UNIVERSITI TEKNOLOGI MARA

PCR AMPLIFICATION OF INTERLEUKIN 2 GENE IN ALOPECIA AREATA

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Dissertation submitted in partial fulfilment of the requirements for the Bachelor of Pharmacy (Hons.)

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ACKNOWLEDGEMENTS

First and foremost, I praise God the Almighty, only by His grace and faithfulness, this thesis was completed within stipulated time.

I would like to convey my sincerest thank to Faculty of Pharmacy, Universiti Teknologi MARA (UiTM) for granting me an opportunity to conduct this study and for giving permission to use facilities available in Brain Research Laboratory.

With this opportunity, I would like to express my deepest gratitude to Dr. John Shia Kwong Siew; my supervisor, Associate Professor Dr. Hamid Fauzi, Dr. Fazleen Haslinda Mohd Hatta, Dr. Rosmadi Mohd Yusoff and Madam Siti Nooraishah Hussin for their patient, guidance, feedbacks and continuous support throughout the study.

I would also like to thank my team members; Nur Adeena Abdullah, Nurfatin Ahmad Rosdi and Radin Mardhiah Radin Abdul Rahman for their help and support in completing this study.

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ABSTRACT

Introduction. Alopecia areata or AA is a disease that targets hair follicles in the skin, causing disfiguring hair loss that is histologically marked by accumulation of infiltrating immune cells aroung the affected hair follicles. IL-2 gene has a role in regulating the survival and proliferation of T regulatory cells (Tregs). Tregs play an important role in preventing autoantigen-mediated immune response. Any perturbation in differentiation and proliferation of Tregs might impair their regulatory role which leads to an uncontrolled immune response which destroys follicular cells of hair bulbs. There is limited study on exon 2 of interleukin 2 gene of AA in Malaysia, thus leading to this study which was to design a set of primer to amplify exon 2.

Methodology. The study utilised the availability of pooled DNA sample from healthy volunteers at the facility hence, no ethic approval needed. The primer was designed using NCBI Primer BLAST. Next, the DNA was amplified and best annealing temperature was optimised or determined by gradient PCR. The amplicon was subjected for agarose gel electrophoresis. Then, the amplicon was sent for purification and DNA sequencing. The DNA sequencing result obtained was analysed for presence of SNP.

Result. The DNA concentration was 35.5µg/mL and its purity at A260/280 was 1.83. The best annealing temperature is 57.0 °C. The SNP was absent in the exon 2 of the pooled DNA sample.

Conclusion. The exon 2 of Interleukin-2 (IL-2) gene was successfully amplified using newly designed primer set.

CHAPTER ONE: INTRODUCTION

1.1 BACKGROUND OF STUDY

Hairs or pili classify as the accessory structure of the skin together with skin glands; sudoriferous and sweat glands and nails. They are develop from the embryonic epidermis. Hair follicles started to develop at about 12 weeks after fertilization. During puberty, androgens cause development of vellus hairs to form terminal follicles in the axilla and external genitalia in both sexes, and on the face, chest and extremities in men which leads to formation of a moustache, a beard, hairy arms and legs and a hairy chest (Kaufman, 2002).

Normal hair growth cycle made up of three stages namely; anagen catagen and telogen respectively. Once after each hair follicle enter telogen phase, that hair strand falls off and re-enter anagen phase once again given that there is no problem with that individual hair follicle (Amin & Sachdeva, 2013). Androgens are mediators of terminal hair growth throughout the body. Without androgens or their activity, scalp hair grows constitutively while body hair growth is inhibited (Kaufman, 2002).

According to American Skin Association, alopecia is a general medical term used to described all type of hair loss either they are localised or diffuse regardless at which part of the body this alopecia takes place. Alopecia have two major classifications; scarring and non-scarring alopecia. Alopecia areata or also known as AA is a type of non-scarring alopecia also become the common cause of non-scarring

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