POTENTIAL OF ANTI-TUMORIGENICITY AND ANTI-METASTASIS OF ANNONA MURICATA (SOURSOP LEAVES) ON MCF-7 AND MDA-MB231 BREAST CANCER CELLS LINE

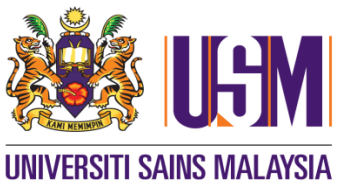
By

DR HUSNA SYAKIRAH BINTI AB RAHMAN

(MBBS IIUM)

Dissertation Submitted In Partial Fulfilment of Requirement for the Degree of Master of Medicine

(General Surgery)



2017

ACKNOWLEDGEMENT

Alhamdulillah, **all praise and thanks to Allah**, The Al-Mighty for blessing me and giving me time and strength to sustain and complete this dissertation. Its completion was supported by many important surrounding me. Special thanks to my beloved husband, **Mohamad Izham Bin Md Yusof**, the one who is non-stop giving encouragement and full support to me throughout the master program and also **my family** who always never stopped praying for my success.

I would like to express my appreciation and thank you to my **supervisor, Dr Mohd Ridzuan Abd Samad** for his advice and guidance. He never stops give encouragement to complete this dissertation. Not to forget to all **my co-supervisor, Dr Wong Pak Kai (Michael) (Department of Surgery), Dr Aidy Irman Bin Yajid (Department of Pathology) and Dr Norzila Binti Ismail (Department of Pharmacology)** for their guidance and help. Special thanks also to **Mr Imi Sairi Abd Hadi (Breast & Endocrine Consultant)**, my co-supervisor from Hospital Raja Perempuan Zainab II, person that gives me idea to do this topic of dissertation and help in providing the materials.

My special gratitude to **Dr Zaidi Zakaria, Head of Department, Department Surgery USM**, for his faith, reinforcement and continuous support in my effort to finish this dissertation.

My special thanks to **Puan Halijah, Laboratory Assistant at Pharmacology Laboratory** that helps me during preparation of extract and **Encik Jamar,**

Laboratory Assistant at Immunology laboratory who help me in managing and conducting the flow cytometer machine for flow cytometer analysis. I also want to thank **Prof Madya Dr Che Maraina, Head Department of immunology** allowed me to use flow cytometer machine. Also thanks to **Ms Mardhiah Kamaruddin, statistician USM**, for helping me in statistical analysis.

Special thank also to Research and Development Unit, Pusat Pengajian Sains Perubatan for USM Short Term Grant 2016 (Grant No: 304/CIPPT/6313322) approval that make this study become reality.

Last but not least my deepest appreciation to all my lecturers and friends that directly or indirectly involved in completion of this dissertation. Indeed only Allah will repay all your kindness to me.

May Allah bless us all.

ABBREVIATIONS

Age-Standardised Rate	ASR
Breast Cancer	BC
Dulbecco's Modified Eagle Media	DMEM
Estrogen receptor	ER
Ethyl Acetate	ETAC
Fetal Bovine Serum	FBS
GasChromatography-Mass Spectometry	GC-MS
International Agency for Research in Cancer	GLOBOCAN
Michigan Cancer Foundation-7	MCF-7
National Cancer Registry	NCR
National Centre for Complementary and Integrative Health done a survey	NCCIH
National Health Interview Survey	NHIS
Progesterone receptor	PR

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

BACKGROUND: Breast cancer is the leading cancer in Malaysia and among women. *Annona Muricata* or Graviola leaves or Soursop leaves also known as 'the cancer killer' has been used worldwide as a complementary for treatment of cancer. Many studies have shown that *Annona Muricata* has a potential of anti-tumorigenicity and chemoprevention to treat cancer. Hence, in this study, we are trying to proof the cytotoxic effect and metastatic effect of Annona Muricata on breast cancer cell lines.

OBJECTIVE: This study aims to determine the cytotoxic effect of the extract of the soursop (Annona Muricata) leaves, the apoptotic effect and effect on cell cycle after treatment of the extracts on the MDA-MB 231 and MCF-7 breast cancer cell lines.

MATERIALS AND METHODS: Extracts from Annona Muricata was prepared using soxhlet method using different solvents (hexane, Ethyl acetate, methanol and water). MDA-MB 231 (ER/PR negative) and MCF-7 (ER/PR positive) breast cancer cell line used in the study. The cytotoxic effect was analysed by counting the number of cells inhibition and identified the IC50 (the percentage of cell populations inhibited by 50% after treatments) of each extracts, the cell proliferation was observed under microscope. The apoptotic effect of MDA-MB 231 and MCF-7 breast cancer cell line

was done by using Annexin V-Fitc Apoptosis Dtec Kit (6140592[1] (31.10.2017). While, the effect of cell cycle of MDA-MB 231 and MCF-7 breast cancer cell line was done by using Cycletest plus DNA Reagent Kit (6193798[1] (31.07.2017). Both tests are then analysed using flow cytometer.

RESULTS: From the experiment, ETAC was the best extract for observing cytotoxic effect for MDA-MB 231 cells line and hexane was the best extract for MCF-7 cells line. There was a decreased of the number of cells populations for both MDA-MB 231 and MCF-7 cells line after treatment with Annona Muricata in different concentrations and time. ETAC and hexane gave best apoptotic effect at late phase of apoptosis, while Tamoxifen gave best apoptosis effect during early phase. Besides, there was significant G1 phase arrest of MDA-MB 231 and MCF-7 cells line after treatment with ETAC and hexane, respectively, as well as Tamoxifen. There was suppression for migration for MDA-MB 231 and MCF-7 cells lines after treatment with ETAC and hexane.

CONCLUSIONS: Annona Muricata has a potential of anti-tumorigenicity on MCF-7 and MDA-MB 231 breast cancer cell lines. It gives changes in morphology of the breast cancer cell, as well as cytotoxic and apoptotic effect. Moreover, Annona Muricata also induces G1 cell cycle arrest in breast cancer cell lines. In addition, soursop leaves can help inhibit breast cancer migration that suggested of metastatic prevention. Thus, Annona Muricata can be recommended for use as complement in

breast cancer patient and as prevention for tumour occurrence. Even though the potential anti-tumorigenicity of Annona Muricata can be observed in this study, Tamoxifen still gave better result compared to Annona Muricata in term of cell inhibition and metastatic effect.

ABSTRAK

LATAR BELAKANG: Kanser payudara adalah kanser yang paling utama dikalangan wanita di Malaysia. ‘Annona Muricata’ atau daun ‘Graviola’ atau daun durian belanda, juga dikenali sebagai “daun pembunuh kanser’ sudah digunakan di. seluruh pelusuk dunia. Banyak kajian dijalankan telah membuktikan bahawa ‘Annona Muricata’ mempunyai potensi untuk menyahtumor dalam merawat penyakit kanser. Oleh itu, kajian ini dijalankan untuk mengenalpasti kesan sitotosik dan ‘metastatik’ daun ini ke atas sel-sel kanser payudara.

OBJEKTIF: Kajian ini dijalankan bertujuan untuk menentukan kesan ‘sititosik’, kesan apoptosis dan juga kesan kitaran pembahagian sel-sel selepas sel-sel ‘MDA-MB 231’ dan ‘MCF-7’ kanser payudara mendapat rawatan ektrak ‘Annona Muricata’

BAHAN DAN CARA: Ektrak- ekstrak dari ‘Annona Muricata’ dihasilkan melalui kaedah ‘soxhlet’ dengan menggunakan ‘solvent’ yang berbeza (‘hexane’, ‘ethyl acetate’, ‘methanol’ and air). ‘MDA-MB 231’ dan ‘MCF-7’ sel-sel payudara telah digunakan di dalam kajian ini. Kesan sitotosik telah dianalisa menggunakan cara pengiraan sel-sel dan IC50 (peratusan populasi apabila sel-sel berkurangan menjadi 50%) bagi setiap ekstrak dikenalpasti, pembahagian sel telah diperhatikan di bawah mikroskop. Kesan apoptosis sel-sel kanser payudara ‘MDA-MB 231’ dan ‘MCF-7’

dilakukan dengan menggunakan ‘Annexin V-Fitc Apoptosis Dtec Kit’ (6140592[1] (31.10.2017). Manakala, kesan kitaran pembahagian sel-sel kanser payudara ‘MDA-MB 231’ dan ‘MCF-7’ dilakukan dengan menggunakan ‘Cycletest plus DNA Reagent Kit’ (6193798[1] (31.07.2017). Kedua-dua ujian ini kemudian dianalisa dengan menggunakan mesin ‘flow cytometer’.

KEPUTUSAN: Daripada eksperimen yang telah dijalankan, ‘ETAC’ adalah ekstrak yang terbaik untuk sel-sel ‘MDA-MB 231’ dan ‘hexane’ adalah ekstrak yang terbaik untuk sel-sel ‘MCF-7’. Terdapat penurunan di dalam jumlah populasi sel-sel ‘MDA-MB 231’ and ‘MCF-7’ selepas diberi rawatan di dalam kepekatan dan masa yang berbeza. ‘ETAC’ dan ‘hexane’ telah memberi kesan apoptosis yang terbaik pada peringkat akhir dalam proses apoptosis. Walaubagaimanapon, Tamoxifen memberi kesan apoptosis di peringkat awal proses apoptosis. Selain dari itu, selepas rawatan oleh ‘ETAC’ dan ‘hexane’, telah terbukti proses kitaran pembahagian sel terbantut pada fasa G1, begitu juga Tamoxifen. Melalui eksperimen ini juga terbukti bahawa ‘Annona Muricata’ bertindak menghalang migrasi sel-sel kanser payudara.

KESIMPULAN: ‘Annona Muricata’ mempunyai potensi penyah-tumor untuk sel-sel ‘MDA-MB 231’ dan ‘MCF-7’. Annona muricata juga memberi kesan di dalam perubahan sel-sel, mempunyai kesan yang baik di dalam proses apoptosis dan menghalang kitaran pembahagian sel pada fasa G1, di samping menghalang migrasi sel-sel kanser payudara. Oleh itu, ‘Annona Muricata” boleh disyorkan untuk

diberikan kepada pesakit – pesakit kanser tahap empat dan digunakan sebagai pencegahan sebelum mendapat kanser. Di dalam kajian ini, walaupon kesan penyahtumor oleh Annona Muricata telah terbukti, Tamoxifen tetap memberi kesan yang terbaik jika dibandingkan dengan ekstrak dari Annona Muricata dari segi pengurangan sel-sel dan kesan ‘metastasis’.

TABLE OF CONTENTS

1. INTRODUCTION

1.1 Background of Study

Breast cancer (BC) is imposing life-threatening issue in the health care of women in this era. From World Health Organization WHO data, BC has increased in incident and has become the highest among the other cancer types in women (National Breast Cancer Foundation, 2015).

Studies in genetic molecular genetic has shown that mutation within genes such as p53, BRCA1 and BRCA2 are the main cause of the development of BC in women, even though the pathophysiology of occurrence of BC still debatable (Schumaker, 2006; Yip *et al.*, 2014). The unhealthy life-style and dietary practice could be the contributing factors towards the observation of increase incidence of BC (Yip *et al.*, 2006).

The prognostic factors of BC are determined by tumour histological grading, nodal and organ involvement and immunohistochemistry (IHC) of the tumour. In northern region of Malaysia most of the BC patients presented at advanced stage of disease (Norsa adah *et al.*, 2005). The management of advanced disease would involve in palliative chemotherapy in the effort of palliation. The chemotherapy imposes risks and unwanted side effects to the patients.

In Malaysia, complementary medicine is widely practiced and favourable among the Malaysian. It does not cure but it provides an improvement to the quality of life where as our conventional chemotherapy prolonging the life expectancy of these advanced BC patients. More emerging studies are required to support the practice of complementary medicine especially in natural products and herbal medicine in oncology patients.

Many studies have shown that complementary medicine has benefits to help in such of patients' condition, thus making such treatment a popular and alternative option to treat illnesses (Mantena *et al.*, 2006). Hence, this study was done to prove that Annona Muricata has cytotoxic effect and anti-metastatic effect on breast cancer cells line.

1.2 Rationale of Study

The management of BC is depending to the TNM staging of the disease. The conventional treatment is surgery followed by adjuvant chemotherapy and radiotherapy. In certain stage of the disease, neoadjuvant chemotherapy may be offered to the patient prior to the definitive surgery. The prognosis is significantly better when treatment is given at the early stage of the disease.

However, the unwanted or unpleasant systemic side effects of the chemotherapy impose a wrong impression to patients which leads them to stigmatize towards chemotherapy.

The reports from previous study shows that the BC patients who uses complementary medicine during and beyond their conventional treatment manage better in terms of their symptoms, prevention of toxicities, pain control and quality of life (Greenlee *et al.,* 2014). The introduction of complementary medicine and herbal medicine gives a new episode in the management of BC.

With more promising study published, complementary medicine should be offered together and adjunct along with the conventional medicine to improve quality of life of these BC patient. It was shown to act synergistically with chemotherapy, increasing the efficacy of chemotherapy (Cheng *et al.,* 2016). Furthermore, it may also act as chemoprevention supplement to prevent from development of cancer (Moghadamtousi *et al.,* 2014b).

This research was designed to study the potential anti-tumour effect of Annona Muricata on MCF-7 and MDA-MB 231 breast cancer cell line. The study will evaluate the apoptotic effects, cell growth arrest and anti-metastatic effects of Annona Muricata on both BC cell lines.

Soursop leaves or Graviola leaves or Annona Muricata has been chosen for this study in view of its potential of anti-tumour effect that was already well-

known world-wide (Moghadamtousi *et al.*, 2014a). These leaves were used as complementary medicine since decades.

Many studied have been done proved that Annona Muricata has good cytotoxic effect on cancer cells (Rachman *et al.*, 2012). It can induce apoptosis and also arrest G1 phase of cell-cycle (Moghadamtousi *et al.*,2014b*)*. Furthermore, Annona Muricata also inhibits cells migration, hence; prevent the metastasis of cancer cells (Moghamtousi *et al.*, 2014b).

All the above properties render the leaves suitable as cancer prevention and for usage in advanced cancer patients.

2. LITERATURE REVIEW

2.1 Breast Cancer

The incidence of cancer is increasing in trend in Malaysia. Based on latest Health Facts 2013, released by Ministry of Health Malaysia, cancer is one of the highest causes of hospitalisation and among the five highest causes of death in Malaysia (Ferlay, 2015). In 2006, breast cancer (BC) was leading cancer in Malaysia and was reported to be the highest among the Malaysian women (Yip *et al.*, 2006).

Figure 1 showed that BC (17.7%) is the highest cancer among other cancers in Malaysian population, followed with colorectal cancer (13.2%) and lungs (10.2%) (National Cancer Registry, 2011). Furthermore, BC is three times higher compared to colorectal and cervical cancer among women in Malaysia, as showed in Figure 2.

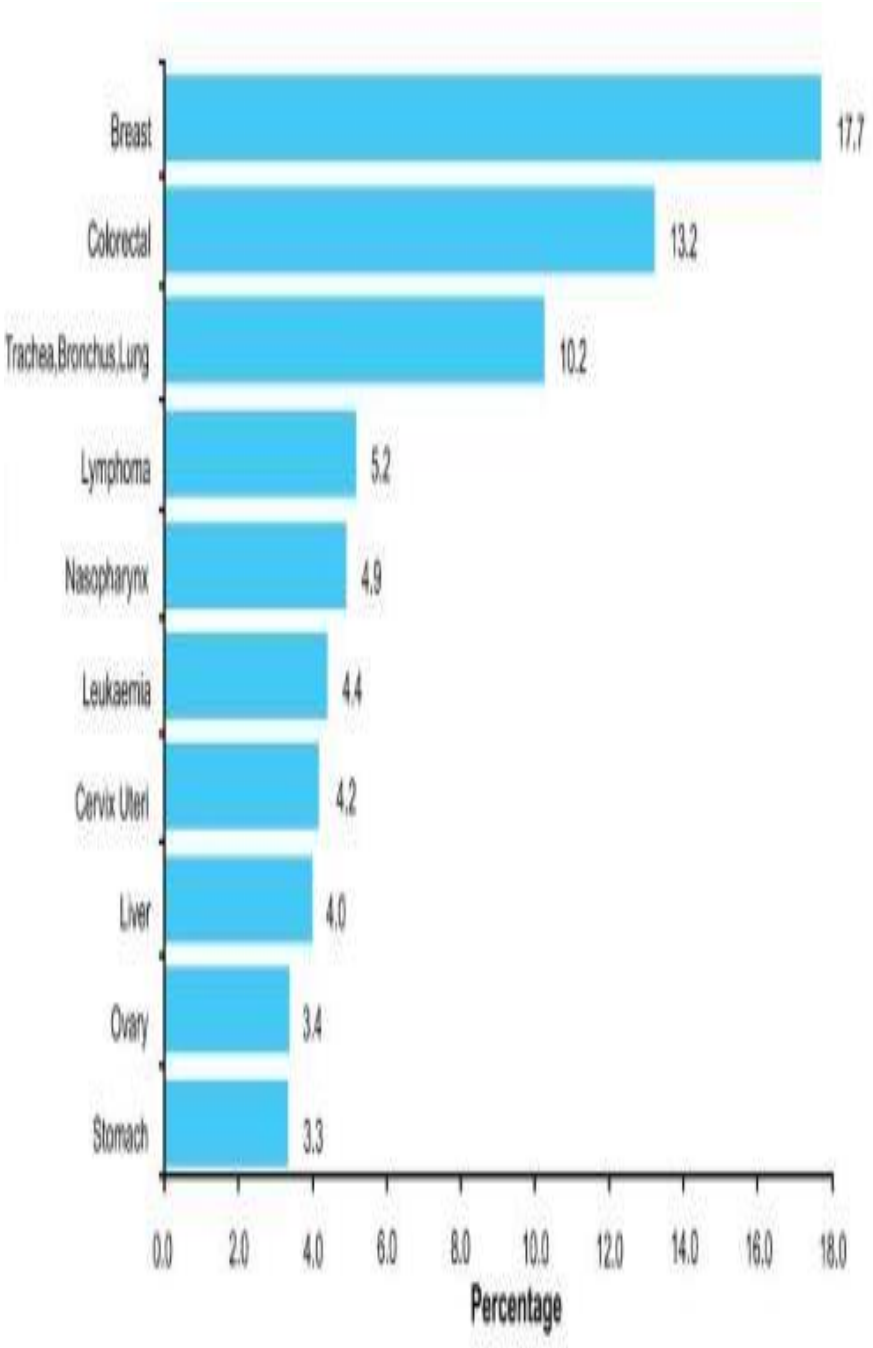


Figure 1: Ten most frequent cancers in Malaysia 2007-2011. (Adapted from; National Cancer Registry Report; Malaysia Cancer Statistic 2007-2011)

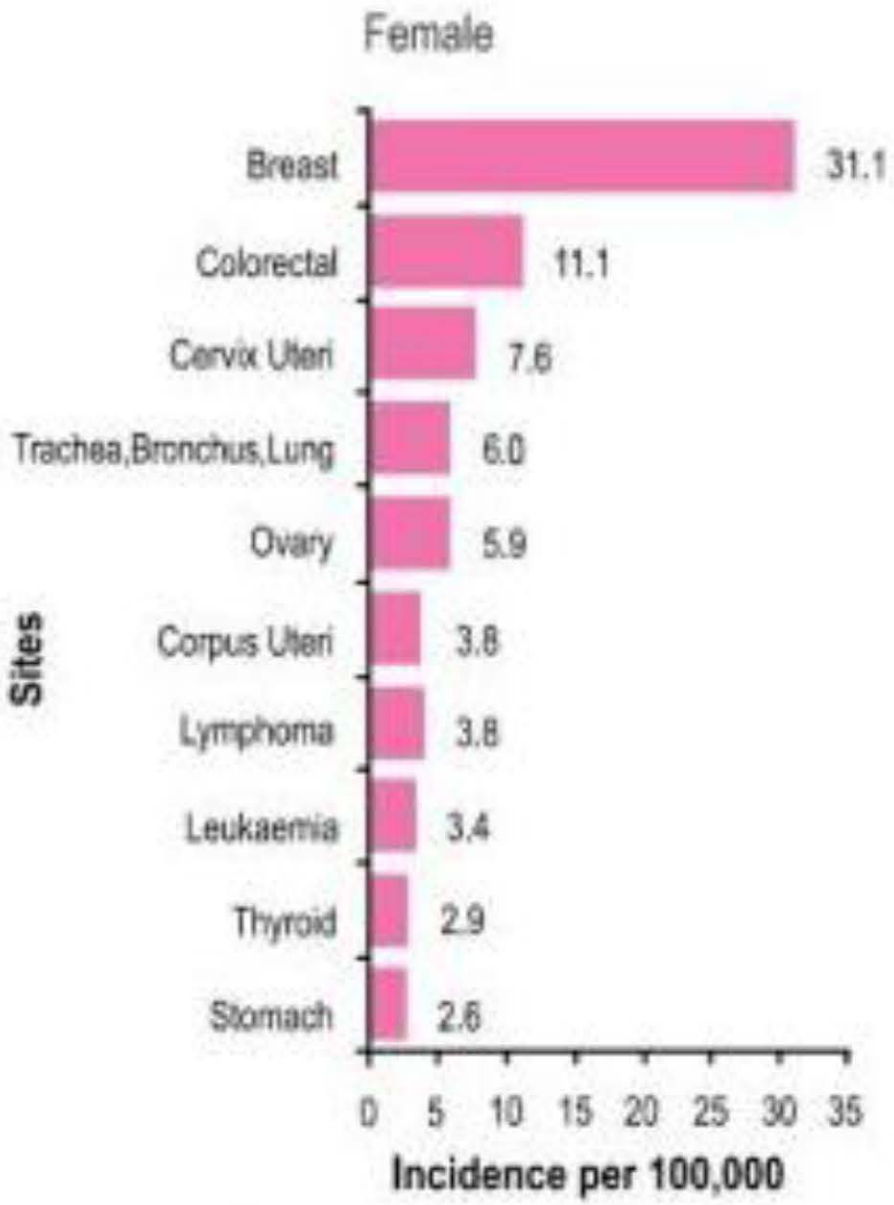


Figure 2: Ten most frequent cancers in females, Malaysia 2007-2011. (Adapted from; National Cancer Registry Report; Malaysia Cancer Statistic- Data and Figure; 2007-2011)

Unfortunately, the rate of BC in Malaysia is increasing by years. The report from GLOBOCAN 2012 showed further increment in the incidence of BC in Malaysia to 28% compared to 6 years ago which was 18% as shown in the Figure 3. While, the mortality rate of BC patients is around 24.7% (Figure 4) (Ferlay, 2015).

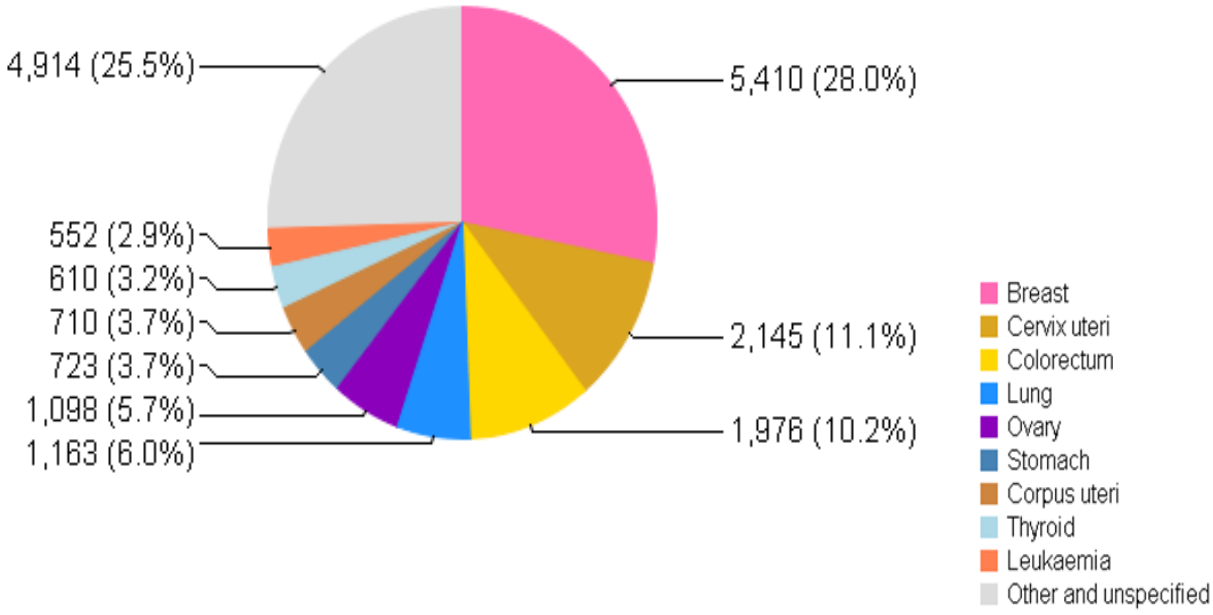


Figure 3: The incidence of cancers in Malaysia in 2012. (Adapted from; International Agency for Research in Cancer (GLOBOCAN, 2012)

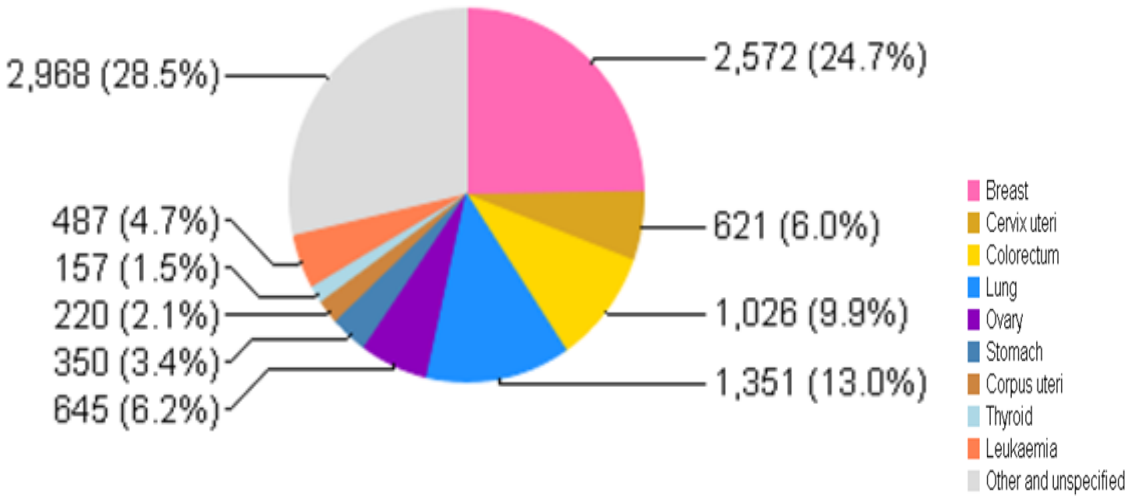


Figure 4: The percentage of mortality case of cancers in Malaysia in 2012. (Adapted from; International Agency for Research in Cancer (GLOBOCAN, 2012)

The National Cancer registry (NCR) 2003-2005 reported as Age-Standardised Rate (ASR) of 47.3 per 100,000 (Malaysia Cancer Statistics, 2006). The International Agency for Research in Cancer (GLOBOCAN) 2012 estimated the ASR of BC in Malaysia as 38.7 per 100000 with 5410 new cases in 2012 (Yip *et al.*, 2014) (Figure 5).

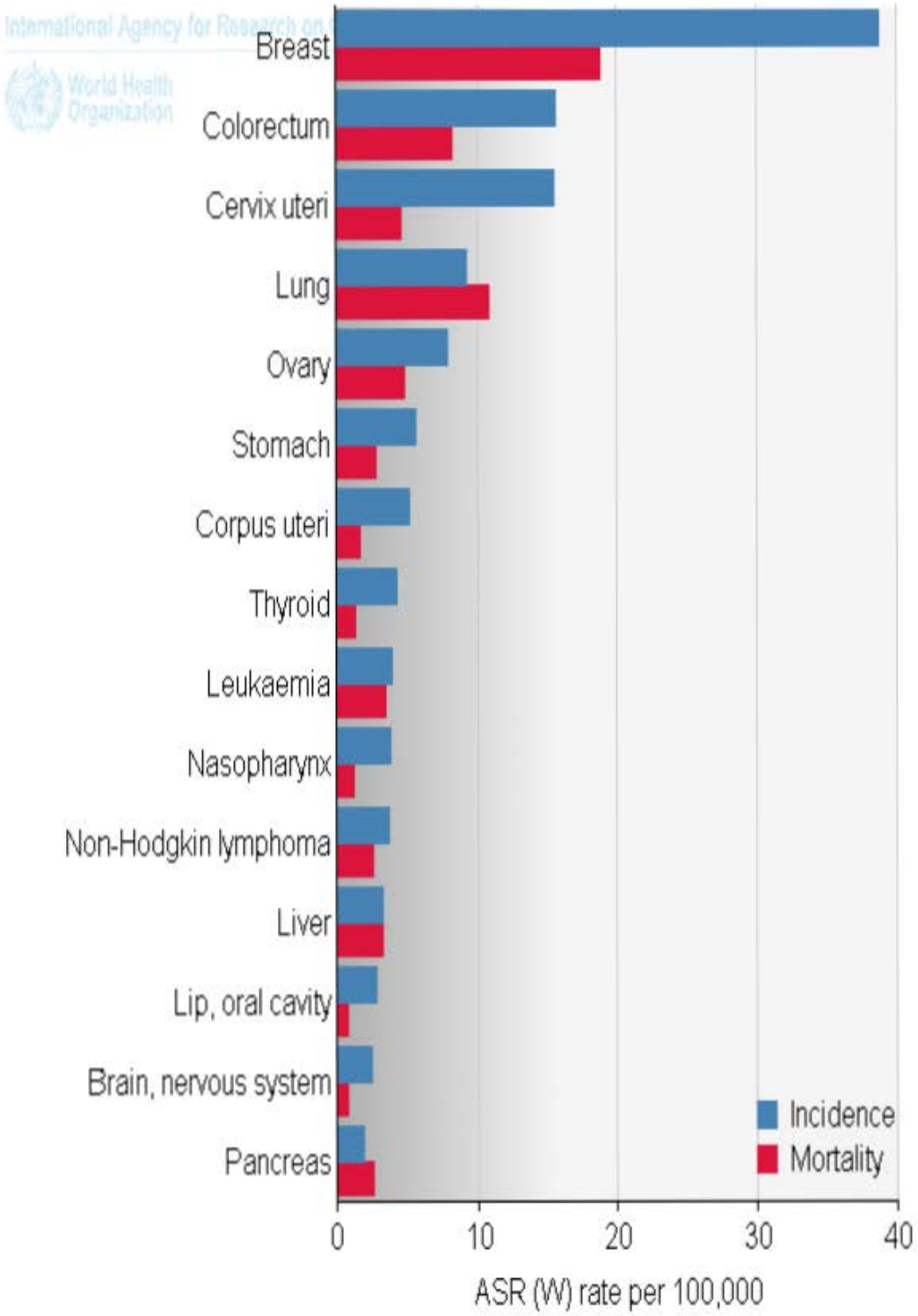


Figure 5: Estimated age-standardised rate and mortality in Malaysia. (Adapted from;

International Agency for Research in Cancer (GLOBOCAN, 2012)

BC is more common found in the Chinese population in comparison to the Indian and Malay population. According to Clinical Practice Guidelines of management of breast cancer, Chinese women had the highest incidence with an ASR of 46.4 per 100 000 populations followed by Indian women with an ASR 38.1 per 100 000 populations and Malay women with an ASR 30.0 per 100 000 populations (Khatcheressian *et al.*, 2013). This is probably due to genetic predisposition among the Chinese. Furthermore, it was known that Chinese has better awareness about BC compared to Malay.

Table 1: Incidence of breast cancer per 100 000 populations (CR) and Age-Standardised Incidence (ASR), by Ethnicity and Sex, Peninsular Malaysia 2006 (Khatcheressian *et al.*, 2013).

	Incidence			
Ethnic Group	No	%	CR	ASR
Malay	1,539	47.6	25.3	30.4
Chinese	1,375	42.5	53.2	46.4
Indian	320	9.9	34.9	38.1

Most of Malaysian women have poor survival from BC and it is estimated that half of the death due to BC could be prevented (Yip and Taib, 2012). Table 2 showed the list of study that had been done by various researches concerning the risk factors of BC among Malaysian.

Table 2: Risk factor of breast cancer in Malaysia. (Adapted from; Yip, C. H., Bhoo Pathy, N. & Teo, S. H. (2014). A review of breast cancer research in Malaysia. Med J Malaysia)

Author (year)	**Controls (n)**	**Cases (n)**	**Recruitment**	**Factors that reduce risk**	**Factors that increase risk**	**Factors that are not significant**
Matalqah et al (2011)	150	150	Penang General Hospital	Low fat diet, education >11 years, breast feeding, being employed	Family history, benign breast disease, menstrual irregularity, use of oral contraceptive (OCP)	
Razif et al (2011)	216	216	HKL and UKMMC	Higher number of life births	Family history	Age at first child birth and menarche not signficant
Norsa'adah et al (2005)	147	147	Kelantan	Breast feeding	Nulliparity, overweight, family history, use of OCP	
Hejar et al (2004)	89	89	Chinese, HKL and UMMC	Breast feeding		
Kamarudin et al (2006)	203	203	HKL	Exercise, low fat diet, longer duration of breast feeding		
Rejali (2007)	62	62	Malayan Hospital	Higher intake of selenium	Nulliparity, exposure to cigarette smoke, use of OCP	
Shahar et al (2010)	70	138	Klang Valley	Higher intake of selenium	Abdominal obesity, physical inactivity, low serum adiponectin	
Sulaiman et al (2011)	382	382	Kuala Lumpur			Total fat and fat subtypes not associated
Suzana et al (2009)	64	127	Klang Valley	Higher intake of selenium, vit A, Vit E		

CON'T

Author (year)	Controls (n)	Cases (n)	Recruitment	Factors that reduce risk	Factors that increase risk	Factors that are not significant
Sharhar et al (2008)	57	139	Klang Valley		Poor antioxidant status and oxidative stress measured by higher levels of malondialdehyde (MDA)	
Shahril et al (2013)	382	382	Kuala Lumpur	Higher Healthy Eating Index-2005 (HEI-2005)		
Ho et al (2009)	37pre-menopausal 68 post-menopausal	36pre-menopausal 66 post-menopausal	Kuala Lumpur		Higher serum progesterone and testosterone levels in postmenopausal women	

Table 3: Risk factor for breast cancer in Malaysia (Modified from; Yip, C. H., Taib, N. A. & Mohamed, I. (2006). Epidemiology of breast cancer in Malaysia. Asian Pac J Cancer Prev)

Increasing age
Geographic location
Family history
Reproductive factors
Early menarche less than 11 years
Late Menopause more than 55 years
Nulliparous
Late first child-b irth more than 30 years
Carcinoma of uterus
Carcinoma of ovary
dietary factors – diet rich in animal fat
Exogenous hormones – oral contraceptives
Hormonal replacement therapy
Alcohol – more than 2 drinks per day
Postmenopausal obesity
Higher socioeconomic group
Limited breast feeding (for long periods is a protective factor)

2.2 Management of breast cancer

The common practice for diagnosis of BC is via triple assessment, which consist of clinical history and physical examination, tissue biopsy and radiological assessment.

Table 4: TNM staging for breast cancer (7th Edition) (Adapted from American joint Committee of Cancer, (Giuliano *et al.*, 2017).

Staging	Description
Tx	Primary cannot be ruled out
T0	No evidence of primary tumor
Tis	Carcinoma in situ
Tis (DCIS)	DCIS
Tis (LCIS)	LCIS
Tis (Paget)	Paget disease of nipple NOT associated with invasive carcinoma and (DCIS and or LCIS) in the underlying breast parenchyma. Carcinomas in the breast parenchyma associated with Paget disease are categorized based on the site and characteristic of the parenchymal disease, although the presence of Paget disease should still be noted
T1	Tumor ≤20mm in greatest dimension
T1mi	Tumor ≤ 1mm in greatest dimension
T1a	Tumor > 1mm but < 5mm in greatest dimension
T1b	Tumor > 5mm but < 10mm in greatest dimension

Cont. Table 4

T1c	Tumor > 10mm but < 20mm in greatest dimension
T2	Tumor > 20mm but < 50mm in greatest dimension

T3	Tumor >50mm in greatest dimension
T4	Tumor of any size with direct extension to the chest wall and/or to the skin (ulceration or skin nodules)
T4a	Extension to the chest wall, NOT including only pectoralis muscle adherence/invasion
T4b	Ulceration and/or ipsilateral satellite nodules and/or edema (including peau d'orange) of the skin, which do not meet the criteria for the inflammatory carcinoma
T4c	Both T4a and T4b
T4d	Inflammatory carcinoma
Nx	Regional LN cannot be assessed (e.g.: previously removed)
N0	No regional LN
N1	Metastases to movable ipsilateral level I, II axillary LN
N2	Metastases in ipsilateral level I, II axillary LN that are clinically fixed or matted OR metastases in clinically detected ipsilateral internal mammary in the absence of clinically evident axillary LN
N2a	Metastases in ipsilateral level I,II axillary LN fixed to one another (matted) or to other structures
N2b	Metastases only in clinically detected ipsilateral internal mammary node and in the absence of clinically evident level I, II axillary LN
N3	Metastases in ipsilateral infraclavicular (level III axillary) LN with or without level I, II axillary LN involvement OR metastases in clinically detected ipsilateral metastases OR metastass in ipsilateral supraclavicular LN with or without axillary or internal mammary LN involvement
N3a	Metastases in ipsilateral infraclavicular LN
N3b	Metastases in ipsilateral internal mammary LN and axillary LN
N3c	Metastases in ipsilateral supraclavicular L
Mx	Metastases cannot be assessed (e.g.: previously removed)
M0	No metastases
M1	Metastases

The management of BC involves the commitment from multidisciplinary team approach depending on the stage of the disease. Surgery is considered the mainstay of treatment for BC, with chemotherapy, radiotherapy and hormonal therapy utilised as adjunctive therapy (Yip *et al.*, 2014).

The Surgical Guidelines for the Management of BC stated that there are two teams that should be involved in the management of BC patient. First team is the diagnostic team; consist of breast specialist clinician (a consultant surgeon), radiologist, and pathologist breast care nurse. Second team is the cancer treatment team, which include the diagnostic team, oncologist, plastic and reconstructive surgeon and/or onco-plastic breast surgeon, medical prosthetist, psychologist and palliative care team (BASO, 2009).

After staging the disease, the patients are categorised into two categories, operable or inoperable. For inoperable disease, the option is neoadjuvant chemotherapy to downstage the tumour followed by surgery. For operable disease, surgery is the gold standard followed by adjuvant radiotherapy, chemotherapy, hormonal therapy and targeted therapy.

2.3 Tumorigenicity

Tumour literally means “new growth”. It is defined as an abnormal mass of tissue growth which exceeds and is coordinated with that of the normal tissues. The tissue growth persists in the same excessive manner after the cessation of the stimuli that lead to tumour development. As we know, tumour can be benign or malignant. Benign tumours are composed of well differentiated cells that closely resemble their normal counterparts, slow growth and have no invasion or metastasis characteristic. In the other hand, malignant tumours are opposite characteristics where they are usually undifferentiated cells, rapid growth and has characteristic of invasiveness and metastasis (Kamb, 1995).

Tumorigenicity is a process of a cells/tissues becoming tumour. This process happens on the intracellular level due to faulty to repair or error in growth signalling in the genetic level. According to study done by Astirin, O.P et al (2013), incidence of cancer is associated with the increase in the expression or mutation of gene that trigger cancer and the decrease in expression of cancer suppressor gene (Astirin *et al.*, 2013). The absence of DNA-repair enzymes also plays an important role in the raise of cancer incidence. As we know, cancer suppressor gene has a crucial function in cell homeostasis to prevent tumour occurrence (Astirin *et al.*, 2013). Deregulation of cancer suppressor gene can lead to cancer progression.

P53 is a tumour suppressor gene that regulates the normal cell cycle. It is an essential protein to suppress cancer. The function is to arrest cell growth by arresting the cell cycle at the G1/S regulation point upon DNA damage recognition. This allows the cell to have time to fix the damage. In addition, p53 also can initiate the apoptosis, if DNA damage proves to be irreversible (Sheikh *et al.*, 1998). Thus, the incidence of cancer also associated with the abnormal process of apoptosis.

Hence, literally, anti-tumorigenicity is a reversible process to prevent or counteract the formation of tumour. Mode of cell death can be implemented through necrosis, apoptosis and aging. Necrosis and apoptosis have different entity and mechanism of action, even though there is certain characteristic of overlap properties.

2.3.1 Necrosis

Necrosis is an irreversible process of cell death that triggered by external factor such as hypoxic, acidic environment, toxic and injury. There will be changes in morphology of the cell, where the cells become swollen with formation of cytoplasmic vacuoles, blebbed cytoplasm and also condense and swollen mitochondria (Cotran, 2010).

2.3.2 Apoptosis

Apoptosis is defined as programmed cell death that is important to maintain equilibrium in tissue (Peter, 2011). Apoptosis is a crucial process in the human body. If the process fails, the tissue will continuously proliferate and will result in the formation of tumour. The characteristics of cells during apoptosis are similar to necrosis, except, the cells shrunk rather than swollen. There is presence of apoptotic body with condensation of chromatin and DNA fragmentation in the cytoplasm and nucleus (Cotran, 2010).

Table 5: The differences between apoptosis and necrosis (Adapted from Robin and Contran, Pathology Basis Of Disease, 8th Edition, 2010)

Differential features of apoptosis and necrosis	
Apoptosis	Necrosis
Affects single cells	Affects groups of neighbouring cells
No inflammatory response	Significant inflammatory response
Cell shrinkage	Cell swelling
Membrane blebbing but integrity maintained	Loss of cell integrity
Increased mitochondria membrane permeability, release of proapoptotic proteins and formation of apoptotic bodies	Organelle swelling and lysosomal leakage
Chromatin condensation and non-random DNA fragmentation	Random degradation of DNA
Apoptotic bodies ingested by neighbouring cells	Lysed cells ingested by macrophages

2.4 Cell Cycle

The proliferation of a cell is a regulated process that involves a large number of molecules and interrelated pathways. The replication of cells is stimulated by growth factors by signalling extracellular membrane components through integrin (Cotran, 2010). The proliferation process of cell cycle is to achieve DNA replication and division.

Cell cycle consists of presynthetic (G1), DNA synthesis (S), Premitotic (G2) and mitotic (M) phases. G0 phase is the phase where the quiescent cells that have not entered the cell cycle reside. Each of the transition is important step in cell cycle. The first transition in the process is from G0 to G1. This is where the activation of transcription genes, including various proto-oncogenes and genes required for ribosome synthesis and protein translation. The critical transition is at the G1 to S transition called restriction point, which is a rate-limiting step for replication (Cotran, 2010).

The assessment for damaged DNA occurs twice and there is often referred to checkpoint. First checkpoint is at the G1/S checkpoint that ensures that the damaged DNA or chromosomes do not complete the replication and to monitor the integrity of DNA before replication (Mantena *et al.*, 2006). The second checkpoint is the G2/M checkpoint where it checks the DNA after replication and monitors whether the cell can safely enter mitosis or not (Cotran, 2010). If there is DNA damaged,

checkpoint activation delays the cell cycle and trigger DNA repair. However, if the damaged is too severe, they are eradicated by the process of apoptosis. On the other hand, if the checkpoint is defective, the cell will continuously be replicating and dividing, which is the basis of tumour formation (Kamb, 1995). Figure 6 summarized the process of cell cycle.

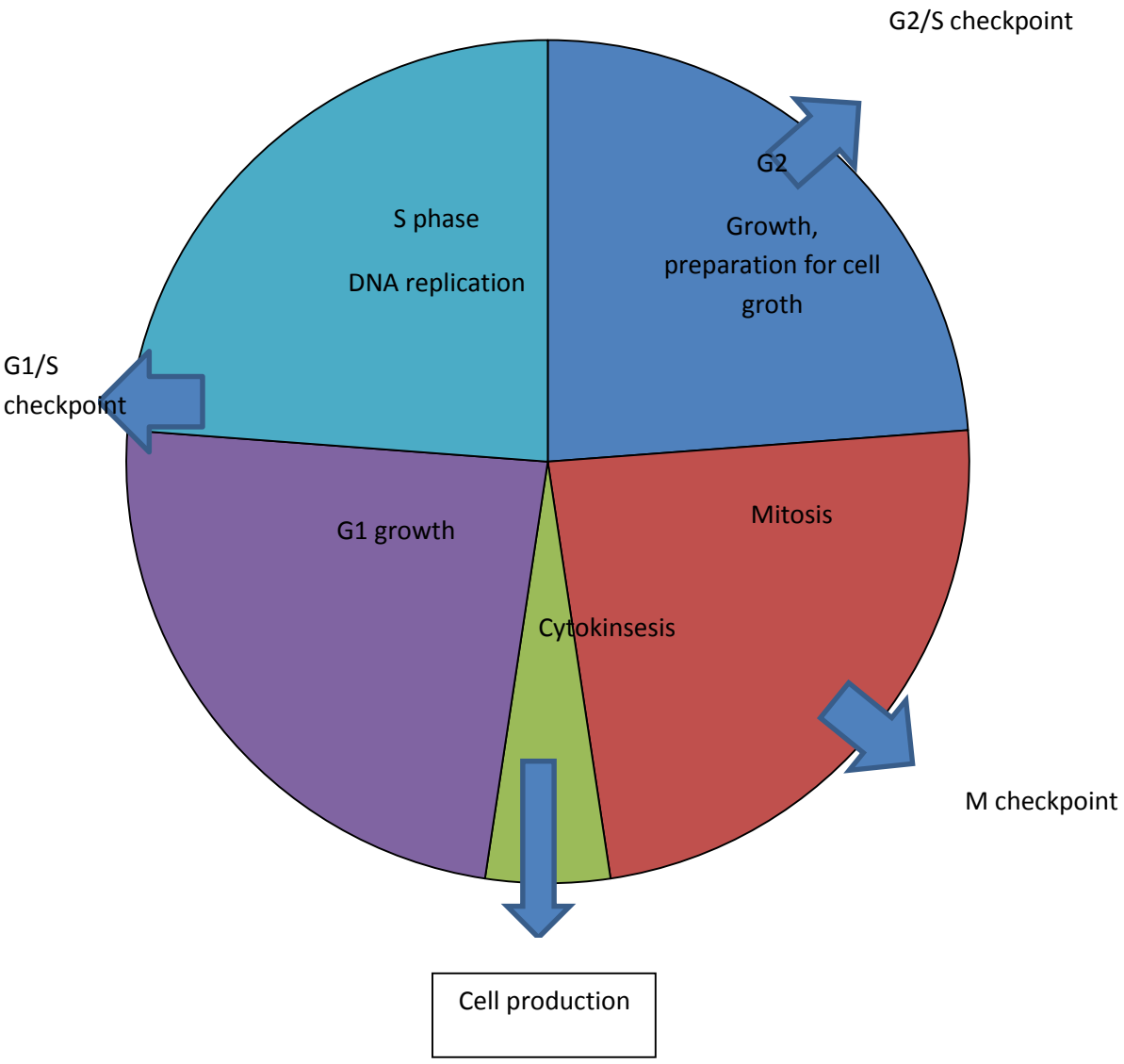


Figure 6: The image above shows the schematic diagram of cell cycle (Adapted from Robin and Contran, Pathology Basis of Disease, 8th Edition, 2010)