GENETIC POLYMORPHISMS OF *XRCC1* ON CERVICAL CANCER SUSCEPTIBILITY RISK

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS ..... **ii**

TABLE OF CONTENTS ..... **iv**

LIST OF ABBREVIATIONS AND SYMBOLS ..... **vi**

ABSTRAK ..... **viii**

ABSTRACT ..... **x**

CHAPTER 1: INTRODUCTION **1**

1.1 Introduction 1

CHAPTER 2: OBJECTIVES OF THE STUDY **6**

2.1 General objectives 6

2.2 Specific objectives 6

CHAPTER 3: MANUSCRIPT **7**

3.1 Title page 7

3.2 Abstract 8

3.3 Introduction 10

3.4 Materials and Methods 15

3.4.1 Study participants 15

3.4.2 Genotyping of XRCC1 Arg194Trp (rs1799782) and Arg399Gln (rs25487) 16

3.4.3 Statistical analysis 17

3.5 Results 17

3.6 Discussion 19

3.7 Conclusion 23

Acknowledgement 23

3.8 References 23

3.9 Tables and figures 29

3.10 Guidelines / Instruction to authors of selected journal 39

CHAPTER 4: STUDY PROTOCOL 41

4.1 Research Proposal Submitted for Ethical Approval 41

4.2 Ethical approval letter 64

CHAPTER 5: APPENDICES 66

5.1 Elaboration of methodology 66

5.2 Additional tables and figures 75

5.3 Raw data softcopy (CD format) 78

LIST OF ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS

BER	Base excision repair
CI	Confidence Interval
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide Triphosphate
MgCl2	Magnesium Chloride
OR	Odds Ratio
PCR	Polymerase Chain Reaction
RFLP	Restriction Fragment Length Polymorphism
SD	Standard Deviation
SNP	Single Nucleotide Polymorphism
Taq	Thermophilus aquaticus
TBE	Tris-borate- Ethylenediamine tetra-acetic acid
XRCC1	X-ray cross complementing group 1
HPV	Human papillomavirus
HIV	Human immunodeficiency virus

SYMBOLS

bp	basepairs
mL	millilitre
mM	millimolar
µl	microlitre
µM	micromolar
rpm	revolutions per minute
U	units
vs	versus
°C	degree Celsius
%	percent
<	less or equal than
≥	more or equal than

ABSTRAK

GENETIK POLIMORFISME *XRCC1* TERHADAP RISIKO KERENTANAN KANSER SERVIKS

Latar belakang: Kanser serviks merupakan kanser yang paling kerap ditemui dalam kalangan wanita dan penyebab kematian daripada kanser keempat tertinggi di serata dunia. Mekanisme pembetulan DNA memainkan peranan penting dalam melindungi sel daripada kerosakan DNA dan karsinogenesis. Umum diketahui bahawa gen pembetulan DNA menjadi penentu penting dalam risiko kerentanan kanser. Genetik polimorfisme merupakan gen yang terlibat dalam pembetulan DNA yang menyumbangkan kepada peningkatan risiko terjadinya kanser serviks. X-ray repair cross-complimenting protein 1 (XRCC1) adalah sejenis protein nuklear dan juga merupakan sejenis protein kunci tanpa enzim sokongan yang terlibat dalam mekanisme pembetulan bes DNA. Variasi genetik antara individu seperti Polimorfisme Nukleotida Tunggal (SNPs) *XRCC1* Arg399Gln G>A (rs25487) dan *XRCC1* Arg194Trp C>T (rs1799782) boleh mengurangkan aktiviti pembetulan kerosakan DNA dan keadaan ini berkemungkinan akan meningkatkan risiko kerentanan kanser. Pengetahuan dan penemuan bukti saintifik mengenai kekerapan polimorfisme (SNPs) *XRCC1* Arg399Gln G>A (rs25487) dan *XRCC1* Arg194Trp C>T (rs1799782) dalam kalangan pesakit kanser serviks masih lagi terhad. Justeru, kajian penyelidikan ini dijalankan bagi memenuhi jurang kekurangan pengetahuan ini dengan menyiasat kesan polimorfisme *XRCC1* terhadap risiko kerentanan kanser serviks.

Kaedah: Seramai 133 orang pesakit kanser serviks dan 133 orang wanita yang sihat sebagai kumpulan kawalan terlibat dalam kajian ini. Penjenisan gen *XRCC1* Arg399Gln G>A (rs25487) dan *XRCC1* Arg194Trp C>T (rs1799782) dilakukan menggunakan teknik Tindak Balas Berantai Polimerase - Polimorfisme Pemotongan Panjang Cebisan (PCR-RFLP). Kemudian, corak genotip dikategorikan kepada homozigot *wildtype*, heterozigot

dan homozigot varian. Frekuensi genotip dan alel kedua-dua polimorfisme *XRCC1* telah dihitung dan dibandingkan antara pesakit kanser serviks dengan wanita sihat kumpulan kawalan menggunakan ujian khi kuasa dua. Seterusnya, analisis regresi logistik yang menerbitkan nisbah ganjil (OR) dengan selang keyakinan (CI) 95% telah dilakukan untuk menilai risiko kerentanan kanser serviks.

Keputusan: Keputusan kajian kami menunjukkan hubung kait yang signifikan antara polimorfisme *XRCC1* Arg399Gln G>A (rs25487) dengan tahap risiko kerentanan kanser serviks. Genotip heterozigot (GA) bagi *XRCC1* Arg399Gln G>A (rs25487) menunjukkan tahap risiko kerentanan kanser serviks yang lebih tinggi dengan nilai nisbah kemungkinan OR: 2.325, 95% selang keyakinan CI: (1.380-3.918) dan nilai p sebanyak 0.002. Namun, tiada hubung kait yang signifikan yang didapati bagi polimorfisme *XRCC1* Arg194Trp C>T (rs1799782) dengan risiko kerentanan kanser serviks.

Kesimpulan: Penemuan kajian kami menunjukkan hubung kait antara polimorfisme genetik dalam salah satu laluan gen pembetulan DNA (BER) *XRCC1* Arg399Gln G>A (rs25487) dengan risiko kerentanan kanser serviks. Hubung kait yang positif daripada kajian ini dapat dipertimbangkan untuk diaplikasi sebagai kaedah penyaringan bagi pengesanan awal pesakit kanser serviks pada masa hadapan. Namun, tiada hubung kait yang signifikan yang didapati bagi polimorfisme *XRCC1* Arg194Trp C>T (rs1799782) dengan risiko kerentanan kanser serviks. Walaupun kajian prospektif dengan jumlah populasi pesakit yang lebih besar dan penglibatan pelbagai SNP dan gen wajar bagi pengesahsahihan penemuan kajian ini, penemuan kami dapat menyediakan data awal tempatan dan peluang untuk kajian pada masa hadapan.

Kata kunci: Kanser serviks, polimorfisme gen *XRCC1*, risiko kerentanan

ABSTRACT

GENETIC POLYMORPHISM OF *XRCC1* ON CERVICAL CANCER SUSCEPTIBILITY RISK

Background: Cervical cancer was ranked fourth as the most frequently diagnosed cancer worldwide and the fourth leading cause of cancer mortality among women. DNA repair mechanism plays a major role in protecting cells against DNA damage and carcinogenesis. It is well known that the DNA repair gene has become an important determinant of cancer risk. Genetic polymorphisms among the genes which are involved in DNA damage response may contribute to an augmented risk of cancer development including cervical cancer. X-ray repair cross-complimenting protein 1 (XRCC1) is a nuclear protein that is one of the key nonenzymatic scaffolding proteins involved in base excision repair (BER). Inter-individual genetic variations such as single nucleotide polymorphisms (SNPs) of *XRCC1* Arg399Gln G>A (rs25487) and *XRCC1* Arg194Trp C>T (rs1799782) may reduce the *XRCC1* activity in repairing DNA damage and thus may increase the cancer risk predisposition. Knowledge and scientific evidence on the frequency of *XRCC1* Arg399Gln G>A (rs25487) and *XRCC1* Arg194Trp C>T (rs1799782) among cervical cancer patients is considerably limited. Therefore, this research was conducted to fill this knowledge gap by investigating the impact of *XRCC1* polymorphisms on cervical cancer susceptibility risk.

Methods: In this study, 133 cervical cancer patients and 133 healthy female control individuals were enrolled. The genotyping of *XRCC1* Arg399Gln G>A (rs25487) and *XRCC1* Arg194Trp C>T (rs1799782) was performed by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Next, the genotype patterns were categorized into homozygous wildtype, heterozygous and homozygous variant. The genotype and allele frequencies of both *XRCC1* polymorphisms were calculated and

compared between cervical cancer patients and healthy individual group using chi-square test. Next, logistic regression analysis deriving Odds Ratio (OR) with 95% CI was performed to elucidate the cervical cancer susceptibility risk.

Results: We found a significant association of genetic polymorphism in *XRCC1* Arg399Gln G>A (rs25487) with cervical cancer susceptibility risk. The heterozygous (GA) genotype of *XRCC1* Arg399Gln G>A (rs25487) showed significantly higher risk for cervical cancer susceptibility with OR: 2.325, 95% CI: (1.380-3.918) and p-value of 0.002. However no significant association was observed for *XRCC1* Arg194Trp C>T (rs1799782) with cervical cancer susceptibility risk.

Conclusion Our study demonstrated an association between genetic polymorphisms in one of DNA repair pathway (BER) gene *XRCC1* Arg399Gln G>A (rs25487) with susceptibility risk of cervical cancer patients. The positive association from this research study may be considered to be applied as a screening tool for early detection in cervical cancer patient in the future. However no significant association was observed for *XRCC1* Arg194Trp C>T (rs1799782) with cervical cancer susceptibility risk. Although a prospective study with a larger patient population and involvement of multiple SNPs and genes necessary to validate this findings, our findings provides preliminary data locally and opportunity for future study.

Keywords: Cervical cancer, XRCC1 gene polymorphism, susceptibility risk

CHAPTER 1: INTRODUCTION

1.1 Introduction

In this modern era, cervical cancer is still one of the major public health issues commonly affecting middle-aged women especially in low- and middle-income countries according to Global Cancer Statistics 2020 (Sung *et al.*, 2021). Cervical cancer was ranked fourth as the most frequently diagnosed cancer and the fourth leading cause of cancer mortality among women with an estimation of 604,000 new cases and 342,000 deaths worldwide (Sung *et al.*, 2021). According to the latest Malaysian National Cancer Registry 2012 – 2016, cervical cancer ranked as the third most common cancer and accounted for 6.2% of all cancers among females after breast and colorectal cancer which was ranked in the first and second position respectively (Manan *et al.*, 2019).

It is now a well-established fact that high-risk Human Papillomavirus (HPV) is responsible for invasive cervical cancer and its precursor form, cervical intraepithelial neoplasia (CIN) (Chen *et al.*, 2020; Wang *et al.*, 2020). HPV is a form of non-enveloped double-stranded DNA virus with approximately more than 100 genotypes. There are about 30-40 HPV types that infect the anogenital tract and among these, 13 are considered high-risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and 5 likely high-risk (53, 66, 70, 73 MM9, and 82 MM4) HPV types (Bhatla and Singhal, 2020). The most aggressive and likely to persist types are HPV 16/18 in which they integrate the host genome and causing cervical neoplasia (Bhatla and Singhal, 2020).

However, HPV alone is not sufficient in contributing to cervical cancer tumorigenesis. This is due to the fact that not all women infected with HPV will definitely progress to cervical cancer since most of the viral infections are self-limited and clears up in a couple of months and only a small proportion of women will exhibit persistent infection which later develops cervical cancer (Charles *et al.*, 2020; Chen *et al.*, 2020;

Wang *et al.*, 2020). Thus, HPV is not the sole determinant of cervical cancer development and other cofactors such as host genetic factor plays a great role in contributing to the progression of CIN into a malignant state. Several environmental and lifestyle factors were recognized as part of the factors associated with cervical cancer development such as cigarette smoking, early and multiple childbirth, multiple sexual partners, immune suppression, consumption of oral contraceptives, and low socio-economic status.

Although significant efforts have been addressed on the role of HPV and other environmental factors in cervical cancer tumorigenesis, the role of host genetic factor in the aetiopathogenesis of cervical cancer is being recognized as an important aspect and allows us to understand better the disease as well as provide potential treatment approach to the patient. DNA molecules (genomic and mitochondrial) are continuously exposed to the damaging activity by various exogenous and endogenous genotoxicants. As part of the natural defence system, DNA damage response functionally act to protect the human genome against various sources of damage (such as DNA lesions, mutations, DNA strand breaks, inter-strand, and DNA-protein links) and this mechanism allows sustaining genomic stability and indirect chromosome maintenance (Kiwerska and Szyfter, 2019). DNA repair mechanisms are a complex process and involves numerous molecules and proteins. Maintaining the integrity of the genome is an extremely important in general and specialized functions of the cells as well as the prevention of the carcinogenesis. DNA repair efficacy in individuals varies in the human population due to the consequence of DNA repair genes polymorphism. This genetic variations in DNA repair genes can affect their efficiency and increase the risk of developing cancer (Abbas *et al.*, 2019).

Generally, there are more than a hundred of molecules implicated in DNA repair pathways in human cells such as base excision repair (BER), nucleotide excision repair (NER), double-strand break repair (DSBR) and DNA mismatch repair (DMR). BER is the major pathway tasked with the removal of oxidative DNA damage because of continuous assault from reactive oxygen species, and, hence maintaining the genomic integrity.

Single nucleotide polymorphisms (SNPs) which are substitutions of single nucleotides along the DNA, are the most common forms of genetic variations associated with susceptibility to diseases. SNPs represent a potentially vast area for the detection of genetic alterations that can be related to different types of diseases across different populations such as various cancers including cervical cancer (Charles *et al.*, 2020). Genetic polymorphisms among the genes which are involved in DNA damage response may contribute to an augmented risk of cancer development (Datkhile *et al.*, 2018). Besides, an individual's DNA repair capacity is genetically determined and results from a combination of multiple genes that display subtle differences in their activity (Colacino-Silva *et al.*, 2017). SNPs may also causing subtle structural alteration in repair enzymes and later modulate the cancer susceptibility (Colacino-Silva *et al.*, 2017).

X-ray repair cross-complimenting protein 1 (XRCC1) is a nuclear protein that is one of the key nonenzymatic scaffolding proteins involved in BER (Whitaker *et al.*, 2017). This nuclear protein was expressed from the *XRCC1* which is located on chromosome 19 (19p13.2). XRCC1 is exclusively required for DNA base excision and strand break repair and eventually maintains genomic integrity (Cai *et al.*, 2017; Yang *et al.*, 2017). *XRCC1* polymorphism may influence the interaction of other enzyme proteins in DNA repair with XRCC1 and results in altered efficiency of the protein which eventually induces the development of cancer (Cai *et al.*, 2017). In addition, XRCC1 is

linked with a scaffolding protein that is directly associated with other proteins such as DNA polymerase β, PARP (ADP-ribose polymerase) and DNA ligase III, in a complex process of BER DNA repair (Colacino-Silva *et al.*, 2017).

In recent past decades, various epidemiological studies proposed the association of the polymorphisms in *XRCC1* with various type of cancers such as lung, gastric, breast, prostate, colorectal, pancreatic, and head and neck (Wei *et al.*, 2011; Dai *et al.*, 2012; Nissar *et al.*, 2015; Datkhile *et al.*, 2016; Hou *et al.*, 2016). According to Zhang and Li, (2021), there has been couple of reports on the relationship between *XRCC1* polymorphism and susceptibility to gynaecological malignancy but many of the findings revealed negative reports. Furthermore, there were several studies have been conducted to evaluate the role of *XRCC1* polymorphisms on cervical cancer risk but the results were inconclusive as well as inconsistent across different populations and ethnicities (Zeng *et al.*, 2017).

Over the years, studies revealed a list of validated SNPs that had been identified and described in the *XRCC1* associated with various conditions such as various cancers as well as non-malignant conditions. Apart from involvement in cancer, polymorphism of XRCC1 were also studied in other non-malignancy condition such as in autoimmune disease specifically systemic lupus erythematosus (SLE) (Mortada *et al.*, 2020), in chronic infectious disorder such as bacterial meningitis (Lopes Pinheiro *et al.*, 2015), hepatitis (Naguib *et al.*, 2020), and also HIV (Liu *et al.*, 2022).

Two common SNPs that have been frequently studied are *XRCC1* Arg194Trp C>T (rs1799782) and *XRCC1* Arg399Gln G>A (rs25487). The transition of nucleotide C>T changes the amino acid arginine to tryptophan at position 26304 in exon 6 of *XRCC* Arg194Trp (rs1799782), while in *XRCC1* Arg399Gln G>A (rs25487) the transition of nucleotide is G>A and changes the amino acid arginine to glutamine at position 28152 in

exon 10 of *XRCC1* (Liu., 2015). There were a few studies that found a significant association between *XRCC1* Arg399Gln polymorphism and the risk of cervical carcinoma (Shuai *et al.*, 2012; Mei *et al.*, 2014; Liu, Liang and Xiao, 2015). Besides, *XRCC1* Arg194Trp polymorphism also demonstrated a significant association with cervical cancer risk in a few previous studies conducted (Ndiaye *et al.*, 2020). In addition, these two SNPs were also found to be associated significantly with other types of cancer such as thyroid, skin carcinoma as well as nasopharyngeal carcinoma (Nedooshan *et al.*, 2017; Wang, Xu and Duan, 2017; Lin *et al.*, 2018).

Even though few studies have shown an association of *XRCC1* polymorphisms with cervical cancer susceptibility risk as well as in other various cancer, those studies were mostly from other countries. The effects of SNPs of other continent populations outside Asia cannot be translated directly to Asian and other populations, and vice versa. There is a considerably low number of studies reported on the distribution of *XRCC1* polymorphisms in the cervical cancer population and to the best available knowledge, there are no reports available on the impact of genetic variations of *XRCC1* on susceptibility risk in Malaysian cervical cancer patients. Malaysia is a known country of multi-ethnicity and consists of a diverse population background thus, it is crucial to have our local study on this particular aspect. Therefore, it is appropriate to address the demand to investigate the host genetic predisposition factors associated with cervical cancer susceptibility risk among the local population. Hence, this study was carried out to investigate the role of SNPs of *XRCC1* Arg399Gln G>A (rs25487) and *XRCC1* Arg194Trp C>T (rs1799782) in modulating cervical cancer susceptibility risk.

CHAPTER 2: OBJECTIVES OF THE STUDY

2.1 General objectives

The main objectives of this study is to elucidate the role of SNPs of *XRCC1* Arg399Gln G>A (rs25487) and *XRCC1* Arg194Trp C>T (rs1799782) in modulating cervical cancer susceptibility risk.

2.2 Specific objectives

The specific objectives of this study are:

1. To determine the genotype and allele frequencies of *XRCC1* Arg399Gln G>A (rs25487) and *XRCC1* Arg194Trp C>T (rs1799782) polymorphisms among cervical cancer patients and healthy female normal controls.

2. To determine the association between the genotype patterns of *XRCC1* Arg399Gln G>A (rs25487) and *XRCC1* Arg194Trp C>T (rs1799782) polymorphisms and cervical cancer susceptibility risk.

CHAPTER 3: MANUSCRIPT

3.1 Title page

Genetic polymorphisms of *XRCC1* on cervical cancer susceptibility risk.

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3.2 Abstract

Background: Cervical cancer was ranked fourth as the most frequently diagnosed cancer worldwide and the fourth leading cause of cancer mortality among women. DNA repair mechanism plays a major role in protecting cells against DNA damage and carcinogenesis. It is well known that the DNA repair gene has become an important determinant of cancer risk. Genetic polymorphisms among the genes which are involved in DNA damage response may contribute to an augmented risk of cancer development including cervical cancer. X-ray repair cross-complimenting protein 1 (XRCC1) is a nuclear protein that is one of the key nonenzymatic scaffolding proteins involved in base excision repair (BER). Inter-individual genetic variations such as single nucleotide polymorphisms (SNPs) of *XRCC1* Arg399Gln G>A (rs25487) and *XRCC1* Arg194Trp C>T (rs1799782) may reduce the *XRCC1* activity in repairing DNA damage and thus may increase the cancer risk predisposition. Knowledge and scientific evidence on the frequency of *XRCC1* Arg399Gln G>A (rs25487) and *XRCC1* Arg194Trp C>T (rs1799782) among cervical cancer patients is considerably limited. Therefore, this research was conducted to fill this knowledge gap by investigating the impact of *XRCC1* polymorphisms on cervical cancer susceptibility risk.

Methods: In this study, 133 cervical cancer patients and 133 healthy female control individuals were enrolled. The genotyping of *XRCC1* Arg399Gln G>A (rs25487) and *XRCC1* Arg194Trp C>T (rs1799782) was performed by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Next, the genotype patterns were categorized into homozygous wildtype, heterozygous and homozygous variant. The genotype and allele frequencies of both *XRCC1* polymorphisms were calculated and compared between cervical cancer patients and healthy individual group using chi-square

test. Next, logistic regression analysis deriving Odds Ratio (OR) with 95% CI was performed to elucidate the cervical cancer susceptibility risk.

Results: We found a significant association of genetic polymorphism in *XRCC1* Arg399Gln G>A (rs25487) with cervical cancer susceptibility risk. The heterozygous (GA) genotype of *XRCC1* Arg399Gln G>A (rs25487) showed significantly higher risk for cervical cancer susceptibility with OR: 2.325, 95% CI: (1.380-3.918) and p-value of 0.002. However no significant association was observed for *XRCC1* Arg194Trp C>T (rs1799782) with cervical cancer susceptibility risk.

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3.3 Introduction

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and other cofactors such as host genetic factor plays a great role in contributing to the progression of CIN into a malignant state. Several environmental and lifestyle factors were recognized as part of the factors associated with cervical cancer development such as cigarette smoking, early and multiple childbirth, multiple sexual partners, immune suppression, consumption of oral contraceptives, and low socio-economic status.

Although significant efforts have been addressed on the role of HPV and other environmental factors in cervical cancer tumorigenesis, the role of host genetic susceptibility is one of the aspects that are currently gaining more insights although its exact mechanism of pathogenicity is not fully understood. Oxidative damage due to the environmental exposure (i.e., physical, and chemical agents) predispose to the oxidative DNA damage in human body and the continuous cellular assaults making the cells prone for the neoplastic transformation. To combat this, DNA repair mechanism is critical in repairing as well as correcting the damages caused by either endogenous or exogenous factors. If left untreated, DNA damage can result in cellular disruption and apoptosis, unregulated cell growth and proliferation as well as further damage leading to development of cancer as described by Hanahan and Weinberg, (2011) in hallmarks of cancer.

Generally, there are more than a hundred of molecules implicated in DNA repair pathways in human cells such as base excision repair (BER), nucleotide excision repair (NER), double-strand break repair (DSBR) and DNA mismatch repair (DMR). BER is the major pathway tasked the with removal of oxidative DNA damage because of continuous assault from reactive oxygen species, and, hence maintaining the genomic integrity.

Single nucleotide polymorphisms (SNPs) which are substitutions of single nucleotides along the DNA, are the most common forms of genetic variations associated with susceptibility to diseases. SNPs represent a potentially vast area for the detection of genetic alterations that can be related to different types of diseases across different populations such as various cancers including cervical cancer (Charles *et al.*, 2020). Genetic polymorphisms among the genes which are involved in DNA damage response may contribute to an augmented risk of cancer development (Datkhile *et al.*, 2018). Besides, an individual's DNA repair capacity is genetically determined and results from a combination of multiple genes that display subtle differences in their activity (Colacino-Silva *et al.*, 2017). SNPs may also causing subtle structural alteration in repair enzymes and later modulate the cancer susceptibility (Colacino-Silva *et al.*, 2017).

X-ray repair cross-complimenting protein 1 (XRCC1) is a nuclear protein that is one of the key nonenzymatic scaffolding proteins involved in BER (Whitaker *et al.*, 2017). This nuclear protein was expressed from the *XRCC1* which is located on chromosome 19 (19p13.2). XRCC1 is exclusively required for DNA base excision and strand break repair and eventually maintains genomic integrity (Cai *et al.*, 2017; Yang *et al.*, 2017). *XRCC1* polymorphism may influence the interaction of other enzyme proteins in DNA repair with XRCC1 and results in altered efficiency of the protein which eventually induces the development of cancer (Cai *et al.*, 2017). In addition, XRCC1 is linked with a scaffolding protein that is directly associated with other proteins such as DNA polymerase β, PARP (ADP-ribose polymerase) and DNA ligase III, in a complex process of BER DNA repair (Colacino-Silva *et al.*, 2017).

In recent past decades, various epidemiological studies proposed the association of the polymorphisms in *XRCC1* with various type of cancers such as lung, gastric, breast, prostate, colorectal, pancreatic, and head and neck (Wei *et al.*, 2011; Dai *et al.*, 2012;

Nissar *et al.*, 2015; Datkhile *et al.*, 2016; Hou *et al.*, 2016). According to Zhang and Li, (2021), there has been couple of reports on the relationship between *XRCC1* polymorphism and susceptibility to gynaecological malignancy but many of the findings revealed negative reports. Furthermore, there were several studies have been conducted to evaluate the role of *XRCC1* polymorphisms on cervical cancer risk but the results were inconclusive as well as inconsistent across different populations and ethnicities (Zeng *et al.*, 2017).

Over the years, studies revealed a list of validated SNPs that had been identified and described in the *XRCC1* associated with various conditions such as various cancers as well as non-malignant conditions. Apart from involvement in cancer, polymorphism of XRCC1 were also studied in other non-malignancy condition such as in autoimmune disease specifically systemic lupus erythematosus (SLE) (Mortada *et al.*, 2020), in chronic infectious disorder such as bacterial meningitis (Lopes Pinheiro *et al.*, 2015), hepatitis (Naguib *et al.*, 2020), and also HIV (Liu *et al.*, 2022).

Two common SNPs that have been frequently studied are *XRCC1* Arg194Trp C>T (rs1799782) and *XRCC1* Arg399Gln G>A (rs25487). The transition of nucleotide C>T changes the amino acid arginine to tryptophan at position 26304 in exon 6 of *XRCC* Arg194Trp (rs1799782), while in *XRCC1* Arg399Gln G>A (rs25487) the transition of nucleotide is G>A and changes the amino acid arginine to glutamine at position 28152 in exon 10 of *XRCC1* (Liu., 2015). There were a few studies that found a significant association between *XRCC1* Arg399Gln polymorphism and the risk of cervical carcinoma (Shuai *et al.*, 2012; Mei *et al.*, 2014; Liu, Liang and Xiao, 2015). Besides, *XRCC1* Arg194Trp polymorphism also demonstrated a significant association with cervical cancer risk in a few previous studies conducted (Ndiaye *et al.*, 2020). In addition, these two SNPs were also found to be associated significantly with other types of cancer such

as thyroid, skin carcinoma as well as nasopharyngeal carcinoma (Nedooshan *et al.*, 2017; Wang, Xu and Duan, 2017; Lin *et al.*, 2018).

Even though few studies have shown an association of *XRCC1* polymorphisms with cervical cancer susceptibility risk as well as in other various cancer, those studies were mostly from other countries. The effects of SNPs of other continent populations outside Asia cannot be translated directly to Asian and other populations, and vice versa. There is a considerably low number of studies reported on the distribution of *XRCC1* polymorphisms in the cervical cancer population and to the best available knowledge, there are no reports available on the impact of genetic variations of *XRCC1* on susceptibility risk in Malaysian cervical cancer patients. Malaysia is a known country of multi-ethnicity and consists of a diverse population background thus, it is crucial to have our local study on this particular aspect. Therefore, it is appropriate to address the demand to investigate the host genetic predisposition factors associated with cervical cancer susceptibility risk among the local population. Hence, this study was carried out to investigate the role of SNPs of *XRCC1* Arg399Gln G>A (rs25487) and *XRCC1* Arg194Trp C>T (rs1799782) in modulating cervical cancer susceptibility risk.

3.4 Materials and Methods

3.4.1 Study participants

This study was performed using archived DNA samples which had been previously extracted from peripheral blood samples of previous research study. The original research entitled "Roles of selected genetic variations and molecular alterations in Human Papillomavirus mediated cancer of uterine cervix" was carried out starting in August 2012 and completed in January 2016. The research study had received ethical approval from the Human Research Ethics Committee (HREC) of Universiti Sains Malaysia (reference numbers: USMKK/PPP/JEPeM [253.3.(7)] and USM/JEPeM/14100325) as well as the Medical Research and Ethics Committee (MREC) of Ministry of Health, Malaysia (reference numbers: KKM/ NIHSEC/08/0804/P12-380, and KKM/NIHSEC/P15-1214). The study subjects were previously recruited from (i) Hospital Universiti Sains Malaysia, Kubang Kerian, Kelantan, (ii) Hospital Raja Perempuan Zainab II, Kota Bharu, Kelantan, and (iii) Hospital Sultan Ismail, Johor Bahru, Johor between August 2012 and January 2016.

The present study received ethical approval obtained from the Human Research Ethics Committee of USM (USM/JEPeM/21120779) which complies with the Declaration of Helsinki. A total number of samples used in this study were 133 cervical cancer patients and 133 normal female control. The selection criteria for cervical cancer cases were clinically and histopathologically confirmed cases of cervical cancer female and their age ranges between 18 to 70 years old. Subjects who have had a personal history of previous or concurrent malignancy, subjects diagnosed with metastatic cancer to the cervix, subjects who have prior treatment before undergoing surgery and those who were found to have a history of autoimmune or chronic infectious disease were excluded from this study. For the normal healthy female control group, the selection criteria were cancer-

free healthy female volunteer who was age-matched to the case group, biologically unrelated to the case, and age range between 18 to 70 years old. Individuals with a family history of cervical cancer and those who were found to have a history of autoimmune or chronic infectious disease were excluded.

3.4.2 Genotyping of *XRCC1* Arg194Trp (rs1799782) and Arg399Gln (rs25487)

The DNA samples were previously extracted from a peripheral blood samples of the study subjects using commercial QIAamp DNA Mini Kit (QIAGEN). The genotyping of both SNPs *XRCC1* Arg194Trp (rs1799782) and Arg399Gln (rs25487) was performed by using PCR – restriction fragment length polymorphism (RFLP) technique. PCR reaction was performed in 20µl of the final volume containing 0.2µM of each forward and reverse primer, 50ng extracted genomic DNA, 2mM $MgCl_2$, 0.2mM dNTPs, 1.0 unit of Taq DNA polymerase and 1X of 5X Promega Green GoTaq™ Flexi Buffer. The PCR master mix for both SNPs was similar. Amplification was performed in Mastercycle thermocycler for the PCR reaction. The PCR condition started with an initial denaturation at 95°C for two minutes, followed by 30 cycles of denaturation at 95°C for 45 seconds, annealing at 58°C for 45 seconds (for *XRCC1* Arg194Trp rs1799782) and 54°C for 45 seconds (for *XRCC1* Arg399Gln rs25487), and extension at 72°C for 45 seconds, then a final extension at 72°C for five minutes. Next, PCR products were incubated for fast digestion for one hour at 37°C with the restriction enzyme *Pvull* (Fermentas, Vilnius, Lithuania) (for *XRCC1* Arg194Trp rs1799782) and restriction enzyme *Mspl* (Fermentas, Vilnius, Lithuania) (for *XRCC1* Arg399Gln rs25487) according to the manufacturer's instructions, and subsequently analyzed by electrophoresis on a 2% agarose gel. The restriction enzyme and the length of digested fragments for both SNPs are shown in Table I and II. After genotyping, the genotypes were categorized into three groups: homozygous wild type (major), heterozygous and homozygous variant (minor).

3.4.3 Statistical analysis

Statistical analysis was performed by using SPSS version 27.0 for statistical calculation. The genotype and allele frequencies for each SNPs of *XRCC1* were compared among cervical cancer patients and normal healthy controls using Pearson's chi-square (X^2) test or Fisher exact test. Next, the association of genotype and allele frequencies of *XRCC1* polymorphism (either singly and in combination) between cervical cancer patients and normal healthy controls were evaluated using logistic regression analysis by deriving odds ratio (ORs) and 95% confidence interval (CI) using unconditional logistic regression analysis. For all analyses, $p<0.05$ was considered as statistically significant.

3.5 Results

3.5.1 Genotype and allele frequencies of *XRCC1* Arg399Gln G>A (rs25487) in cervical cancer patients and controls

The genotype and allele frequencies of *XRCC1* Arg399Gln G>A (rs25487) in cervical cancer patients and controls are shown in Table III. Among the 133 cervical cancer patients, 66 (49.6%) showed heterozygous, 54 (40.6%) showed homozygous wild-type and 13 (9.8%) showed homozygous variant genotypes. In controls, the genotype frequencies were 78 (58.7%) for homozygous wild-type, 41 (30.8%) for heterozygous and 14 (10.5%) for homozygous variant. The heterozygous (GA) genotype was significantly higher in cervical cancer patients as compared to the controls. On the contrary, the homozygous wild-type (GG) was significantly higher in controls compared to the cervical cancer patients. The frequencies of G allele and A allele were 65.4% and 34.6% in cervical cancer patients and 74.1% and 25.9% in controls, respectively. Allele A was found to be significantly higher in cervical cancer patients (34.6%) compared to controls (25.9%) with a p-value <0.05.

3.5.2 Genotype and allele frequencies of *XRCC1* Arg194Trp C>T (rs1799782) in cervical cancer patients and controls

The genotype and allele frequencies of *XRCC1* Arg194Trp C>T (rs1799782) in cervical cancer patients and controls are shown in Table IV. Out of the 133 cervical cancer patients, 73 (54.9%) showed homozygous wild-type, 54 (40.6%) showed heterozygous and 6 (4.5%) showed homozygous variant genotypes. In controls, the genotype frequencies were 68 (51.1%) for homozygous wild-type, 54 (40.6%) for heterozygous and 11 (8.3%) for homozygous variant. In cervical cancer patients, 75.2% showed C allele and 24.8% showed T allele, whereas in controls, 71.4% showed C allele and 28.6% showed T allele. No significant difference in the frequencies of these genotypes and alleles were found between cervical cancer patients and controls.

3.5.3 Risk association of *XRCC1* Arg399Gln G>A (rs25487) with cervical cancer susceptibility

Table V showed the associated risk of *XRCC1* Arg399Gln G>A (rs25487) polymorphism with cervical cancer susceptibility. The heterozygous (GA) genotype showed significantly higher risk for cervical cancer susceptibility with OR: 2.325, 95% CI: **(**1.380-3.918) and p-value of 0.002. The homozygous variant (AA) showed higher risk values with OR: 1.341, 95% CI: 0.584-3.079, but were not statistically significant with p-value of 0.489.

3.5.4 Risk association of *XRCC1* Arg194Trp C>T (rs1799782) with cervical cancer susceptibility

Table VI shows the associated risk of *XRCC1* Arg194Trp C>T (rs1799782) polymorphism with cervical cancer susceptibility. No significant risk association was

found between the heterozygous and homozygous variant genotypes with cervical cancer susceptibility.

3.6 Discussion

Study on the *XRCC1* polymorphisms has become an area of interest for intensive research as the resultant functional alterations that impair the DNA damage cellular repair mechanism and genome stability, impose their possible role in cancer susceptibility risk. There are numerous studies done to investigate the role of *XRCC1* polymorphism in cancer development including cervical cancer. However, most of the study pertaining to *XRCC1* polymorphism and cervical cancer were conducted outside Malaysia. To the best of our available knowledge, there are no available reports on the association of *XRCC1* polymorphism with cervical cancer in Malaysia.

In our study, we investigated two commonest SNPs of XRCC1 (*XRCC1* Arg399Gln G>A (rs25487) and *XRCC1* Arg194Trp C>T (rs1799782) and their association with cervical cancer susceptibility risk. We found a significant association of genetic polymorphism in *XRCC1* Arg399Gln G>A (rs25487) with cervical cancer susceptibility risk. Our study involved 133 cervical cancer patients and 133 healthy female control individuals revealed that carriers of heterozygous (GA) genotype of *XRCC1* Arg399Gln G>A (rs25487) showed significantly higher risk for cervical cancer susceptibility. Our findings on *XRCC1* Arg399Gln G>A (rs25487) association with cervical cancer risk are in agreement with other previous studies.

A meta-analysis by Liu *et al.*, (2015) found a significant association between XRCC1 Arg399Gln polymorphism and the risk of cervical carcinoma risk in both Caucasians and Asians. Study by Roszak *et al.*, (2011) on *XRCC1* Arg399Gln polymorphism by PCR-RFLP in 189 patients with advanced cervical cancer and 308

controls. Their study revealed that patients with advanced cervical cancer having the Gln/Gln or Gln/Arg vs Arg/Arg genotype displayed a 1.726-fold increased risk of cervical cancer (95% confidence interval [CI]=1.158-2.572, p=0.007). The odds ratio for Gln/Gln vs Gln/Arg or Arg/Arg was 1.742 (95% CI=1.073-2.827; p=0.0236). They also discovered a significantly higher frequency of the *XRCC1* Arg399Gln allele in patients with cervical cancer than in controls, with OR=1.489 (95% CI=1.148-1.930, p=0.0026). Other studies also demonstrated a strong association of the *XRCC1* Arg399Gln polymorphism with an increased risk of cervical cancer (Shuai *et al.*, 2012; Mei *et al.*, 2014). In addition, study by Datkhile *et al.*, (2018) also in agreement with other findings where he found out *XRCC1* Arg399Gln were significantly increased in relation to the relative risk of cervical cancer (OR= 2.99; 95% CI= 1.60-5.56). Study by Al-Harbi *et al.*, (2017) also revealed patients harbouring variant allele *XRCC1* Arg399Gln have approximately 1.5-fold increased risk to develop cervical cancer.

On the other hand, our study observed no statistical association for *XRCC1* Arg194Trp C>T (rs1799782) with cervical cancer susceptibility risk. However, in other studies *XRCC1* Arg194Trp has been showed to be significantly associated with cervical cancer risk. A meta-analysis by Li *et al.*, (2012) suggested *XRCC1* Arg194Trp was associated with significant cervical cancer risk (Trp/Trp vs Arg/Arg, OR = 2.21, 95% CI = 1.60–3.06; Arg/Trp vs Arg/Arg, OR = 1.23, 95% CI = 1.02–1.49; dominant model, OR = 1.36, 95% CI = 1.14–1.63; recessive model, OR = 2.06, 95% CI = 1.51–2.82). Besides, Ndiaye *et al.*, (2020) found a significant association between *XRCC1* Arg194Trp and cervical cancer (OR= 2.696; 95% CI= 1.181-6.154; p= 0.018, using an additive model), (OR=2.989; 95% CI= 1.078-8.283; p= 0.035, using a dominant model). A few other studies also discovered a positive association of the *XRCC1* Arg194Trp with cervical cancer development risk (Shuai *et al.*, 2012; Mei *et al.*, 2014)

Genetic polymorphism of *XRCC1* was also shown to be associated with various other cancers as well. A study by Nedooshan *et al.*, (2017) found a statistical association between *XRCC1* Arg194Trp and thyroid cancer risk with the homozygote genetic model (TT vs. CC: OR = 1.815, 95% CI = 1.115-2.953, p= 0.016) and the recessive genetic model (TT vs. TC+CC: OR = 1.854, 95% CI = 1.433-2.399, p= <0.001). He also found a significant association between *XRCC1* Arg399Gln polymorphism risk of thyroid cancer among Caucasians with allele genetic comparison (A vs. G: OR= 0.882, 95% CI = 0.794-0.979, p= 0.136) and dominant genetic comparison (AA+AG vs. GG: OR=0.838, 95% CI = 0.728-0.965, p= 0.014). In addition, Wang, Xu and Duan, (2017) suggested that the *XRCC1* Arg399Gln might be a risk factor for non-melanoma skin cancer in Asian populations, and the *XRCC1* Arg194Trp might be a protective factor for patients with squamous-cell skin cancer cases. Besides, a meta-analysis by Lin *et al.*, (2018) *XRCC1* Arg399Gln is a potential predictor for susceptibility to nasopharyngeal carcinoma, especially for Asians**.**

Although our study shows a significant association of one of the SNP studied *XRCC1* Arg399Gln G>A (rs25487) with cervical cancer risk, there are a few limitations that needs to be highlighted. Firstly, our study examined the association of only two SNPs of *XRCC1* with cervical cancer risk. Other SNPs or genes that are involved in all pathways of DNA repair were clearly not covered and this can be considered for the future study. DNA repair pathway mechanism is a very complex process and the molecular target focus for research study is massive and obviously our findings does not conclusive and representative of all. To get better clearer and broader picture, future research need to incorporate multiple genes studies involved in DNA repair pathway and its association in cervical cancer development. With the advent of massively parallel sequencing technologies such as whole genome sequencing or whole exome sequencing, this research

question has a huge potential to be answered and the application in the research need to be considered. In addition, we acknowledge the limitation of the time for the research conducted, research budget as well as the limited research resources and skills. In addition, this study was conducted using achieved DNA samples that was extracted from peripheral blood of the previous researcher. Thus, there are limitations in getting the details of clinicopathological data that was conducted earlier due to missing data. Because of this limitation, we are not able to discuss further details on the other aspect particularly related to demographic background such as ethnic background, age, and other socio-demographic and salient clinical information of each participants.

The advances in SNP mapping utilizing high throughput DNA sequencing could facilitate the analysis of variants in DNA repair pathway. This may contribute to the advancement of knowledge on genetic predisposing factors related to cervical cancer susceptibility risk in Malaysian population. This, in turn may help to identify the high risk individuals with cervical cancer susceptibility risk genotype in the population and devise appropriate preventive strategies as well as incorporate this molecular genetic approach in the patient's clinical management.

One of the significant potential outlook of the study of XRCC1 in cervical cancer is the potential implementation of this molecular biomarker for gynaecologic cancer screening. According to a meta-analysis by Zhang and Li, (2021), XRCC1 genotype detection is expected to be a molecular marker for gynaecologic cancer screening. At the moment there are not many supporting data discussing the role of XRCC1 genotype detection as part of the gynaecologic cancer screening implementation yet. Thus, by further expanding the research on this particular issue is crucial for us to be able to understand better the role of XRCC1 genotype in particular related to cervical cancer and other gynaecological malignancy in our local population. The implementation of

personalized therapy pertaining to XRCC1 in cervical cancer and other gynaecologic cancer is one of the potential that should not be overlooked. This potentially can be applied clinically in gynaecologic cancer screening, diagnosis or prognostication of the disease. We are hoping in future this approach may assist the treating physician to incorporate this genetic testing in the clinical management of the cervical cancer as well as other gynaecologic cancer.

3.7 Conclusion

Our study demonstrated an association between genetic polymorphisms in one of DNA repair pathway (BER) gene *XRCC1* Arg399Gln G>A (rs25487) with susceptibility risk of cervical cancer patients. The findings of the current research might contribute to a screening tool for early detection in cervical cancer patient in the future. However no significant association was observed for *XRCC1* Arg194Trp C>T (rs1799782) with cervical cancer susceptibility risk. Although a prospective study with a larger patient population and involvement of multiple SNPs and genes necessary to validate this findings, our findings provides preliminary data locally and opportunity for future study.

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3.8 References

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