Available online at **www.ijmrhs.com**

ISSN No: 2319-5886

International Journal of Medical Research & Health Sciences, 2016, 5, 9:203-210

Exponentially Decaying Electric Pulses for Improving Radioactive Iodine uptake in human thyroid cancer cells

Amr A. Abd-Elghany[1,2]

[1] *Radiology and Medical Imaging Department, College of Applied Medical Sciences, Prince Sattam Bin Abdul-Aziz University, KSA*
[2]*Biophysics Department, Faculty of Science, Cairo University, Egypt*
Corresponding E-mail: amrabdelghany25@gmail.com

ABSTRACT

Radioactive iodine (radioiodine) is an effective nuclear medicine treatment used to eradicate thyroid cancer cells. The problem was the inability of thyroid cells to retain radioiodine which causes the thyroid cancer cells to be resistant to radioactive iodine treatment. Therefore, there are different methods that have been established to enhance the radioiodine uptake within the thyroid cancer cells for therapeutic purposes. Exposure of human cells to exponentially decaying high intensity, short duration electric pulses permeabilizes the plasma membrane to impermeable molecules. Electroporation is a physical modality that involves high intensity, short duration electric pulses to facilitate the entry of impermeable molecules by increasing the plasma membrane permeability. The purpose of this study was to use exponentially decaying high intensity, short duration electric pulses in incorporating radioactive iodine into non-iodine retaining follicular thyroid carcinoma cell line FTC133. Results showed that the uptake of radioiodine by electroporation has a dependence on the electric field, external concentration of the iodine, time and the temperature of incubation. The incorporated radioiodine was retained over a period of 24 h. The permanent concentration of the incorporated iodine may have a significant effect on the tumoricidal properties if approved in vivo.

INTRODUCTION

Differentiated follicular adenocarcinoma thyroid cancer is deriving from the follicular epithelium and retaining the major biological features of healthy thyroid tissue, including expression of the human sodium iodide symporter(hNIS), the key cellular regulator for specific iodine uptake[1].Radio-iodine uptake and concentration inside the thyroid cells via sodium iodide symporter represents the most important process normally in the production of thyroid hormones[2] as well as killing of cancer cells[3].Differentiated thyroid cancer (DTC) is an uncommon disease clinically, but worldwide, its incidence shows a noticeable increase[4]. Radio-iodine therapy (RAIT)is performed by the administration of radioactive sodium or potassium iodide (^{131}I) intravenously for selective post-surgical irradiation of thyroid residuals of non-resectable or incompletely resectable DTC[5]. The main therapeutic defy is the inability of iodine uptake by some metastatic lesion which have not been fully understood and give the chance of recurrence. To increase the therapeutic outcome of RAIT it is necessary to develop a new and easy outpatient strategy to increase the radioiodine uptake by thyroid cells.

Exposure of cells to short intense electric pulses above a critical threshold results in dramatic temporary increase in the electrical conductivity and permeability of the cell membrane allowing the entry of impermeable exogenous molecules. The increase in the membrane permeability is attributed to pores formation in the plasma membrane. These pores could be resealed without losing the cytoplasmic macromolecular contents, and most cells survived after pore resealing. This phenomenon is called electroporation or electropermeabilization[6-8].The process of

electroporation is often used for yeast and bacterial transformation [9], electrochemotherapy (transdermal drug delivery)[10-15],cell electrofusion[16, 17] and electro-insertion of proteins[18].The permeability changes depend on various experimental parameters such as, intensity, number and the width of the electrical pulses, pre- and post pulse incubation temperature, as well as the composition of the exposure medium[19].

The aim of this work was to study the possibility to incorporate radioiodine into refractory thyroid cells in vitro using single exponential electric pulse as a physical drug delivery tool. It has been observed that a substantial amount of radioiodine was incorporated into the electropermeabilized cells and remained inside the cells for a considerable time. These results may form the basis to achieve enhanced concentration of radioiodine in thyroid tissue in pathological situations, which are promising in treating thyroid disorders.

MATERIALS AND METHODS

Cell Culture

Human follicular thyroid carcinoma cell line (FTC133) were cultured in modified essential medium 1x (MEM 1x) (Life technologies, Grand Island, USA) supplemented with L-glutamine, 10% fetal calf serum (FCS) (Life technologies), 100units/ml of penicillin and 125µg/ml of streptomycin (Invitrogen Corporation, Green Island, USA). The cells were grown in 75cm^2 tissue culture flasks at 37°C, in a humid atmosphere of 5% CO_2 in air. Cells were trypsinized (0.05% trypsin-EDTA) (Invitrogen Corporation) and suspended in serum-free MEM for 5min. at 37°C then centrifugation at room temperature (200 g, 6 min)[20]. Cells were resuspended (1x10^6 cells/ml) in either low conductive medium serum-free MEM 1x (1mS/cm) or physiologically conductive medium Phosphate Buffer Saline (PBS) pH 7.4 (10mS/cm).

Exposure of cells to exponentially decaying electrical pulses

Each 400µl cell suspension was placed in a disposable electroporation cuvette with gap distance between the electrodes 2 mm and exposed to an exponentially decaying electric pulses using cell electroporator (MicroPulser, Bio-Rad, USA).The cells were electroporated using single exponential pulse, exponential decay time constant 1 ms and field strength (0.3-5.7kV/cm) at room temperature. The temperature measurements at the end of the exposure were measured using digital thermometer. All the previous exposure conditions were done in duplicate using either serum free MEM 1x or PBS. Cell viability was assessed using cloning efficacy test to get the field intensity for optimum viability.

Measurement of ^{125}I uptake:

^{125}I radioactive doses used in the experiment (Nordion Sciences Advancing health) has 59 days half-life, the chemical form was^{125}I as iodide in dilute NaOH solution with pH8.0 – 11 and the radiopurity≥ 99.9% gamma (^{126}I ≤ 0.005% ^{137}Cs + ^{134}Cs ≤ 0.0001%).The gamma counter used in the study was (Packard Cobra Model 5005) it is a multi-detector system with five 1.5 inch NaI through-hole detectors with an energy range of up to 1000 keV has the capability to count five samples at a time and can count 12x75 mm RIA tubes, 16x100mm and a variety of smaller micro-tubes using an available adapter. It include an LCD display for output, Dot Matrix Printer, tube racks, protocol ID tags and operation manuals. Specific calculations include calibration curve display-, linear-, polynomial regression-, log-, logit-, 4 PL-, auto spline-, manual spline- and cubic spline curve fitting algorithms.

A part of the thyroid cell suspension (400 µl) was mixed with 10 µCi of carrier-free ^{125}I and the mixture was exposed to exponentially decaying electric pulses with field strengths ranging from (0.3-5.7kV/cm). Then the suspension was incubated at 37ºC for 1h with their respective controls. Electroporation parameters were preserved constant throughout the experiments. At the end of the incubation the cells were in the form of pellet by centrifugation and then washed with serum-free MEM 1x. Each pellet was counted in a gamma counter.

Foroptimization of ^{125}I concentration, different concentrations of ^{125}I ranging from 1 to 40µCi were mixed with 400 µl of the cell suspension. Each was subjected to field strength 3.5kV/cm and then incubated at 37ºC for 1 h. The cells became in the form of pellet, then washed and counted in the gamma counter.

Radioactive ^{125}I retention measurement

The retention of the incorporated iodide in the cells was monitored over periods of 1, 6 and 24 h. The same amount (400 µl) of the cell suspension was exposed to single exponential electric pulse with field intensity of 3.5kV/cm in the presence of 10 µCi of Na^{125}I. After exposure to electric pulse the cells were incubated at 37ºC. At the end of the

incubation the cells were pelleted by centrifugation and the cell pellets were retained, washed then counted over the previously mentioned intervals and the retainability was measured.

Trichloroacetic acid (TCA)protein precipitation assay

To measure the protein-bound iodine, TCA precipitation of the cell pellet was used. First, the cells were lysed in a lysis buffer (10 mMTris pH 7.5, 250 mM sucrose, 160 mMKCl, 5% Triton X-100, 0.1mM PMSF, 2.5 µg/ml Aprotinin) and stored on ice for 10 min. Then the suspension was centrifuged and10% chilled TCA was added to the aspirated supernatant by equal amounts (v/v). The suspension was centrifuged at 2400 rpm and each pellet was counted in the gamma counter.

Statistical analysis

Iodine uptake in the cells was expressed as TCA perceptible counts. Each data point represents the mean ± standard error S.E. of three independent experiments, each experiment being performed in triplicate. For each figure, the individual values of the controls of all the corresponding independent experiments were used to determine the control average value and the standard deviation. The statistical significance of the data (exposed vs. control cells) was determined using the unpaired Student's *t*-test (**P*<0.05, ***P*<0.01, ****P*<0.001).

RESULTS

Effect of field intensity on cell viability

Cells suspended in either low conductive medium MEM or high conductive medium were subjected to single pulses of different field intensities from 0.3 to 5.7kV/cm. The viability of the cells was measured by cloning efficacy test. Fig. 1 shows the loss of cell viability as a function of field strength. No significant change in the cell viability was observed at field intensities less than 3.5kV/cm for cells suspended in either MEM or PBS. It was observed that, post-pulseincubation of the cells at 37^{o}C recovered normal cell viability.It is supposed that at 37^{o}C the pores of the cell membrane were resealedand the original membrane integrity was maintained without the loss ofintracellular components which enable cells to remainviable.

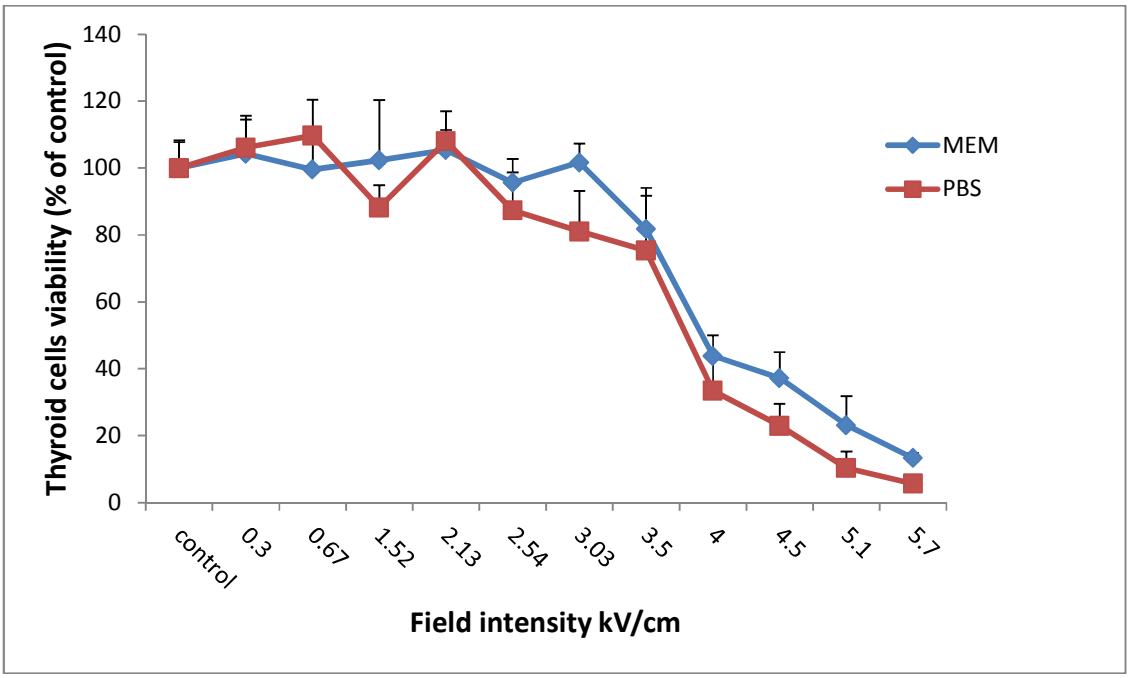

Fig. 1: Effect of both field strength and conductivity of the medium on the thyroid cells viability. The cells were exposed to electric pulse at room temperature. The viability was measured using cloning efficacy test.

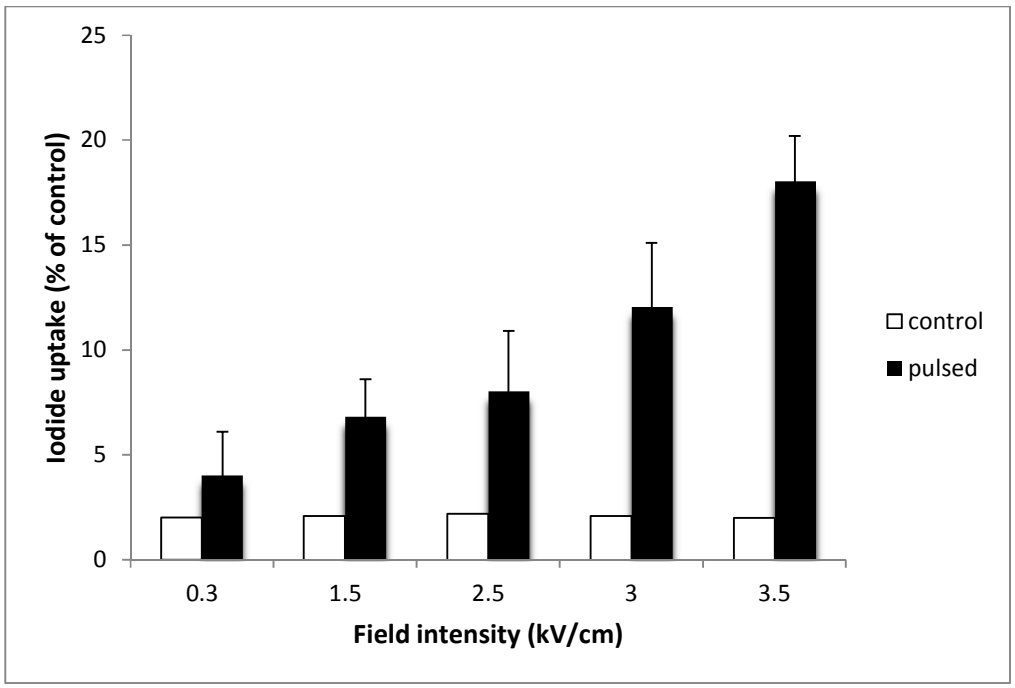

Fig. 2: Effect of field strength on radioactive iodine uptake by thyroid cells. The cells were exposed to electric pulse in the presence of 10µCi $Na^{125}I$ and incubated at 37oC for 1 h. The radioactivity in the cells was measured after TCA precipitation.

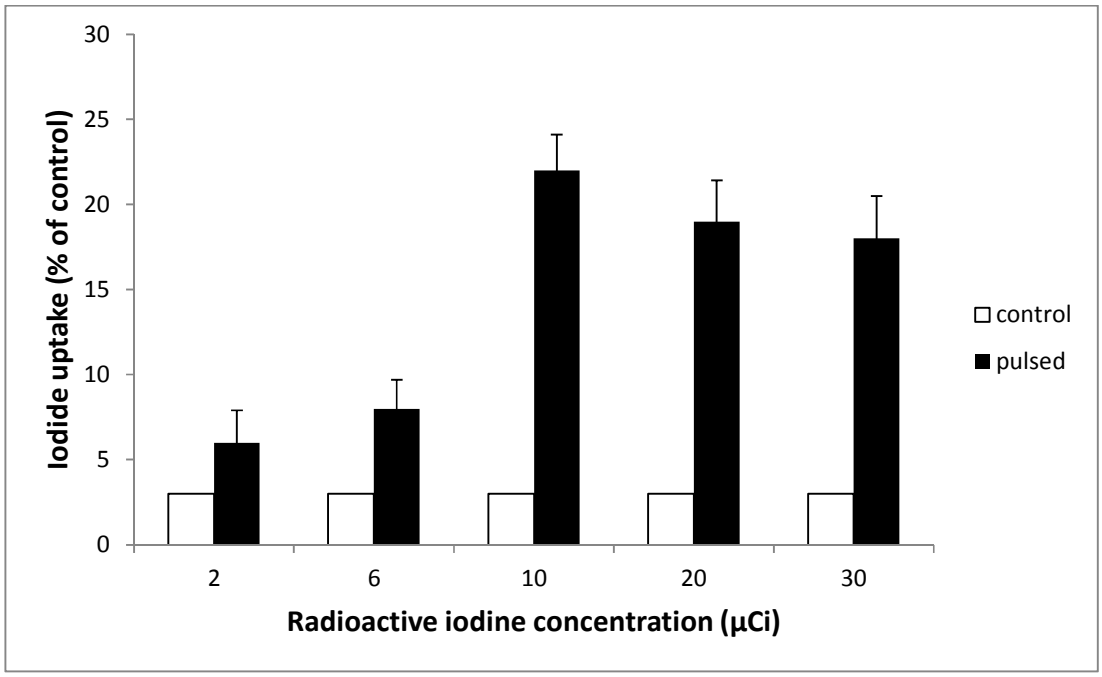

Fig. 3: Effect of exogenous radioactive iodine concentration on iodide uptake by thyroid cells. The cells were exposed to electric pulse of field intensity 3.5kV/cm in the presence of different $Na^{125}I$ concentrations (2-30µCi) and incubated at 37°C for 1 h. The radioactivity in the cells was measured after TCA precipitation.

Incorporation of ^{125}I in electroporated thyroid tumor cells

Cells suspended in MEM containing ^{125}I were exposed to a single exponential electric pulse at room temperature. It was observed that field intensity was the crucial parameter in the uptake percent of ^{125}I in the TCA precipitate of the cells incubated at 37^{o}C(Fig. 2). The maximum uptake percent of^{125}I uptake in the cells was obtained at field intensity of 3.5kV/cm, being 9–11fold more than measured in control cells (p<0.001). Also, it was noticed that the uptake of^{125}I by the electroporated cells was a concentration dependent for tested concentrations (2–30 µCi). The dependence of ^{125}I on the concentration follows a plateau response for a saturation concentration of 10µCi (Fig. 3).Ata concentration of 10 µCi, ^{125}I uptake was increased by9–11 folds over the control cells; beyond this concentration it seems to be saturating.

Retention of radioactive iodine electropermeabilized into the cells

Retention of the electropermeabilized radioactive iodine across thyroid cells was assessed by measuring the released^{125}I in the supernatant. Fig. 4, showed the retention of the incorporated ^{125}I into the pulsed cells substantially over a period of 24 h.

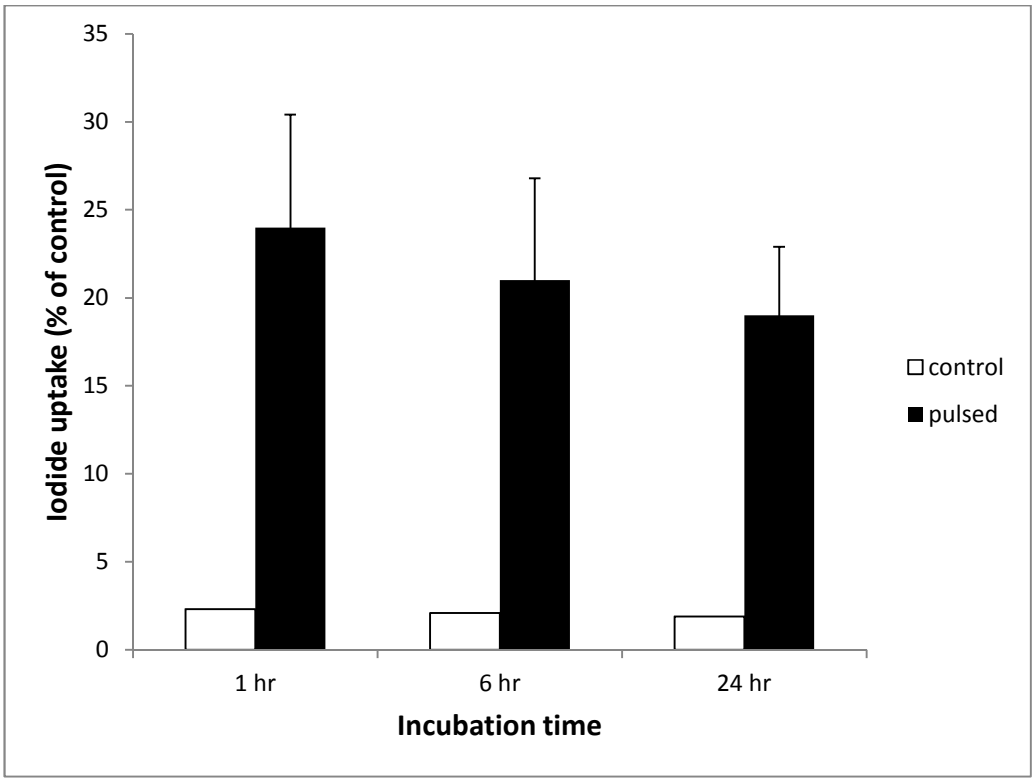

Fig. 4: Retainability of the electropermeabilized radioactive iodine in the thyroid cells. The cells were exposed to electric pulse of field intensity 3.5kV/cm in the presence of 10µCi Na^{125}I and incubated at 37^{o}C for 1 h. The radioactivity in the cells was measured after TCA precipitation. The retainability measurements were repeated after the incubation at 37^{o}C for 6 and 24h.

DISCUSSION

Electroporation or electropermeabilization is a physical approach that uses high intensity, short duration electric pulses of different shapes to increase the cell membrane permeability. This allows the entry of impermeable exogenous molecules into cells. It has been reported that the increase in membrane permeability is due to the formation of new and reversible aqueous pathways called electropores. My present study used an exponentially decaying electric pulse to increase the efficacy of the uptake of radioactive iodine into cancer cells without the loss of cell viability. A lot of experiments used exponential pulse generators for skin electroporation [21-23].The potential advantage of exponentially decaying electric pulses is to maintain the electropermeabilization efficient by drifting the drug molecules through the skin by electrophoresis [24] so it could be useful for in vivo applications. Our previous work showed the possibility of enhancing the transdermal delivery of insulin using exponential pulses[25].

It has been reported that differentiated thyroid cancer cells can be controlled by radioiodine. However, some metastatic sites fail to concentrate radioactive iodine and this puts a lot of constrains on the therapeutic trials. It is unclear whether restricted entry or lack of retainability is the reason for the lack of concentration of radioiodine by these cells. Attempts have been made to explore and develop alternate treatments like chemotherapy and external radiotherapy[26, 27]. But these approaches have been of limited success. Lithium has been used as a potential adjuvant in ^{131}I therapy to enhance the retainability of the radioiodine[28], while retinoic acid has been used for the re-differentiation therapy of non-iodine concentrating thyroid metastases[29, 30]. Misaki and his colleges (1996)used tumoricidal cytokines to enhance the uptake of radioiodine in in vitro cultures of human thyroid cancer cells. The uptake observed was only1.5–3 times more than that ofthe controls.

The present study demonstrated that exponential electroporation allows the entry of a substantial amount of radioactive iodine into the thyroid cells. The follicular thyroid carcinoma FTC 133 cell line was selected as an efficient model to study the incorporation of iodide by electroporation. The iodide uptake was increased with the pulse strength of3.5 kV/cm pulse; the % uptake was 9–11 times greater in pulsed cells than in control ones(Fig. 2). It was obvious that the field intensity was the crucial and deciding parameter in iodide uptake. Interestingly, iodide incorporated into the cells was retained to 24 h, suggesting that electroporated membrane was resealed to its impermeable state by incubation at 37^{o}C. The size of the incorporated iodide ion is very small when compared to the size of the drug molecules that have been widely used. It was expected that a reverse transport of the iodide ion mayoccur and may lead to some leakage. But my experiments showed that this process did not occur because iodide molecules entered into the cell conjugated with the intracellular proteins, as was evident by the TCA precipitation assay.

Nowadays, electrochemotherapy using bleomycin as an anticancer drug became a well-established technique for the treatment of head and neck cancer, basal cell carcinoma, malignant melanomas and metastases of bladder transitional cell carcinoma[31-33]. Electropermeabilization has been performed using different types of electrodes, e.g. metallic plate electrodes (non-invasive)for the treatment of easily accessible small surface tumors; needle array (invasive) electrodes for the treatment of large superficial or deep seated tumors; electrodes integrated into catheters for cardiovascular and hollow organ applications; and flow-through electrode systems for ex vivo therapy[34]. The successful trial for exponentially decaying electric pulse highlights the possibility of using electroporation as a transdermal radioiodine delivery using metallic plate electrodes.

CONCLUSION

The present work showed the possibility of using exponentially decaying electric pulses in radioiodine therapy of thyroid cancer. The amount of radioiodine incorporation into a thyroid cell can be controlled by the intensity of the electric pulse. The retainability of radioiodine strongly suggests the usefulness of this modality for in vivo experiments for treating of thyroid cancer.

Acknowledgements
This work was supported by National Cancer Institute and faculty of Science, Cairo University, Egypt and College of Applied Medical Sciences, Department of Radiology, Prince Sattam Bin Abdulaziz University, KSA.

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