BIOLOGICAL EFFECTS OF DIFFERENT MRI EXPOSURE ON SINGLE-STRAND DNA OF RABBITS: AN IN VIVO STUDY

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by

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Dedication

This research is dedicated to the

NABI MOHAMMAD  **BIN ABDULLAH BIN ABDUL MUTTALIB,**

HIS FAMILY, AND FRIENDS.

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I am profoundly thankful to Allah for granting me the strength and courage to successfully complete this research. My heartfelt appreciation extends to several individuals who have played a pivotal role in this journey. First and foremost, I would like to express my deepest gratitude to my principal supervisor, Dr. Nik Noor Ashikin Binti Nik Ab Razak. Her unwavering support, insightful guidance, and motivating presence have been instrumental throughout the project. I also value her enthusiastic collaboration and the invaluable advice she provided, not to mention her generous efforts in evaluating this work. I must also extend my thanks to Professor Dr. Muhammed Mizher Radhi, whose recommendation and supervision on this topic were invaluable. His contributions significantly shaped the direction of this research. Dr. Nur Sakinah Binti Suardi deserves special mention for her valuable advice and unwavering support, which greatly enhanced the quality of this work. I am deeply grateful to my parents, Mustafa Shaheen and my mother, for their trust, unyielding support, and constant prayers, which have been a source of strength for me. My husband, Dr. Basil, also merits my appreciation for his trust and support. I would like to acknowledge the academic and non-academic staff at USM's School of Physics for their assistance and support, which have been invaluable throughout this study. Finally, my heartfelt gratitude goes out to all the staff in the MRI department at Alkarkh Hospital, Neuroscience Center, and Alkarama Hospital for their cooperative and helpful contributions to this research.

Ramziyah Mustafa Shaheen

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LIST OF SYMBOLS

$\nabla^{\rightarrow}$	The tissue moves with a velocity
A/m	Amperes per meter
dB	Decibel unit
G	Gauss
GHz	Giga hertz
Hz	Hertz
kHz	Kilo hertz
MHz	Megahertz
mM	milli moll
mT/m	Milli-tesla per meter
mV/sec^{-1}	millivolts/second
T	Tesla
W/m^2	Watt per square meter
μT	Microteslas
$\vec{E}$	Induce Electric field

LIST OF ABBREVIATIONS

B_0	Expression static magnetic field
B_1	Expression radiofrequency field
CA	Chromosomal aberrations
CV	Cyclic voltammetry
CBC	Complete blood count
dH_2O	Expression deionized water
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
dsDNA	Double-strand DNA
EDTA	Ethylenediaminetetraacetic acid
EDX	Energy dispersive x-ray
EDS	Energy Dispersive Spectrometer
ELF	Extremely low frequency
EMFs	Electromagnetic fields
EM	Electromagnetic
EMA	European Medicines Agency
FDA	Food and Drug Administration
FESEM	Field emission scanning electron microscope
FID	Free induction decay
GBCA	Gadolinium-based Contrast Agent
GCE	Glass carbon electrode
Gd	Gadolinium
GMF	Gradient magnetic fields
Hb	Hemoglobin

IARC	International Agency for Research on Cancer
ICNIRP	International Commission on Non-Ionizing Radiation Protection
IF	Intermediate frequency
LMA	Low-melting agarose
MF	Magnetic field
MRI	Magnetic resonance imaging
$MgCl_2$	Magnesium chloride
Min	Minute (min)
MN	Micronuclei
PLTs	Platelets
RBCs	Red blood cells
RF	Radiofrequency
RF-EMF	Radiofrequency electromagnetic fields
RFR	Radiofrequency radiation
RNA	Ribonucleic acid
ROS	Reactive oxygen species
rpm	Revs per minute
SAR	Specific absorption ratio
SSB	Single-strand break
SMF	Static magnetic field
ssDNA	Single-strand DNA
TL	Tail length
TM	Tail moment
T1	Spin-lattice relaxation is the time-constant
T2	Spin–spin relaxation
TVMF	Time-varying magnetic fields
WBCs	White blood cells

WHO	World Health Organization
ZnO NPs	Zinc Oxide Nanoparticles
I_T	The threshold intensity
10X RBC	10X = 100 ml of Red Blood Cell lysis
1XPBS	1X refers to 10 ml, and PBS is Phosphate-buffered saline.

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KESAN BIOLOGI DARI DEDAHAN MRI YANG BERBEZA KE ATAS DNA UNTAI TUNGGAL ARNAB: KAJIAN IN VIVO

ABSTRAK

Sistem pengimejan resonans magnetik (MRI) moden menggunakan gabungan canggih medan magnet statik (SMF), medan magnet kecerunan (GMF), dan medan frekuensi radio (RF). Beberapa kajian menyiasat potensi kesan MRI pada DNA, yang masih diperdebatkan, terutamanya dari segi keselamatan MRI. Walaupun agen kontras gadolinium (Gd) biasanya digunakan dalam MRI, ia boleh menyebabkan kesan sampingan yang serius seperti fibrosis sistemik nefrogenik. Kajian in vivo ini dijalankan ke atas 45 ekor arnab jantan untuk menyiasat kesan penggunaan variasi kekuatan magnetik MRI pada masa pendedahan yang berbeza terhadap asid deoksiribonukleik (DNA). Selain itu, pengukuran juga dilakukan terhadap kesan Gd dan kontras alternatif seperti magnesium klorida ($MgCl_2$) dan nanozarah zink oksida (ZnO NPs) pada DNA bebenang tunggal selepas pendedahan MRI. Ujian komet digunakan untuk mengkaji pecahan bebenang tunggal DNA (SSB) dengan mengukur tiga parameter komet: panjang ekor (TL), % ekor DNA, dan momen ekor (TM). Selain itu, parameter hematologi seperti sel darah putih (WBC), sel darah merah (RBC), hemoglobin (Hb), platelet, dan limfa% (limfosit ialah sel imun) turut dikaji selepas pendedahan kepada MRI pada tempoh yang berbeza. Sebagai tambahan, kajian tindak balas redoks turut dijalankan. Kajian ini mendapati hubungan yang signifikan antara kekuatan magnetic, tempoh pendedahan, dan parameter kerosakan DNA yang diperhatikan. Apabila kekuatan magnetik meningkat daripada 0.5T kepada 3.0T, dan dengan pendedahan yang lebih lama, terdapat aliran menaik dalam purata % nilai DNA ekor, TL dan TM, yang menunjukkan peningkatan kerosakan DNA. Pada 0.5T, tidak

terdapat perbezaan yang ketara selepas 10 dan 20 minit pendedahan kerana tenaga yang tidak mencukupi untuk menyebabkan kerosakan DNA. Perubahan hematologi melibatkan peningkatan WBC semasa pendedahan lanjutan kepada 0.5T dan 1.5T, dan menurun semasa pendedahan kekuatan magnetic 3.0T yang lebih tinggi. Apabila tempoh pendedahan berpanjangan dan kekuatan magnetik meningkat daripada 0.5T kepada 3.0T, ia mengakibatkan penurunan dalam RBC dan Hb yang lebih rendah. Peningkatan platelet yang ketara telah diperhatikan dengan pendedahan yang lebih lama dan kekuatan magnetik yang lebih tinggi. Peratusan limfa menurun apabila kekuatan magnet meningkat daripada 0.5T kepada 3.0T dalam jangka masa yang panjang. Kumpulan Gd dan ZnO NP kedua-duanya menunjukkan kesan yang ketara terhadap SSB DNA selepas 20 minit pendedahan kepada 1.5T, dengan ZnO NPs menjadi kurang oksidatif berbanding Gd, seperti yang diukur melalui voltametri siklik. Sebaliknya, $MgCl_2$ mempunyai kesan yang tidak ketara terhadap SSB DNA disebabkan oleh sifat antioksidannya, menunjukkan bahawa ia mungkin merupakan alternatif yang lebih selamat berbanding Gd untuk aplikasi MRI pada masa hadapan.

BIOLOGICAL EFFECTS OF DIFFERENT MRI EXPOSURE ON SINGLE-STRAND DNA OF RABBITS: AN IN VIVO STUDY

ABSTRACT

Modern magnetic resonance imaging (MRI) systems rely on a combination of static magnetic fields (SMF), gradient magnetic fields (GMF), and radiofrequency fields (RF). The potential impact of MRI on DNA remains a subject of debate, particularly regarding its safety. Additionally, gadolinium (Gd) contrast agents, commonly used in MRI, have been associated with adverse side effects such as nephrogenic systemic fibrosis. This in vivo study, conducted on 45 male rabbits, aimed to investigate the effect of different MRI magnetic strengths and exposure durations on single-strand DNA (ssDNA). Furthermore, it explored the effects of Gd and alternative contrast agents, such as magnesium chloride ($MgCl_2$) and zinc oxide nanoparticles (ZnO NPs), on ssDNA after MRI exposure. The comet assay was used to assess DNA single-strand breaks (SSB), measuring three parameters: tail length (TL), percentage of DNA in the tail (% DNA tail), and tail moment (TM). The study also examined hematological parameters, including white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), platelets, and lymphocyte percentage (Lymph%), following exposure to different MRI strengths over varying durations, alongside an analysis of redox reactions. Results indicated a significant relationship between magnetic field (MF) strength, exposure duration, and DNA damage. As MF strength increased from 0.5T to 3.0T, and with longer exposure, there was a notable increase in % DNA tail, TL, and TM, reflecting greater DNA damage. At 0.5T, short exposures (10 and 20 minutes) did not show a significant difference due to insufficient energy to cause DNA damage. Hematological changes included an increase in WBCs

during prolonged exposure at 0.5T and 1.5T, while WBCs decreased at 3.0T. Prolonged exposure also led to a reduction in RBC and Hb levels, with a corresponding increase in platelets. Lymphocyte percentage decreased with higher magnetic strength and longer exposure. The Gd and ZnO NPs groups both exhibited significant effects on SSB DNA after 20 minutes of exposure to 1.5T, with ZnO NPs being less oxidative than Gd, as measured by cyclic voltammetry. In contrast, $MgCl_2$ had an insignificant effect on DNA SSB due to its antioxidant properties, suggesting it may be a safer alternative to Gd for future MRI applications.

CHAPTER 1

INTRODUCTION

1.1 Background

Electromagnetic fields (EMFs) are classified as ionising or non-ionising based on their ability to ionise an atom or molecule (Tan et al., 2018). The non-ionising part of the electromagnetic spectrum includes static electromagnetic fields,radiofrequency, radio waves, infrared, visible light, and ultraviolet. Typical sources of EMFs in medical applications employing non-ionising radiation include magnetic resonance imaging (MRI) scanners, diathermy, and transcranial magnetic stimulation (Fatahi et al., 2017). The MRI is a highly effective, non-invasive diagnostic technique for investigating the anatomical structures and functioning of the body. The most widely employed nucleus in MRI is hydrogen (proton), which has a pretty strong signal and is found in biological tissue at 99% concentration. When subjected to a strong magnetic field (MF), most of these protons become connected with water and, to a lesser extent fat (Nowogrodzki, 2018). Radiofrequency (RF) pulses excite the nuclear spin energy transition, whereas gradient system localise the signal in space. Diversifying parameters of the pulse sequence can create unique differences between tissues based on the relaxation characteristics of hydrogen atoms (Weishaupt, 2013). With the growing number of MRI systems being regarded a safe technology because they can deal with atoms without affecting their structure, composition or properties, there are many studies and research that have appeared regarding concerns that their use may cause harmful biological effects (Collins & Wang, 2011; Coskun, 2011; Sharma & Jagannathan, 2022). However, other studies have reported an increase in DNA damage induction following exposure to MRI (Gonzalez et al., 2015; Knuuti et

al., 2013a). The combination of these three fields are static magnetic field (SMF), gradient magnetic field (GMF), and RF field during imaging poses potential health risks (Sammet & Sammet, 2015). Can the non-ionizing radiation in MRI cause DNA damage?

MRI interacts with the human body via three well-established physical mechanisms: magnetic induction, magneto-mechanical interactions, and electronic interactions (Non-Ionizing Radiation Protection & others, 2009). Recombination of free radicals plays a significant role in the interaction between the SMF and biological systems. Exposure to SMF is known to increase the concentration, activity, and lifespan of paramagnetic free radicals. This, in turn, can influence paramagnetic free radicals, potentially leading to gene mutations, oxidative stress, and sometimes apoptosis (Ghodbane et al., 2013a). In addition, the GMF vibrates mechanically because of the rapidly fluctuating currents passing through the coils, giving rise to three potential MR concerns nerve and cardiac stimulation occasionally causing discomfort (Stafford, 2020). RF fields with frequencies greater than 10 GHz are highly absorbed by the skin, and only a small portion of the energy reaches the human body's internal organs. These fields are measured in terms of power intensity (W/m^2). For frequencies above 10 GHz, power levels greater than 1000 W/m^2 are required to cause harmful effects, such as skin burns or ocular cataracts (Gherardini et al., 2014). Several studies have reported an increase in DNA damage or genotoxicity associated with MRI exposure, and some published research (Reddig et al., 2015; Simi et al., 2008b). However, they did not support it because other research contradicts these results (Fatahi & Networks, 2017; Szerencsi et al., 2013b), this raises the question of whether MRI in EMFs constitutes a health risk (Brand et al., 2015). This issue has lately resurfaced, with contradictory reports of DNA damage in human cells and immediate

blood cell count alterations after cardiac MRI (Lancellotti et al., 2015) reported in some published studies. The gadolinuim (Gd) contrast medium is very common used in MRI also has several side effects that impact the patient's health, a previous study reporting the presence of cytotoxicity and genotoxicity (Cho et al., 2014). The World Health Organization (WHO), responsible for cancer research, has categorized RF fields (International Agency for Research on Cancer. Radiation, 2013). It is potentially carcinogenic to humans' class IIB (cancer-causing to humans). The European Parliament has endorsed the ICNIRP guidelines as part of the 2013/35/EU Directive that establishes restrictions on exposure to electric fields generated by human activities in SMF and GMF below 1 Hz.

No one has studied and compared the effects of different MRI scans (0.5T, 1.5T and 3.0T), which are the most widely used types of devices to see if there is an effect on DNA, and sometimes a type of examination requires time. We chose multiple exposure times for each of the three MRI systems to see when and how much of an effect on DNA single-strands (SS) and blood cell parameters.

1.2 Research problems statement

MRI is a non-intrusive diagnostic technology that offers detailed information about the anatomical structure and functions of the human body. However, there's an ongoing debate in the literature about whether MRI may cause DN A damage to human blood cells in individuals exposed to it (Fiechter et al., 2013; Lee et al., 2011; Zradziński, 2015). While others present contradictory findings (Foster et al., 2017a; Szerencsi et al., 2013a). The evidence regarding MRI and increased DNA damage is inconclusive. and although some studies suggest that might harm DNA, there is a lack

of definitive proof, especially concerning patient health. Therefore, more research is needed to comprehensively investigate the genetic effects of MRI.

Many studies described the effects of each of the three magnetic fields (SMF, GMF, and RF), such as how exposure to SMF affects specific human structures such as the retina, the penile gland, and cells in the sinuses (Magnetic Resonance Imaging, 2016). Also influences blood properties, after one minute of exposure to the SMF (Magnetic Field, 2011). In addition, the GMF vibrates mechanically because of the rapidly fluctuating currents passing through the coils, giving rise to three potential MR concerns nerve and cardiac stimulation occasionally causing discomfort (Stafford, 2020). Although RF has no direct biophysical effects, they do stimulate energy transfers into tissues, resulting in localized heating (Fatahi & Networks, 2017). It is important to evaluate the extent to which MRI affects DNA during the examination. This is a very important issue for the patient's health. DNA damage is a major health problem, leading to genetic diseases as well as genetic changes or mutations in the cell, which may eventually lead to cancer.

An MRI could cause alterations in blood counts, an increase in WBC count from MF exposure could lead to up to a 65% increase in the chance of dying from ischemic heart disease (IHD) (Lassale et al., 2018). MRI 1.5T exposure resulted in a rise in blood viscosity due to altered Hb concentration responsible for blood oxygenation and could alter the blood flow leading to IHD (Sirajuddin et al., 2021).

Besides effect contrast agents are frequently used in MRI. Gd is paramagnetic, toxic in its free state makes chelation bound to another chemical when used and affects relaxation times (T1 and T2) (Lin & Brown, 2007). Few studies (Cho et al., 2014; Yildiz et al., 2011), have explored the effects during MRI scanning when contrast

agents have used genotoxicity effects of Gd in conjunction, making it essential to investigate their impact and explore alternative safe contrast agents such as $MgCl_2$ and ZnO NPs.

Some chemical substances can affect the behavior and composition of blood components. These effects may be indirect on cellular oxidative stress levels, affecting blood parameters. It is also necessary to know the electrochemical behavior of the redox reactions in the blood medium after exposure to MRI when using contrast media.

This in vivo study evaluated the effects of different MRI exposures on SS DNA at different exposure times. Additionally, investigate the potential of alternative safe contrast agents, including $MgCl_2$ and ZnO NPs. Blood samples were used to investigate changes in five blood parameters, such as WBCs, lymph%, RBCs, Hb, and PLT, following exposure to MRI at different durations. Also, redox reactions were studied for different contrast media after MRI exposure.

1.3 Objectives of the research

i) To assess the impact of different MRI scanning systems on the incidence of single-stranded DNA breaks at varying exposure times.

ii) To investigate the effects of different MRI systems and scanning durations on hematological parameters.

iii) To compare the effects of MRI contrast agents and alternative contrast agents on DNA damage.

iv) To analyze the redox reactions of MRI contrast agents and alternative contrast agents using cyclic voltammetry.

1.4 Scope of research

This study looked at the biological effects of MRI scanning on DNA single strands using three distinct MRI systems: 0.5T, 1.5T, and 3.0T exposure at intervals of 10, 20, 30, and 40 minutes. Furthermore, the investigation impact of three different contrast media including Gd, alternative contrast $MgCl_2$, and ZnO NPs on DNA single-strand. With the evaluation of five blood parameters, such as WBCs, RBCs, Hb, PLT, and lymph%, following exposure to MRI at different durations. Also, redox reactions were studied for different contrast media after injection and MRI exposure.

The experiment was conducted in vivo as a non-randomized controlled trial (interventional study). This study used 45 male rabbits of limited weights and ages of New Zealand white species. They are then divided into three groups: control group, exposure group without contrast material, and exposure group with contrast material. Before and after MRI, 4 cc of blood was taken from the rabbit's ear by venipuncture. All irradiated and non-irradiated samples (control samples) were examined using the alkaline comet test for DNA monitoring (single-series). The data was statistically examined for DNA damage using the program Tri Tek Comet Score™ processing images and measuring DNA damage metrics such as tail length, percentage DNA in the tail, and tail moment. Furthermore, statistical data was collected utilising more sophisticated systems such as microscopes, cameras, and computer analysis SPSS software packages, and analysis of five blood parameters (WBC, RBCs, Hb, PLT, and lymph%) by an automated haematology analyze. Cyclic voltammetry was used to investigate redox responses in blood media for various contrast media including Gd, alternative contrast $MgCl_2$, and ZnO NPs following injection and MRI exposure.

1.5 Outline of the thesis

The content of this thesis is divided into five chapters can be summarised as follows: The first chapter defines the background of the effects of the magnetic field in MRI on the biological effects, the problem statement, objectives, scope of research, and provides an overview of the thesis.

Chapter two presents a comprehensive literature review that covers fundamental theories and concepts of MRI, discusses the effects of interaction mechanisms on patients and various factors, explores the effect of alternative contrast agents ($MgCl_2$ and ZnO NPs) on DNA SS and provides insights into comet analysis.

In chapter three, the methodology is detailed, including the equipment and materials used in the experimental setup, the grouping of rabbits for different MRI exposures, the use of Gd and alternative contrasts ($MgCl_2$ and ZnO NPs), blood sample collection, conducting the comet assay (alkaline), reagent preparation, DNA study, blood tests for five parameters, and statistical analysis.

Chapter four discusses the statistical analysis results. It describes the findings from FESEM, EDX, and UV-vis characterisation of ZnO NPs and investigates the in vivo effects of three types of MRI exposure on single-stranded DNA over time. The chapter discusses the effect of the Gd contrast medium on SS DNA and compares it with the alternative contrast media, $MgCl_2$ and ZnO NPs. Additionally, it elaborates on the effects of MRI at different intervals on Hematology parameters such as WBCs, lymph%, Hb, RBCs, and PLT. The chapter concludes with a discussion of the redox reactions of different contrast media on blood components through cyclic voltammetry. Chapter five summarises the study's findings and makes recommendations for future research.

CHAPTER 2

LITERATURE REVIEW AND THEORY

2.1 Introduction to MRI

MRI uses MF and RF signals to create images of anatomical structures, the presence of disease, and other biological functions within the human body. If a pathologic process does not change one tissue characteristic and produces contrast, it may be visible in an image due to its effect on other traits. Consequently, the MRI process is somewhat more complicated than other imaging methods. The signals are generated by the magnetisation that occurs in the tissue when the patient is exposed to a powerful magnetic field. During an MRI examination, three major magnetic fields are employed to generate 3D images: SMF, GMF, and high RF. Studies have demonstrated that exposure to MRI components (SMF, GMF, and RF) can cause biological effects (Albert et al., 2009). A study discovered that after 15 mins of exposure to 0.2T, the cells from a normal human neural cell culture had significant morphological changes, forming branched dendrites with synaptic buttons (Pacini, 1999). Furthermore, as the scanner's strength increases, the exposure levels also increase suggesting substantial development of transient health symptoms that may lead to semipermanent health impacts (Schaap et al., 2014). Each of these, individually or collectively, may cause significant bio-effects, if applied at sufficiently high exposure levels.

In recent times, the contradictory evidence for DNA damage in human lymphocytes following MRI has resurfaced (Brand et al., 2015). While some studies found increased DNA damage and genotoxicity (Knuuti et al., 2013b). Similarly,

found that MRI 1.5T significantly affects the DNA double-strand break of human lymphocytes (Fiechter et al., 2013).

2.2 MRI energy fields

The main magnet of an MRI scanner creates a strong and uniform magnetic field around the patient being scanned, which is typically in the range of 0.5T to 3 T. Certain atomic nuclei can absorb RF energy when placed in an external magnetic field; the resultant evolving spin polarization can induce an RF signal in an RF coil and thereby be detected (Caverly, 2015).

Radiobiological studies already suggest that such fields are harmless at the levels routinely employed in MRI. Regardless of the type of MRI energy field employed, there is a certain intensity level known as the threshold intensity (I_T) below which no response is produced. MRI is entirely safe under I_T. Above I_T, the reaction to MRI exposure develops gradually initially, then more rapidly, until a complete response is recorded. A deterministic reaction has a threshold and a dose-dependent severity (Lancellotti et al., 2015). In addition to a threshold intensity-response relationship, these fields exhibit a time-intensity relationship (Biological Effects of Magnetic Resonance Imaging, 2016).

I_T is applicable to continuous exposure. Shorter exposure times produce the same response above this threshold. What is not clear is whether these MRI energy fields have negative health effects when combined. Table 2.1 shows how the three MRI energy fields interact with matter in different ways (Biological Effects of MRI, 2016).

Table 2.1 Mechanisms of the interaction between the three MRI energy fields and tissues.

MRI Energy Field	Mechanism of Interaction
SMFs (B0)	Polarization
Transient GMFs (BSS, BΦ, BR)	Induced currents
RF field (B1)	Thermal heating

The technology used in MRI procedures has continuously developed over the past four decades, resulting in MRI systems with strong SMFs, faster GMFs, and more powerful RF transmission coils. As shown in Table 2.2, the three types of MFs are generated by the following basic MRI system components (Fatahi & Networks, 2017):

Table 2.2 Typical range of magnetic fields used in MRI scanners.

Field	Range	Frequency	Applies
Static magnetic field	0.2- 7 T	0 Hz	Always
Spatial gradient	0- 25 T/m	0 Hz	Always, movement within it acts like a time-varying field
Gradient fields	0- 70 mT/m	0-10 kHz	During image acquisition, multiple trapezoidal pulses of a few milliseconds duration
Radiofrequency fields	0- 50 μT	10-300 MHz	During image acquisition, amplitude-modulated pulses of a few milliseconds duration

At 1.5T and 3.0T, Typical field strengths for clinical MRI, the difference between high and low energy, and enhancements to increase MRI sensitivity include increased MF strength and hyperpolarization via dynamic nuclear polarization. There are also a variety of chemical exchange-based signal amplification schemes that increase sensitivity (Gallagher, 2010). The principal cause of MRI-associated acoustic noise is the fluctuating gradients that impose Lorentz forces and vibrations, which are mechanically related to the system's structure. Patients often report heightened sound

pressure levels of 80 –119 dB (Heismann et al., 2015). This noise is produced when the gradient coils' internal currents change quickly.

2.2.1 Static magnetic field (SMF) B_0

The SMF represented by the symbol B_0 is the main MF generated by the MRI scanner, the central point is known as the iso-centre, and its strength (B_0) is measured in Tesla (T). One of the distinguishing characteristics of superconducting magnets is that they are typically cylindrical or solenoid coils with a strong field in the internal bore, as shown in Figure 2.1. Some patients may experience claustrophobia due to the comparatively small diameter and long bore of the MRI using a non-electrical permanent magnet (Elster, 2023). Even when the MR scanner is not imaging, there is a significant MF present (Hartwig et al., 2009), it causes net magnetisation in the human body and is in charge of nuclei alignment. Its utilised to align all hydrogen atoms in the body in the same spatial direction. In comparison, a 1.5 T MRI machine 15.000 Gauss is magnitudes higher than 30,000 times stronger than the earth's magnetic field (Stikova, 2012), while 1T = 10,000 Gauss, with the earth's magnetic field ≈ 0.5 Gauss (Mc Robbie, et al.,2006).

These interactions align the molecules to increase the attraction. In a magnetic dipole, the two magnetic poles create a MF running north to south (Atkins et al., 2023). In MRI, the item of interest is usually a molecule, atom, nucleus, or subatomic particle, also known as the magnetic dipole moment. The stronger the magnetic moment, the greater the MF and torque. The magnetic dipole moment is a vector because it combines strength and orientation/direction (Mumford, 2021).

This interaction is known as a dipole-dipole interaction, and it is the most fundamental single mechanism underlying T1 and T2 relaxation in biological tissues.

Four primary elements impact the strength of the dipole interaction: (1) spin types; (2) spin distance; (3) spin angle; and (4) spin relative motion (Petronek et al., 2021), within tissues, when one polar molecule interacts with the negative ends of another polar molecule, a trace of heat is produced.

As a result, they can interact directly with moving charges (ions, proteins, and so on) and magnetic materials found in tissues via a variety of physical methods, including magnetic induction, magneto-mechanical, and electronic interactions (Fatahi & Networks, 2017). Resonant pictures cannot be obtained in SMFs at this strength, except for hydrogen atoms. Numerous results indicate that powerful SMF effects significantly influence both endogenous and external ROS production (Tain et al., 2018). The potentially harmful effect of SMF on living beings is that it can enhance the activity, concentration, and duration of paramagnetic free radicals, resulting in oxidative stress, genetic mutation, and/or death. This is based on advanced investigations of SMF's impact on oxidative stress responses (Ghodbane et al., 2013a). This was shown in one of the studies: blood counts altered in response to an external magnetic field, with variations in numbers, clumping, viscosity changes, and DNA strand breakage (Mustafa et al., 2019). Another study explores how a strong SMF could control blood velocity and pressure and can be useful for treating arterial diseases, such as hypertension (Akar et al., 2019). Numerous investigations suggest that the effects of SMF exposures occur in animals, at levels ranging from mT to T through recognised interaction pathways, and are more likely to occur in fields above 2 T (Leitgeb et al., 2016).

2.2.2 Gradient magnetic fields (GMF)

The gradient's force is quantified as the change in field strength per unit distance. Millitesla per metre (mT/m) is a commonly used unit. The highest gradient strength that may be achieved is a design element of a particular imaging system. Some imaging systems require high gradient strengths of 200 mT/m or more for good performance (Elster, 2023b). Most 1.5T and 3.0T superconducting whole-body scanners have maximal gradient strengths in the region of 30-45 mT/m, whereas lower field (< 0.5T) permanent scanners range from 15-25 mT/m (Klein, 2015). Cycling power via gradient coils produces GMF, which is utilised to control the selective excitation of the patient's protons. They are orientated so that three gradient coils can be formed in three orthogonal directions (known as the x, y, and z directions). Additionally, two or more gradient coils can be coupled to provide a gradient in any desired direction (Elster, 2023a). Figure 2.1 illustrates how the gradient coils provide a changing MF in the Z direction (Stikova, 2012).

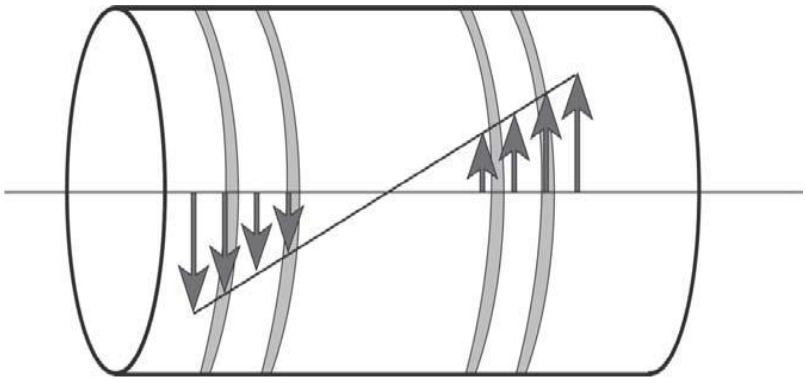

Figure 2.1 A diagram of the magnet bore and the time-varying magnetic field in the Z direction (Stikova, 2012).

GMFs, which serve as spatial localisation in the picture reconstruction process, are frequently turned on and off, switch quickly during scanning, and generate spatial fluctuations in the SMF (B_0). It is applied by switching MFs, usually in the range of

10-100 kHz (Fatahi & Networks, 2017). For this reason, MFs are time-varying ranging from extremely low frequency (ELF) to infrared (IF) (Makinistian & Vives, 2024).

Most available studies address potential associations between residential ELF and cancer (Grellier et al., 2014). After reviewing the scientific evidence, the IARC categorised ELF as "possibly carcinogenic" for humans (WHO and IARC, 2002). Exposure to ELF has been shown to cause a significant increase in DNA strand breakage. Furthermore, long-term exposure to ELF has been shown to contribute to the development of some neurodegenerative disorders by generating ROS (Lai & Singh, 2004). Exposure to ELF fields can produce symptoms such as skin redness, tingling, and burning, as well as headaches, fatigue, nausea, and palpitations (Mihai et al., 2014).

The GMF in the MRI system functions to provide position-dependent variation in MF strength, to induce electric currents that could be sufficiently large in tissues to interfere with the normal function of nerve cells and muscle fibres, leading to ventricular fibrillation, a more serious reaction to electric currents flowing through the body (Non-Ionizing Radiation Protection & others, 2010). Riley employed electrode numerical simulation models to forecast the electric field amplitude needed for stimulation as a function of waveform (pulse duration, waveform, and pulse train length), as well as to calculate gradient-induced brain and cardiac stimulation thresholds (Formica & Silvestri, 2004), as shown in Figure 2.2.

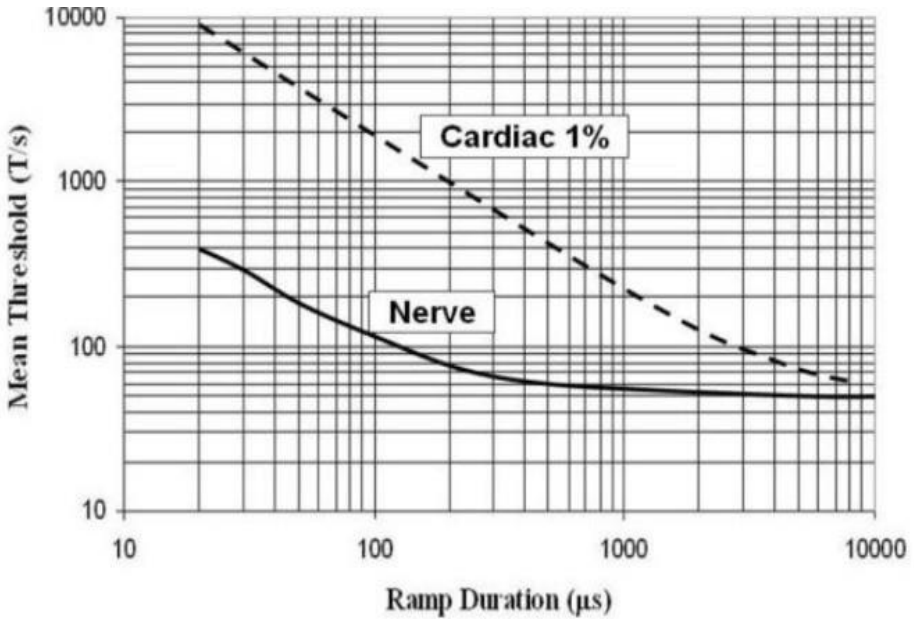

Figure 2.2 The mean peripheral nerve stimulation thresholds and cardiac stimulation thresholds for the most sensitive population percentile estimated by Reilly (Schaefer et al., 2000).

2.2.3 Radiofrequency fields (RF) B1

The RF field is responsible for stimulating the patient's protons inside the stable MF and placing nuclei during their use to energise and excite the hydrogen (proton) at the Larmor resonance frequency. This energy is re-emitted by the nucleus (relaxation) and detected by a receiver coil immediately following the termination of the pulsed RF. The generate used circularly polarised RF fields with frequencies ranging from 42, 64, 128, and 298 MHz (0.5T, 1.5T, 3.0T, 3.5T) respectively, depending on the magnetic flux density (Fatahi & Networks, 2017), using MRI scanners, the intensity of the RF field is assessed by calculating the MF strength.

EMF can be thought of as consisting of two independent components: (a) elec tric, represented by a state of space known as the electric field, where Coulomb force s act on stationary electrically charged objects, and (b) magnetic, represented by a sta te of space known as the MF, where Lorenz forces act on nonstationary (movin) elect rically charged objects (representing electric currents) (Lewczuk et al., 2015).

EMFs can alter physical objects, such as biological organisms, within their effect range. A lot of elements influence how this impact manifests itself. The most important are the following: (i) field intensity—the intensity of an electric field is measured in volts per meter (V/m), while the intensity of the MF is expressed in amperes per meter (A/m), (ii) distance from an object expressed in meters (m), (iii) the frequency of radiated energy is measured in hertz (Hz) for time-dependent fields and zero for time-independent fields. (iv) Surface power density (specific power) is the relationship between the intensity of radiated energy (power) and the area over which it is radiated, expressed in watts per square metre (W/m^2) (Lewczuk et al., 2015). The RF coil that surrounds the patient inside the magnet bore is the source of this radiation.

The degree to which RF affects the body can be determined by the interaction between the frequencies and wavelengths associated with RF and the organs. The wavelengths associated with 10 and 200 MHz are 30 and 1.5 meters, respectively. Shorter wavelength RF penetrates less deeply, causing higher surface heating (Hartwig et al., 2009). Numerous investigations to ascertain the thermoregulatory responses to tissue heating brought on by RF radiation at common magnetic resonance frequencies. It may also induce currents to flow through intra-cardiac leads, resulting in inadvertent cardiac pacing. Regardless of the surroundings, the device gives a feeling of discomfort. Prolonged exposure to such energy absorption can result in the development of neurological disorders and symptoms of electromagnetic hypersensitivity, including lethargy, skin problems, and digestive issues (The Effects of Radiofrequency, 2019), due to result in a high amount of energy absorbed within the body organs (Zielinski et al., 2020). Some researchers have used RF to prevent colorectal cancer cells from metastasizing to the liver, but have found that it causes

changes in DNA in nearby cells, leading to abnormal cell proliferation (Wong et al., 2010).

2.3 Genetic material

DNA is a molecule that functions as the biological instructions found in the genetic material that give each person and other organism its distinct characteristics adult organisms pass on their DNA and set of instructions to their progeny during reproduction (Portin, 2014). In eukaryotic organisms, the nucleus is a specific region of the cell that contains DNA. Because cells are small and organisms contain many molecules of DNA per cell, each one must be tightly coiled. Tightly coiled DNA is known as chromosomes, and when unwound it allows for replication. The term "genome" refers to an organism's entire set of nuclear DNA which contains the instructions needed to develop, survive, and reproduce. A gene is any sequence of DNA that contains instructions for making a protein. To perform these functions, the DNA sequences must be translated into signals that can be used to generate proteins (Roth, 2019). DNA is a double-stranded molecule held together by weak connections between two base pairs of nucleotides that contain the genetic instructions needed in the growth, development, reproduction, and function of all living creatures. These two chains are linked together by hydrogen bonding between the bases on the various strands all of the bases are inside of the double helix, while the sugar-phosphate backbones are on the outside (Behm, 2019). It turns out that DNA is held together by weak bonds. So, any outside influence affects the chain of bonds. Whereas, MRI produce high MF, GMF, and RF energy that absorption and interactions with tissues, lead to heating, due to molecular vibrations within cells, potentially leading to the

impact of weak hydrogen bonds that maintain the integrity of DNA strands and may cause DNA damage.

Some studies demonstrate DNA SSB after exposure to an MRI scan (Lee et al., 2011; Yildiz et al., 2011). There are several mechanisms for interacting with living systems such as the proton exchange rate mapping, high GMF, RF intensity, time exposure, etc. They can directly interact with moving charges (ions, proteins, etc.) and magnetic substances within tissue through physical mechanisms (Tain et al., 2018, 2019). This generates heating within tissues, which leads to increased ROS generation. It is well understood that cells in the body do not act in isolation, with intercellular signalling essential for sustaining tissue multicellular organisation and optimal cell activity. ROS along with reactive nitrogen species (RNS), are produced as a result of both normal cell metabolism and inflammation, as well as environmental exposure (Swenberg et al., 2011), prolonged exposure can effectively produce ROS and cause havoc with the DNA repair process mechanisms (Demirhan, 2021). However, MRI uses a high MF strength, such as 1.5T and 3.0 T, to induce a resonance effect of some atomic nuclei in the body (Nagel & Narula, 2013). So, since MRI directly causes DNA lesions, it may disrupt the internal balance within cells for some time, resulting in greater internal damage (Hill et al., 2016).

2.3.1 Single-strand DNA (ssDNA)

Single-strand DNA (ssDNA) and double-stranded DNA (dsDNA) are two distinct forms of double-stranded DNA. DNA SS is produced during DNA replication (Alberts et al., 2002).

The replisome, a loose complex made up of numerous proteins, works together to more correctly duplicate DNA (Duderstadt et al., 2014). The helicase protein separates duplex DNA into two complementary single strands. A DNA polymerase protein traverses each strand, generating the complementary daughter strand as well. Because DNA synthesis proceeds in a 5-to-3 direction, one of the two polymerase proteins must 'backstitch' every 100 to 1000 nucleotides on one of the strands (the lagging strand) to keep up with the helicase motion. As a result, a large amount of DNA SS exists between the helicase and the DNA polymerase. Because DNA SS is sensitive to enzymatic and oxidative degradation, it can be difficult for high-fidelity DNA replication (Bochkarev & Bochkareva, 2004). Re-examining the DNA strands that emerge from the helicase or the self-complementary regions of the lagging strand can also stop replication or result in deletion errors. To defend themselves from these implications, all organisms possess proteins that sequester DNA SS. These proteins are known as SS DNA binding proteins in bacteria (Maffeo & Aksimentiev, 2017).

DNA damage, particularly molecular degradation, has long been a subject of investigation. Because DNA is the storehouse of genetic information in all living cells, its integrity and stability are essential for survival. However, DNA is not inert; it is a chemical entity vulnerable to environmental attack (Clancy, 2008). Endogenous and external forces constantly damage it, while DNA repair enzymes fix the damage. Any flawed repair process, imbalance in damage and repair, or repair faults can all lead to mutation, illness, and cell death (Phillips et al., 2009). No single genotoxic endpoint can accurately predict the genotoxic potential and consequent cancer risk of occupational and environmental agents (Rossner et al., 2005).

In general, any unrepaired or repaired DNA damage is a big concern when evaluating genetic risks. When primary DNA damage increases in cells exposed to

MRI (Reddig et al., 2015). MFs may not be the direct cause of cancer, but they may contribute to the production of ROS and oxidative stress, which may promote or activate the expression of oncogenes. As a result, scientists are divided on the issue of whether exposure to MFs causes cancer (Maffei, 2022).

2.4 Haematology (Complete blood count)

The complete blood count (CBC) is a series of tests that count the number of RBCs, WBCs, and PLT in the blood. A complete blood count (CBC) can check your overall health and detect a range of diseases, including infections, anaemia, and leukaemia, whereas blood cells are mostly generated and matured in the bone marrow before being released into the bloodstream as needed under normal conditions. Our study analysed five parameters: WBC, lymph%, RBC, Hb, and PLT.

Several investigations have revealed that MRI can have vital effects on human health (Maffei, 2022), it also has a share of these effects in the changes that occur in blood parameters and their nature. In a study conducted during MRI 1.5T, significant immediate blood cell alterations or activations figured inflammatory response and DNA damage in lymphocytes (Lancellotti et al., 2015). Another study affected the blood properties, after one minute of exposure to 1.5T MRI, the viscosity of blood decreased. Upon additional exposure to the field, the viscosity increased only slightly with increasing exposure (Magnetic Field, 2011). Blood is a liquid which has a different magnetic permeability, where WBCs behave as diamagnetic microparticles while RBCs exhibit diamagnetic or paramagnetic behaviour that is because of oxygenation of their Hb (Seah et al., 2016). That is, the iron acts as a tiny magnet, destroying the homogeneity of the MF in iron-laden tissue (Wood, 2011).

In recent study of short-time exposure, the exposed group to MRI 1.5T showed a significant increase in neutrophils, lymphocytes, and monocytes except for WBCs which decreased. When increased time exposure a significant decrease in lymphocytes except for WBCs and neutrophils which increased, monocytes were non-significant (Babalola et al., 2024). Parameter measurements were carried out in a study to exposure of MRI 1.5 T, and 3 T for 10 minutes for normal (healthy) subjects recorded a decrease in parameters RBCs, Hb and PLT, while an increase in WBCs and high biochemical measurement (Abdullah, 2023), abnormal blood cell counts can cause cardiovascular problems (Maulood, 2018). These changes that occur may be due to the increased generation of free radicals in the work of cell membranes, which causes a malfunction in the functions of cell membranes and thus the effect of the cumulative magnetic field. Thus, can affect biological systems including DNA SSB. Several studies on the biological effects of MFs, most of the studies assessed genotoxic effects, with limited and inconsistent findings for blood parameters. Therefore, this study investigates the effects of different MRI MF strengths and scanning durations on hematological parameters.

2.5 Effects of MRI on biological system

2.5.1 Effects of static magnetic field

Over the last two decades, numerous research has been undertaken to evaluate the potential dangers of exposure to high SMF levels such as 0.5T, 1.5T, and 3.0T. SMFs are difficult to protect and can easily enter biological tissue (Hashish et al., 2008). Thus, SMFs can interact with biological systems by exerting forces on molecules and cells with diamagnetic susceptibility. They can also affect enzyme kinetics and act on moving charges (including moving fluids). The metabolic functions

of human tissues require a large number of chemical reactions, so it is reasonable to assume that a strong influencing factor may alter the rates or equilibrium conditions of these reactions (Formica & Silvestri, 2004). These primary causes of cell changes after incubation in external SMF are disruption of free radical metabolism and elevation of their concentration. This disorder leads to oxidative stress and, as a result, damages ion channels, leading to changes in cell morphology, expression of various genes and proteins, and ultimately programmed cell death and proliferation (Ali, 2007). Some authors concluded exposure to SMF triggers an iron-mediated mechanism that promotes free radical production in brain cells, resulting in DNA strand breakage and cell death (Ghodbane et al., 2013a).

Additionally, SMFs have been shown to affect a variety of human tissues, including the retina, pineal gland, and certain cells in the paranasal sinuses. However, the effects are not the same as dangerous or teratogenic/carcinogenic (Magnetic Resonance Imaging, 2016). In a recent in vitro study of humans, different periods of exposure to SMF 1.5T induced DNA damage at 50 minutes (Abdullah et al., 2016). Approved other in vitro studies of humans, and exam procedures of the brain, using three-channel head coils for 22, 45, 67, and 89 minutes during MRI scanning. Following 3 T MRI scanning, there was a significant increase in the percentage of DNA damage discovered (Lee et al., 2011). Most of these studies conclude that SMFs have minimal biological effects (Schwenzer et al., 2007). According to other studies, no induction of DNA damage (Amara et al., 2011; Szerencsi et al., 2013b).

Nonetheless, various common cellular effects of SMFs, including cell orientation, cell proliferation, and cell cycle distribution pattern (Zhang et al., 2017), can produce ROS levels when SMFs are exposed to different intensities (Calabrò et al., 2013; Vergallo et al., 2014). Highly active radicals, irons and molecules that have

a single unpaired electron in their outer shell (Wang & Zhang, 2017), influences the spin of electrons in free radicals, which may lead to changes in chemical reaction kinetics and possibly alter cellular function (Albuquerque et al., 2016). Thus, the increased creation of free radical activity in cells might damage cells by destroying macromolecules including DNA, protein, and membrane lipids (Ghodbane et al., 2013b; Wang & Zhang, 2017).

As for the haematology parameters, in vitro study, upward WBCs and reduced lymphocytes after exposure to SMF 128 mT (Milovanovich et al., 2016), another study exposure to SMF128 mT increased WBCs, RBCs Hb, and Hb level (Amara et al., 2006). Some study negative findings have been reported in the literature (Belyaev et al., 2006; Chemeris et al., 2006; Hook et al., 2004). Short-term exposure studies include those on hematologic parameters, circadian rhythms, neuronal activity, cognitive function and behaviour, cell growth and morphology, cell reproduction and teratogenicity, DNA structure, blood-brain barrier permeability, and other biological changes. The majority of these studies concluded that SMFs have limited biological impact (Schwenzer et al., 2007).

The ICNIRP issued a study reviewing in vivo and in vitro studies undertaken to detect biological responses to SMF in the milli T to several T (tesla) ranges, to provide new advice on occupational exposure limits and general public exposure (International Commission Non-Ionizing Radiation Protection, 2009). Most known in vitro investigations found that SMF above 30 μT affected cellular endpoints. It is widely known that static fields less than 1T are not genotoxic (Belyaev, 2006). Nonetheless, the study discovered significant, time- and dose-dependent increases in the frequency of micronuclei in mice subjected to SMFs of 2T, 3T, and 4.7T (Suzuki et al., 2001).

2.5.2 Effects of time-varying magnetic field (TVMF)

The gradients are pulsed during and between RF excitation pulses, using a quicker imaging or spectroscopic process. They are created by power cycling through gradient coils and are used to localise aligned protons inside the body. This allows spatial reconstruction of tissue sections into pictures (Schmitt, 2013).

During an MRI scan, a time-varying electromagnetic field in the RF range can induce effects via multiphoton absorption, causing the molecules in tissues to vibrate or ions and electrons to move, both of which lead to heating (Hartwig et al., 2009). High GMF leads to continuous cellular ROS production which affects the blood and induces cellular stress, increased oxidative stress may occur in leukaemia with increasing exposure time (Zablotskii et al., 2014). Increase ROS levels depending on MF intensity, frequency, and exposure time, lead to induced changes in enzymatic activities (Wang & Zhang, 2017). In a previous study, significant morphological changes, such as enlarged endoplasmic reticulum and mitochondria, increased lysosomes, and distorted microvilli, were detected in cells exposed to high levels of GMFs (Qian et al., 2013). All of the cells as well as the cell membrane are mechanically stressed by high GMF (Zablotskii et al., 2016). It can transmit stress on the cell nucleus leading to DNA changes in gene expression or may lead to an impact on the repair mechanism of DNA damage. Additionally, high GMF can cause either membrane potential depolarization or hyperpolarization (Zablotskii et al., 2016).

Nevertheless, the biological effects of SMF are influenced not only by the field intensity but also by the field's gradient. ROS may be one of the processes behind the biological effects caused by EMF (Lai, 2019; Schuermann & Mevissen, 2021). Low electromagnetic energy has reportedly been shown to be insufficient to break the