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METHODS

A New Rapid Technique for the Fixation of Thyroid Gland Surgical Specimens

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One of the main diagnostic problems in thyroid pathology is to distinguish between follicular adenoma and follicular carcinoma. Thorough sampling of the nodule's capsule is recommended in order to identify capsular invasion. However, during the hardening of the tissue, by the usual fixatives the capsule shrinks and rolls downwards and sometimes the capsule separates from the remaining tissue. The present work evaluates the use of "*Lymph Node Revealing Solution*" (LNRS) for the rapid fixation of different thyroid lesions as compared to that of formalin. Fifty-one unselected consecutive cases of thyroid nodules, which included various benign and malignant lesions, were examined. Each specimen was cut in two equal parts; one was fixed in LNRS, the other in formalin. Fixation in LNRS for 2 hours gave adequate results in sectioning and staining of the tissue, and excellent immunostains. Its advantage over formalin is the conservation of the natural relationship between the capsule and the rest of the tissue, on the same plane, as well as the short time required for the final diagnosis. (Pathology Oncology Research Vol 5, No 1, 70–72, 1999)

Key words: lymph node revealing solution, thyroid, fixation

Introduction

Partial or total thyroidectomies are performed usually because malignancy can not otherwise be ruled out. A rapid diagnosis is essential in order to start the treatment as well as to shorten the anxiety period for the patient and his family while waiting for the definitive pathological report. One step in the tissue preparation is the fixation of the specimen.

The fixatives recommended for thyroid tissue are buffered formalin, Bouin, Zenker formol and Heidenhaim's Susa fluid.[1,2,3] The time required for adequate fixation with the different fixatives is 12-24 hours.

Another problem with thyroid nodules is that during the hardening of the tissue the capsule shrinks and rolls downwards. Thorough sampling and visualisation of the capsule is very important in order to distinguish follicular adenoma from follicular carcinoma and in other cases of thyroid malignancy, in order to be able to report accurately whether there is invasion of the capsule.

We have used a new fixative, which is suitable for paraffin embedding, histochemistry and immunohistochemistry, and has the advantage that it is much quicker und does not distort the microscopic pattern.

Material and Methods

Fifty-one consecutive thyroid specimens were included in this study. The thyroid tissue was sent immediately after excision, on a moistured pad. Each lobe was longitudinally cut into two equal parts, which were submerged, either in LNRS or in 10% buffered formalin, for one hour.

LNRS *("Lymph Node Revealing Solution"),* was developed by one of the authors (Dr. Rumelia Koren)[4] and is a mixture of traditional fixatives and fatty solvents: 65% of 95% ethanol, 20% of diethyl ether, 5% of glacial acetic acid and 10% of buffered formalin prepared under

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Table 1. **The Antibodies Used for Immunostain and the Sources**

Code antibody	*Commercial source*
CKTGS Thyroglobulin	DPC, LA,CA
08-0052 Vimentin	ZYNIED, San Francisco, CA
CK F8S Factor VIII Related antigen	DPC
08-0129 P53	Zymed

hood. After the first hour, each segment was further sectioned into 3-4 mm thick slices and re-immersed for another hour in the respective fixatives. At the end of the second hour, the sections were divided into smaller parts, and examined every 2 hours to see if the fixation was adequate, and then processed and embedded routinely in paraffin. The slices from the LNRS were rinsed for 10 minutes in running water before processing. The paraffin blocks were cut in 4 µm thick sections and stained with H&E and immunostains with the avidin-biotin-peroxidase complex method as required,[5] for thyroglobulin, factor VIII, vimentin, p53 *(Table 1)*.

Results

Table 2 summarizes the histological diagnosis and macroscopical data of all the cases. The patient's age at diagnosis ranged between 20–80 years (mean 48.61). The thyroid lobe diameter ranged between 1.5–17 cm, (mean 6.25).

After 1 hour – with 10% neutral-buffered formalin fixation, the capsule enfolded, pressing out the tissue inside the nodule, so that it protruded several millimetres above. In contrast with LNRS, the capsule and the rest of the tissue were on the same plane so it was easy to make thorough sampling of these areas *(Figure 1)*. The tissues submerged in formalin were almost completely unfixed. The tissue with LNRS was mostly white but still had small-unfixed areas, especially in the larger specimens.

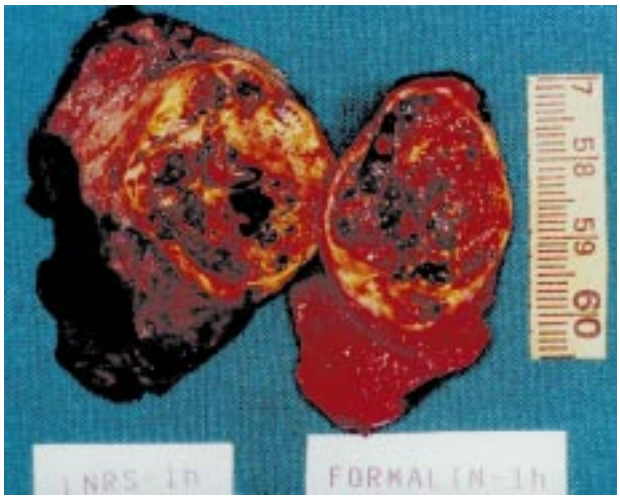

Figure 1. *Macroscopical appearance of papillary carcinoma. Note: the good fixation after 1 hour in LNRS as opposed to almost unfixed tissue in formalin.*

After 2 hours – of fixation the thyroid specimen fixed in LNRS, was ready for processing, while those fixed in buffered formalin were pink and soft. Complete fixation was achieved only after 12 hours. Processing and section-

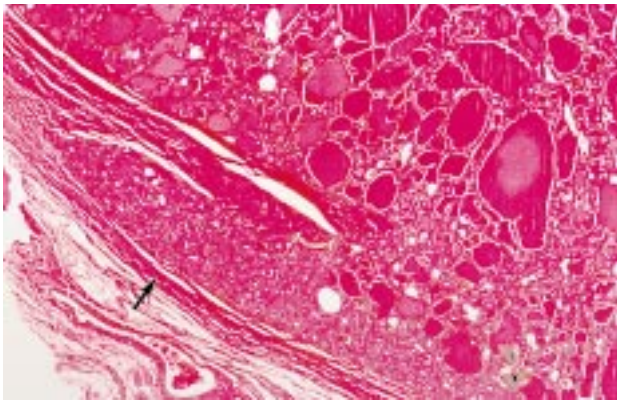

Figure 2. *Follicular carcinoma with LNRS fixation. Note: the capsular invasion (arrow). H&E x100.*

Table 2. **Pathological and clinical data of all the cases**

Diagnosis	*No of cases*	*Sex Male/ Female*	*Age range*	*Weight range*	*Largest diameter range*
Follicular adenoma	9	5/4	21–63 y	1–90 gr	2–10 cm
Follicular carcinoma minimally invasive	5	1/4	29–64 y	7–31 gr	1.5–10 cm
Papillary carcinoma	14	1/13	20–71 y	7–34 gr	3.5–7.5 cm
Metastasis from liposarcoma	1	1/0	63 y	114 gr	11 cm
Dishormonogenetic goiter	1	1/0	34 y	38 gr	10 cm
Hashimoto's thyroiditis	2	0/2	38–54 y	8–40 gr	4–6 cm
Nodular goiter	19	5/14	24–73 y	5–204 gr	4–11 cm

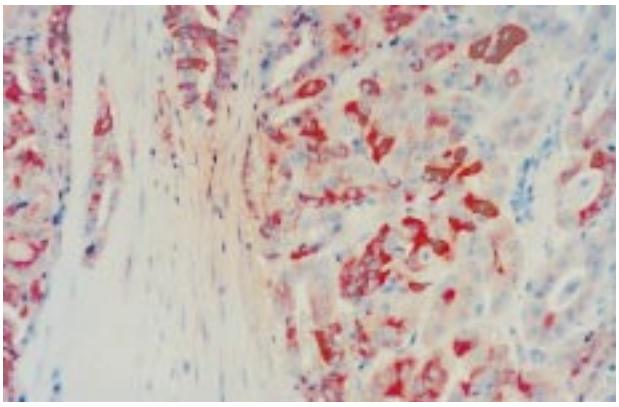

Figure 3. *Follicular carcinoma with LNRS fixation. Immunostain for thyroglobulin x400.*

ing the paraffin blocks of LNRS-fixed tissue was the same as for the formalin fixation. An excellent quality of the sections was achieved with the fixation in LNRS for 2h. The H&E stain in all sections was similar to that achieved with 10% neutral-buffered formalin. There was no cytoplasmatic or nuclear alteration. In the specific cases of papillary carcinoma, the ground glass appearance was maintained and so were the results of special stains. We obtained excellent immunohistochemical results with thyroglobulin, factor VIII, vimentin, that stain mainly the cytoplasm as well as with p53 that stains the nuclei in the carcinoma cases.

Examples of the capsular invasion and of the immunohistochemical stained slides are shown in *Figs 2, 3*.

Discussion

Fixation is the most important factor in the preparation of good histological sections. Its role is to preserve the tissue by stopping autolytic changes, and allowing the tissue to remain unchanged and as close to life status as possible, without shrinkage. A good fixative should slightly harden the tissue without brittling it.[4] With the use of LNRS we obtained all the above mentioned qualities.

In the specific case of thyroid nodules the conservation of the capsule, without altering its relations to the adjacent tissue, is essential, especially for differentiating follicular adenoma from follicular carcinoma, a dominant nodule of nodular hyperplasia and the variant of papillary carcinoma that is both follicular and encapsulated.[6,7] Many of our samples were sent for frozen section examination, so the tissue had to be sliced freshly. The maintenance of the natural relationship is more difficult after having sliced the specimen. This was achieved by LNRS much better than with formalin. The time required for adequate fixation with 10% neutral buffered formalin is usually 12–24 hours. LNRS is superior, requiring only two hours. We think that the quick penetration is due to the combination of ether, glacial acetic acid with ethanol and the buffered formalin.

The fixative should also allow adequate penetration of the immunohistologic reagents and not interfere with the subsequent immunologic and chromogen reaction.[8] LNRS fulfils all of these demands. LNRS contains two dehydration agents, who preserve relatively higher molecular weight DNA and RNA compared with formalin.[9] This combination makes it suitable for morphological studies and further molecular biology analysis, when necessary. On the other hand with microwave-stimulated fixation which is a short process there are artifacts due to the acceleration of diffusional and reactive processes and the staining processes themselves can be sometimes different at elevated temperateres.[10]

In conclusion: LNRS is easy to prepare with cheep solutions avaible in every laboratory, and has all the above mentioned qualities of a good fixative. It's advantage is by the conservation of the natural relationship between the capsule and the rest of the tissue, on the same plane, as well as allowing shorter time interval between the excision of the thyroid and the final report, thus shortening the time of anxiety for the patient and his family while waiting for the pathological report, and shortening the time for operation of the controlateral lobe when necessary.

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