

A. **Project Leader** : Prof. Dr. Nor Hayati Othman

[student – Puan Rusidah Mat Yatim]

Project title : Comparative study between flow cytometric and conventional HPE method in Multinodular Goiter- USM experience

B. **Research Summary Proposal**

Introduction:

In HUSM, the most common thyroid diseases is multinodular goiter (MNG). In an earlier study of 300 consecutive cases it was noted that malignant transformation of MNG in our population was high, 36.8%; higher than what is cited in the literature. We postulated that MNG is a precursor lesion to the development of malignant thyroid diseases. The current study is an extension of the previous work – is to determine if flow cytometry could be used to diagnose early malignant transformation among patients who came in with MNG using the theoretical premise that flow cytometry detects DNA changes in abnormal cells.

Progress in defining the role of flow cytometry in the assessment of solid tumors has been slow, partly because of the greater histological complexity of solid tissues, and partly because of practical difficulties in obtaining suitable material compared to hematological diseases. Over the past decade, great advances have been made and flow cytometry is now used more frequently for measurement of DNA content in solid tumors. Recently, the technique has been extended by the development of flow cytometric assays for other cellular components, such as membrane markers, oncoprotein and growth factor receptors. There can be little doubt that it will become a valuable technique in the study of the biology and treatment of cancer. Quantitative multiparameter flow cytometric analysis of surface antigen and DNA has become a well established and reliable technique in the investigation of lymphoreticular cells.

In solid tumors however, flow cytometry has mainly been used for DNA analysis, and more recently for quantitative assay of nuclear antigens. Locally, no work has been done utilizing flow cytometric determination of multinodular goiter using single cell suspension. We embarked on diagnosing MNG using flow cytometry and detect malignant transformation in thyroid cell suspension based on DNA abnormalities.

[This study encountered a few difficulties – at the beginning of this study, there were very few published reports or literature on flow cytometry on solid lesions. Most were flow cytometry on fluid such as blood or fresh tissue suspensions obtained from fine-needle aspirate. The first 6-8 months was spent on optimizing the technique on getting good readable tissue suspension from formalin-fixed tissue blocks. The major challenge was to develop a method on how to separate thyroid cells from mixture of blood, colloid and fibrous tissues commonly occurring in MNG. Our solution to this problem was using fresh frozen thyroidectomy specimens.

When we managed to overcome the initial difficulty, the flow-cytometry machine broke down. It took a few months for it to be repaired. The alternative was to use the machine available at Hematology Laboratory. However, since the machine was to cater clinical cases, priority were given to them. We then encountered problems concerning the availability of spare-parts to repair the broken machine. Some parts had to be ordered overseas and it took too long to arrive. Training was also a problem. Experts to this field were from Singapore. Because of SARS outbreak, travel was discouraged and the consultants refused to come to provide the training at that time thus delaying the flow cytometry optimization, affecting the project progress. The postgraduate student who was doing the project had personal problem when her child was admitted for chronic health problem which eventually was the main reason why she withdraw from her studies despite completing the project].

Objectives:

1. To establish solid tumor preparation for FCM
2. To compare DNA flow cytometric analysis and conventional histopathology in Multinodular goiter.
3. To utilize DNA flow cytometric analysis in determining malignant transformation of multinodular goiter.

Methodology: (see flowchart)

Material:

Fresh thyroid samples were obtained from surgery department HUSM. Samples were divided into two parts. Half of the tissue was fixed in formalin as routine (current standard procedure) as control/comparison. The second half of the tissue was subjected to this study

1) Mechanical disaggregation

Tissue samples were minced with a sterile blade, and then put into tissue disaggregate machine to prepare single cell suspension.

2) Filtration

Samples were filtered with 35µm pore size filter. The filtrate was centrifuged to obtain cell pellet.

3) DNA labelling

Pellet was tagged with propidium iodide to determine DNA ploidy.

4) Data Analysis

The tagged cells was analysed with flow cytometer

5) Result comparison

The results obtained are compared with those obtained by routine microscopy.

C. The results/ finding

• **Objectives Achieved**

1. To establish solid tumor preparation for FCM

This objective was achieved. The single cell suspension was prepared using automated tissue disaggregation was established. This single cell suspension was then analyzed using flow cytometry. We noted this method is not suitable for paraffin embedded tissue.

2. To compare FCM DNA ploidy and conventional histopathology in Multinodular goiter.

The finding shows that the DNA ploidy alone cannot be used as a tool to diagnose Multi nodular goiter. This is due to the complexity of the tissue. The DNA ploidy could only acts as an adjunct to the existing histopathological examination. Further studies using bigger sample size could be done before final conclusion could be made.

3. To utilize FCM data in determining malignant transformation of multinodular goiter.

The finding shows that the DNA ploidy alone could not be used to determine malignant transformation of multi nodular goiter. Further study should be done for other cellular components such as membrane markers, oncoprotein and growth factor to enhance the flow cytometric result so that a comprehensives result could be made to determine malignant transformation of multi nodular goiter.

Assessment of the programme/Project Schedule

- 1) Methods of optimization and troubleshooting was time-consuming
- 2) Delay in getting special training from Consultant from Singapore due to SAR outbreak
- 3) Delay in purchasing automated tissue disaggregation machine
- 4) Delay in repaired Flow cytometer due to the existing FCM was breakdown.

Assessment of Programme/Project Costs

The approved budget (RM29, 000.00) was appropriate for the entire work

List and status of equipment purchased (Status refers to the present condition of the equipment and its utilization)

- i. Medimachine (Tissue Disaggregation Machine)
- ii. Pipets

All the above equipments are functioning and in good condition as of date undersigned on this form