical manifestations of disease. It has been reported that the circulating antibodies directed against thyroglobulin (TgAb) are principally of the IgG isotype. Serum IgG is a main antibody which comprises 70-80% of total immunoglobulins [3]. The estimation of presence and elevation of this antibody is important in the recurrence and to monitor the follow-up- treatment [3]. In the

present study, we have used absorption spectroscopy for the discrimination of DTC serum samples from that of normal samples. UV/VIS absorption spectroscopy is the study of how a sample responds to light. When a beam of light passes through a substance or a solution, some of the light may be absorbed and the remainder transmitted through the sample [1,2]. Absorbance is proportional to the concentration of the substance interacting with the light (this is known as Beer's Law) [1]. In this spectroscopic technique, spectral changes can be measured with very high sensitivity, and they can be used to monitor biochemical alterations, which are the precursors for many diseases, including cancer. Because light delivery and collection are compatible with optical fibers and data analysis can be achieved in real time, spectroscopic information can serve as a powerful tool for assessing the state of any clinical sample.

2. MATERIAL AND METHODS

Ocean Optics CHEMUSB4-UV-VIS single beam spectrophotometer used for recording absorbance of serum samples. In this pilot study, we have used 15 serum samples, 5 from normal subjects and 10 from patients being monitored for DTC (age range, 18–60 years; mean age, 39 years). All of the patients had histologically confirmed DTC, were postoperative, and had received radioiodine. Serum Tg & TgAb measurements were done using a commercial ELISA assay with analytic sensitivity of 0.2 ng/mL. 5 ml of blood samples were obtained after taking consent from the patients. Blood samples were transported to the lab immediately after collection. The samples were allowed to stand at room temperature in upright position for about 30 min. The serum was then centrifuged at 3000 rpm for 5 minutes. The clear serum thus obtained was diluted 1:2 (for 1 ml serum + 1 ml distilled water) and their absorption spectra's were recorded using the software SPECTRA suite of OCEAN OPTICS.

3. RESULTS

Figure 1 shows typical overlaid spectra of normal individual and a patient recorded in the UV region (200 nm-800 nm). The patient's radioiodine scan report was positive with a small remnant tissue. The measured values of Tg and TgAb levels for this patient through IMA were found to be 300.00ng/ml 151.79 IU/ml respectively. From the figure it is clear that, a prominent peak (N_1) in normal spectrum was found at 290nm which corresponds to human serum albumin (HSA) and two weak peaks at 405nm and 492 nm correspond to immunoglobulins peaks. The patient's absorption spectrum shows five more extra peaks (D_2 , D_3 , D_4 , D_5 , and D_6) at 405nm, 492nm, 540nm, 577nm and 610nm along with HSA peak (D_1). The relative intensity of HSA peak has found to be decreased in the patient's spectrum compared to that of

normal sample. This may be due to variation in albumin: globulin ratio which has affected the viscosity of serum in normal and diseased conditions. In figure 1, the inset shows the commercially available standard serum IgG spectrum with two strong peaks at 405nm and 492 nm. So these two peaks can be related to IgG subclass of immunoglobulins. The intensities of these two peaks were found to be increased from normal to DTC condition, signifying their importance during disease condition. The serum IgG level increases in various disease conditions including inflammatory responses. Since our main objective is to see the change in the concentration of IgG with respect to malignant conditions and its possible role in interfering with the results of thyroglobulin test, we have not incorporated the patients with inflammatory conditions of various organs or sites in this pilot study. It is also clear in figure 1 that, there is no N_4 peak in the normal healthy individuals. The presence of extra peak in the 540 region in almost all the malignant condition (D_4) shows its possible role in malignancy. Since it is a pilot study we have not done the gel electrophoresis method for the characterization of individual peaks obtained with the spectroscopic study of recording serum samples. The other peaks (D_3 , D_5 , and D_6) were not identified in the present study.

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remnant tissue in the neck with TgAb of 151 IU/ml. Inset: The absorption spectra of standard IgG antibody. Figure 2 shows the serum absorption spectra recorded in the UV –VIS region (200 nm-800 nm) (A) normal and (B) DTC patient's serum samples. The Tg level for the normal individuals were < 2 ng/ml and for the patients range of 100 ng/ml to 300ng/ml. Very much increased level of TgAb levels >150 IU/ml, has been observed in the patient's serum with positive scan report. It is clearly seen from the Figure 2 (A) that absorption spectra of all normal samples are similar with the absorption

corresponds to peak N_1 is approximately double than that for D_1 peak in patient's spectrum.

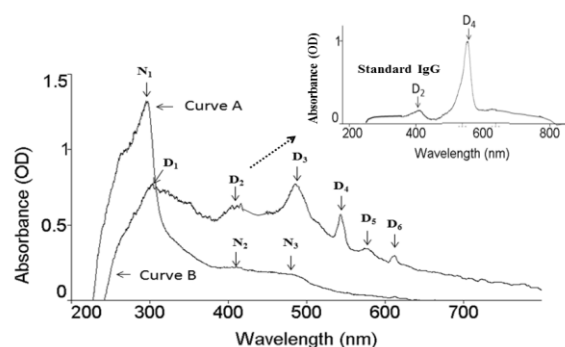


Fig 1: Absorption spectra of serum samples recorded in the UV –VIS region (200 nm-800 nm) (A) normal (B) patient who had remnant tissue in the neck with TgAb of 151 IU/ml. **Inset:** The absorption spectrum of standard IgG antibody.

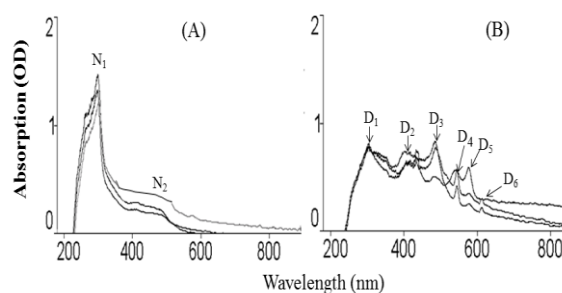


Fig 2: Absorption spectra of normal and DTC serum samples (A) normal with Tg < 2 ng/ml and (B) patients with TgAb levels >150 IU/ml.

From the Figure 2 (B) it is clear that the spectral profile of the DTC patients is totally different from that of the normal conditions with 6 peaks (D_1 to D_6), with varying intensity. Two of these peaks have already been identified as immunoglobulin peaks. The present study shows that the relative intensity of the antibodies in the spectral profile can be directly related to the actual level of antibody present in the serum of individuals suffering from DTC by ELISA test.

4. CONCLUSION

We can conclude that, the present spectrophotometric technique discriminates normal and DTC patient's serum samples. Since, this technique is cheaper, simpler, more specific, be less disturbed by other compounds, it may be used as supplementary technique to already existing ones. Also, small time is needed to record and analyze the absorption spectra which make this technique very attractive for real time applications.

5. ACKNOWLEDGMENTS

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