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NEUTROPHIL FUNCTION TEST: COMPARISON BETWEEN CONVENTIONAL NITROBLUE TETRAZOLIUM (NBT) AND DIRECT NBT ASSAYS

By

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DECLARATION

"I hereby declare that this thesis is my original work and has not been submitted previously or currently for any other degree at UiTM or any other institutions."

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ABSTRACT

Polymorphonuclear neutrophils (PMNs) are white blood cells that play an important role as the first line of immune defence against pathogens. Neutrophils activation will trigger production of oxidative burst which is mediated by NADPH oxidase. The product of oxidative burst is a part of the powerful bacteria killing system in PMNs. Neutrophils function is evaluated based on its ability to produce oxidative burst. The conventional nitroblue tetrazolium (NBT) assay is the gold standard method to evaluate neutrophils function. The NBT test is however laborious, complicated and skill-dependent. An alternative method called direct NBT assay was recently introduced. Thus, in the present study, the performance between conventional and direct NBT assays were compared. A total of 20 heparinized venous blood samples from normal individuals and one sample from chronic myeloid leukaemia (CML) patient were evaluated for neutrophil function by using both methods. There was a good correlation between conventional and direct NBT assays for percentage of NBT positive cell with PMA stimulated cells (r_s=0.97, P<0.01) and unstimulated cells (r_s=0.93, P<0.01). Hence, the results from direct NBT assay is as comparable as that of conventional NBT assay. The direct NBT assay is quicker and simpler than the conventional NBT assay because whole blood is used thus it eliminates the preparation of PMNs cell. In addition, sample volume of as small as 0.1 ml blood can be used which makes the direct NBT assay ideal for evaluation of neutrophil function in children and neonates. Therefore, the direct NBT assay may be a better choice or an alternative approach to evaluate neutrophil function as it is quicker, simpler, easier and less skill needed.

Keywords: Polymorphonuclear neutrophils, Conventional NBT assay, Direct NBT assay and oxidative burst

CHAPTER 1

INTRODUCTION

1.1 Background of study

Polymorphonuclear neutrophils (PMNs) are white blood cells that act as the first line of immune defense against pathogens such as bacteria. According to Wright et al. (2010), neutrophils constitute about 40 to 60 percent of white blood cells in the body. The response to microorganisms will trigger neutrophils adhesion, migration as well as their bactericidal functions. The process of neutrophils ingesting and killing the microorganism is defined as phagocytosis. Based on Gentle and Thompson (1990), the ingestion of microorganism by phagocytosis is continued by degranulation. Neutrophils contain primary and secondary granules that are essential for killing and degradation of microorganisms. As stated by Elbim and Lizard (2009), neutrophils activation will trigger microbicidal mechanisms which include the release of antimicrobial peptides and proteolytic enzymes from the granules. The lysosomal granules may fuse with the phagocytic vacuoles into which they empty their contents.

In addition, neutrophil activation will also trigger production of reactive oxygen species (ROSs) that is also known as oxidative burst. Oxidative burst is the production of highly reactive oxygen derivatives in certain cells such as neutrophils and macrophages. The generation of oxidative burst in PMNs is mediated by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase or oxidative burst oxidase (Elbim & Lizard, 2009). The products of oxidative burst are a part of the powerful bacteria killing system in PMNs. These toxic components are used to kill the bacteria engulfed by the neutrophils. Therefore, the deficiency of NADPH oxidase may cause impaired bactericidal activity (Mandell et al., 1970).