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Photo-Thermal Induced Optical Scattering Modulation Sensor for Malaria Diagnosis

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Abstract

Malaria is one of the leading killer diseases in sub-Saharan Africa. Although the disease is curable, early and accurate diagnosis is key to effective therapy. Existing malaria diagnostic techniques have low detection accuracy especially when the parasite load in the blood is low. In this paper, we report on a simple photo-thermal based technique for detection of the *Plasmodium* parasites' biomarker (hemozoin) in blood smear samples. The technique has demonstrated 100% *Plasmodium* detection sensitivity and specificity from the ten blood smear test samples used.

Keywords

Photothermal Induced Optical Scattering Modulation, Photoacoustic Probing, Hemozoin, Hemoglobin, Probe Beam, Excitation Beam

1. Introduction

Malaria Background

Malaria is a tropical disease that has been a leading cause of deaths in sub-Saharan Africa with children below five years being the most affected. According to World Health Organization (WHO) 2015 malaria report, the disease caused about 0.5 million deaths globally and infected approximately 200 million people during the reported period [1]. The disease is caused by a protozoan parasite of the geneus *Plasmodia*. There are five species of *Plasmodia* that infect humans namely; *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, and *Plasmodium knowlesi*. The parasites are transmitted from infected to non-infected persons through a bite by female

anopheles mosquito. Once the parasites have been launched into the blood stream, they are transported to the liver for incubation [2]. Upon maturity the parasites are released to the blood stream where they attack red blood cells (erythrocytes), feeding on hemoglobin and depositing a by-product known as hemozoin. Hemozoin is an insoluble iron compound. The compound has been reported to possess nano-rod structure and strongly absorbs optical radiation [3]. Hemozoin therefore can serve as *Plasmodium* parasite biomarker in blood.

Early and accurate diagnosis of the malaria is key to effective treatment of the disease. However existing malaria diagnostic technique such as optical microscopy (which is the gold standard method) and *Plasmodium* parasites antigen detection assays also known as Rapid Diagnostic Techniques (RDTs) lack high detection sensitivity and specificity [2], [4]. Both methods are invasive with optical microscopy also being a time consuming process. Therefore, there is an urgent need for development of a rapid malaria diagnostic technique with capability of non-invasive operation.

Limited studies have been reported on development of non-invasive techniques for malaria diagnosis [5]-[11]. The trend from the reported literature points towards use of photothermal related techniques for probing hemozoin presence in infected erythrocytes flowing through blood vessels beneath the skin surface. Photoacoustic flow cytometry has been the most preferred option [2], [8] [9] [10] [11]. This is because photoacoustic detection techniques boast from a number of desirable features which include; high detection sensitivity due to high optical absorption contrast in absorbing molecules. Besides, the emitted acoustic signal is less immune to scattering as and therefore can form high resolution images as compared to purely optical imaging technique such as Diffuse Optical Tomography (DOT) [12], [13]. Third, optically bulk tissue can be probed in reflection mode where both the optical source and the detector can be situated on the same side of the probed sample [14] [15] [16] [17]. Other existing photo-thermal based techniques such as thermal lens effect [18] are considered not suitable for *in vivo* probing of hemozoin since they require use of thin transparent samples (small volumes) and forward mode detection (where the optical sources is in the opposite side of the detector with respect to the sample). These requirements render the techniques unsuitable for *in vivo* probing of human tissue—because of its bulkiness and the strong optical scattering involved.

Figure 1 (obtained from reference [19]) gives the schematic diagrams of photoacoustic flow cytometry and thermal lensing. PBDBEB is the diameter of the probe beam of a thermal lens setup before application of the excitation beam to the sample. PBDAEB is the diameter (divergence) of the probe beam after the application of the excitation beam. Thermal lens effect causes broadening of the probe beam and change of its intensity after the sample has been irradiated by the excitation beam. This is because when the sample absorbs optical energy from the excitation beam the energy is converted to heat and temporary confined within the illuminated region of the absorber. This causes perturbation of

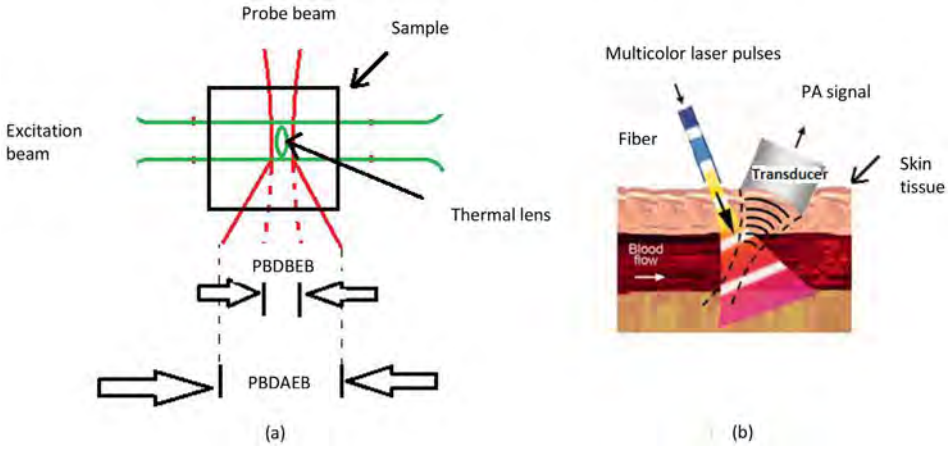

Figure 1. Configurations of thermal lensing and photoacoustic flow cytometry. (a) Perpendicular geometry of thermal lens setup [19], thermal lens effect; (b) photoacoustic flow cytometry, PA flow cytometry.

the absorber's intrinsic optical properties (specifically the refractive index) from the equilibrium (steady) state which in turn causes divergence and change of intensity of the probe beam.

The reported photoacoustic based malaria diagnostic technique on the other hand suffers from a number of limitations which has hampered its clinical adoption. One major challenge has been the problem of wide inter- and intra-variability of detected PA signals. Detected acoustic signals also suffer from low SNR due to acoustic attenuation before the signal reaches the transducer. The conversion efficiency from optical energy to acoustic energy in tissue (water) is also significantly low due to the low value of Gruneisten parameter for water which is estimated to be 0.2 [20]. This requires use of sophisticated hardware to suppress the noise and amplify the detected signal and this makes the setup complex, bulky and expensive.

In this paper, we report development of a novel technique termed as Photothermal Induced Optical Scattering Modulation (PTIOSM) for detection of hemozoin in malaria infected blood. The technique is premised on the fact that photon absorption by chromophores is followed by a relaxation session where the absorber's molecules move from excited state to ground state. If the relaxation process is non-radiative, the absorbed optical energy is released in form of heat. The emitted heat energy takes some time before it diffuses away from where it is deposited (Thermal conferment time). During this phase, the intrinsic optical properties such as the absorber's refractive index and its optical absorption coefficient are temporarily modified.

In the case of PTIOSM technique, modulation in the probe beam signal is detected in the reflection (backward) mode instead of transmission (forward) mode as is the case for thermal lens deflection. Modulation intensity with respect to optical excitation wavelength is expected to carry information on the composition as well as the concentration on chromophores in the probed sample.

2. Materials and Methods

2.1. PTIOSM Set-Up

A common anode, 3 W - 9 W RGB Light Emitting Diode (LED) (product ID 2524, from Adafruit Industries, USA [21]) was used as the optical source for providing both the Probe Beam (PB) and the Excitation Beam (EB). The PB was produced by forward biasing the blue LED segment with a constant 350 mA current to generate blue light whose peak wavelength was 465 nm. The EB beam was produced by driving the red and green LED segments with 1 MHz square pulses having 40 nS pulse width. The input signal for driving the LED segments was from a DDS function generator (KKmoon 40 MHz Function Signal Generator Dual-channel DDS Arbitrary Waveform Pulse Signal Generator 1 Hz - 100 MHz Frequency Meter) that was coupled to a custom made RGB LED driver circuit. **Figure 2** gives the block diagram of the setup. Both the red and green LED segments were overdriven with 2 A forward current during the ON period of the driving waveform in order to produce sufficient optical intensity for generation of PTIOSM signal.

Light from the RGB LED (PB and EB) irradiated samples (stained blood smear slides) and the transmitted light passed through an optical filter (Omega Optical, Inc. 450 nm SP (450 SP) RapidEdge 25 mm Optical Shortpass Filter) that blocked the EB (the red and green pulsed light) but transmitted the probe beam (the blue light) after interacting with the photo-thermally excited sample. A photodiode and trans-impedance amplifier module (OPT101) was used to detect light from the filter and the generated photodiode current was converted to a voltage signal and then pre-amplified using an inbuilt trans-impedance amplifier in the module. To boost the bandwidth of the detected signal, a 100 ohms resistor was externally connected to the trans-impedance module (between pin 2 and 5 while pin 4 was left unconnected) to provide the negative feedback instead of using the inbuilt 1 MΩ resistor that offers a limited bandwidth of 12 KHz [22]. The output of the trans-impedance amplifier was coupled to a digital oscilloscope (OWON SDS1052, 2 channel, 500 MS/s, 10 KB recording length) for digitization. The digitized signal was then loaded to a computer for signal processing. Matlab R2015b software was used for processing the acquired PTIOSM signals. **Figure 3** gives the algorithmic steps used for signal processing.

2.2. Signal Pre-Processing

The aim of this stage was to reduce or possibly eliminate the noise riding in the acquired PTIOSM signal. There were two possible main sources of noise in the acquired signals; the radiative pick-up noise from adjacent switching equipment such as the function generator, and the background (stray) light detected by the photodiode. Noise correction was performed by coherently subtracting the PTIOSM signal due to a blank glass slide (also termed as the reference sample) from a PTIOSM signal obtained from a stained blood smear sample. The resultant differential signal was free of existing common mode noise.

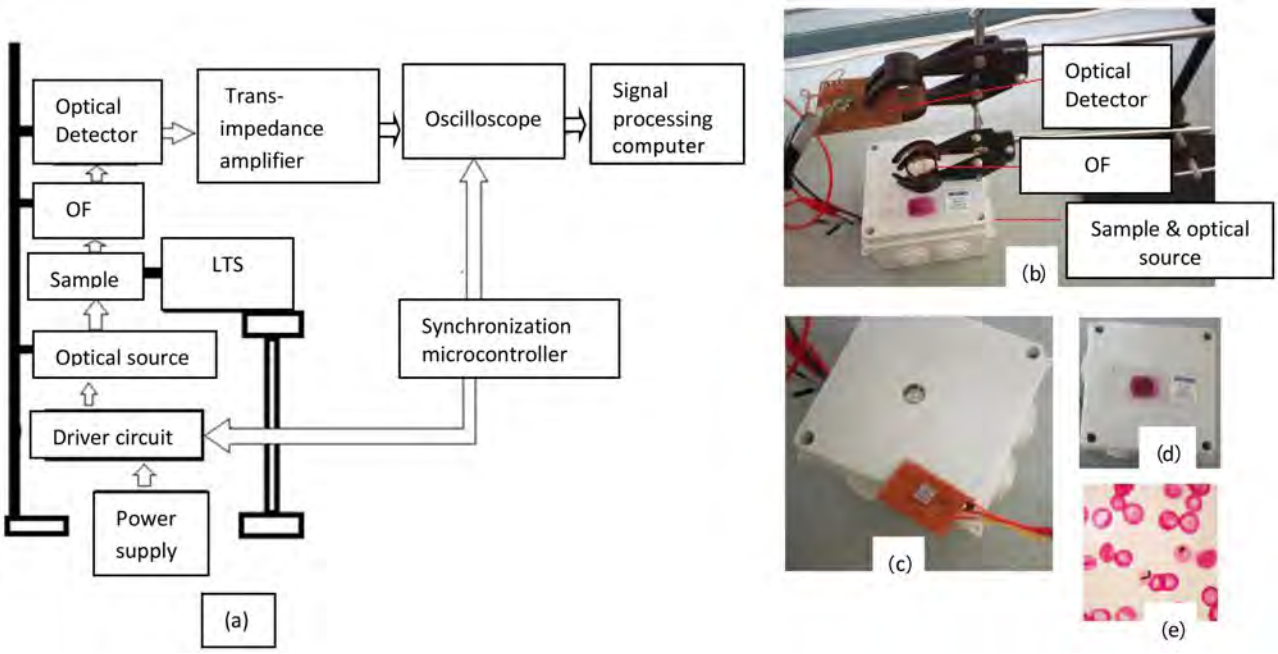

Figure 2. Setup diagram of the PTIOSM probe for malaria diagnosis, (a) setup block diagram: OF (optical Filter), LTS (Linear translational stage); (b) photo of the setup showing optical source, sample, optical filter and photodiode circuit; (c) Optical source and detector; (d) blood smear sample; (e) microscopic view of the sample.

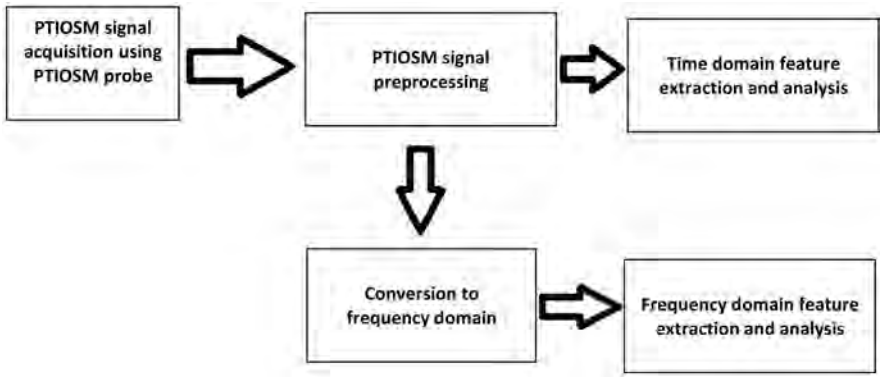

Figure 3. Algorithmic steps for PTIOSM signal processing.

2.3. Frequency Domain Feature Extraction

Fast Fourier Transform (FFT) algorithm implemented in Matlab was used to convert the acquired time domain signals to frequency domain. The amplitude and phase spectrums of the frequency domain signals were plotted in Matlab and visually analyzed with a view of identifying the frequency bands which best differentiated infected samples from non-infected samples based on their signal intensities at different frequency bands.

2.4. Sample Description

Two sets of Geimsa stained blood smear samples were used to test the developed PTIOSM probe. One was *Plasmodium falciparum* infected blood smears samples (acquired from Carolina biological company [23]) and the other was *Plasmo-*

dium non-infected blood smear samples locally prepared. In each of the two sets, PTIOSM signals from five slides were acquired, processed and analyzed as described above.

3. Results

Frequency Domain PTIOSM Signals

After conversion from time domain to frequency domain and noise filtering of PTIOSM signals, scatter plots for the amplitude spectra belonging to the five *Plasmodium* positive and five *Plasmodium* negative samples were made (Figure 4). From the scatter plots the following observations were made: At some frequency bands of the PTIOSM signal, the *Plasmodium* positive PTIOSM signals had more spectral energy compared to *Plasmodium* negative PTIOSM signals and therefore it was possible to find a threshold value that differentiated infected samples from the non-infected ones. In the case of the pulsed red light excitation (Figure 4(a)), 100% classification accuracy of *Plasmodium* positive samples from *Plasmodium* negative samples was possible at frequency band between 1.7 MHz and 1.8 MHz because the signal amplitudes of all infected samples were greater than those of *Plasmodium* negative samples. This means that at this frequency band, the *Plasmodium* parasite detection sensitivity and specificity was 100% (there were no false positives or false negatives). For the PTIOSM signals due to pulsed green light excitation (Figure 4(b)), the best classification accuracy was observed at frequency band between 1.5 MHz and 1.6 MHz. At this frequency band, the signal intensities from all the five *Plasmodium* positive samples were greater than the *Plasmodium* negative signals except for one *Plasmodium* negative sample signal whose amplitude was comparable to those of positive samples. This represents a classification specificity of 100% and sensitivity of 83.3%.

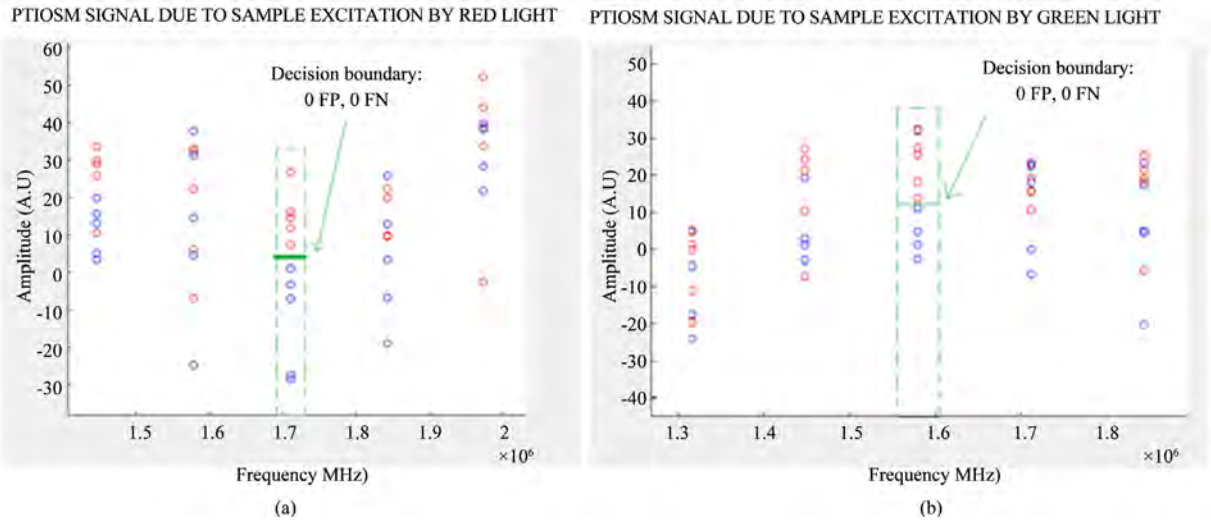

Figure 4. Frequency spectra of PTIOSM signal for malaria infected (red circles) and non-infected samples (blue circles). (a) PTIOSM signal due to red light excitation; (b) PTIOSM signal to blue light modulation. FP is false positive samples and FN is false negative samples.

4. Discussion

PTIOSM technique ability to correctly classify malaria infected blood smear samples from the non-infected samples can be attributed to two factors; change of hemozoin's refractive index during optical excitation hence causing a significant modulation of the PTIOSM signal at frequency band corresponding to its size. Another possible explanation could be that the excitation beam causes generation of photoacoustic waves, which in turn induces vibration of optical scatterer in the medium leading to modulation of diffuse reflected light from the sample. Variations of signal intensity from samples having the same infection status (either infected or non-infected) can be attributed to two possible factors: the concentration of responsible chromophores (in this case hemozoin and hemoglobin) and the optical excitation intensity.

From the reported results, the developed PTIOSM probe has demonstrated excellent capability of malaria diagnosis in blood smear samples. The simple instrumentation involved and its real time operation makes it a suitable candidate for mass screening of the disease in malaria endemic regions. However, further testing using more samples prepared under different conditions (both thin and thick blood smears, stained and non-stained) are required to conclusively determine the techniques sensitivity and specificity. Besides, further experimentation is required to investigate the capability of the probe in quantifying the parasite load (parasitemia) in the samples and the limit of detection of the technique.

5. Conclusion

A novel, simple photo-thermal based technique (PTIOSM) for detection of *Plasmodium* parasites in infected blood smear samples has been described. A RGB LED was used as the optical source to supply both the PB and the EB. Diffusely reflected PB light from the sample was detected by a photodiode, amplified and the signal preprocessed for noise cancellation. Fourier transform was used to convert the signals from time domain to frequency domain and then some specific frequency bands were used to differentiate *Plasmodium* infected samples from non-infected samples based on an empirically determined intensity threshold. The technique yielded 100% detection sensitivity and specificity using red LED light as the excitation beam.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Histomorphological Changes in the Skin and Eye Induced by Sub-Chronic Exposure of Wistar Rats to 3G Cell Phone Radiation

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Abstract

The effects of electromagnetic radiation produced by a 3G cell phone (third-generation) on skin tissues and eyes were investigated in terms of histomorphological parameters. A total of 26 Wistar rats (2 weeks-old, each weighing 40 g at the time of experiment) were used. They were maintained under a control room with water and food continuously available. The animals were divided into two experimental groups: Group A (Exposed) and Group B (Control), each with 13 Wistar Rats kept inside a plexi cage. Group A was exposed to a 3G cell phone radiation while Group B the control group, was not. All animals were generally anesthetized with Ketamine injection and then decapitated. The skin tissue was excised from the dorsal area and eyes samples were taken from all the rats by enucleating of the eye balls, fixed in 10% neutral buffered formalin for a minimum of 72 hours before processing through a graded alcohol and xylene was used as a clearing agent, embedded in paraffin blocks. Tissues were sectioned at 5μm thick and routinely stained with hematoxylin/eosin. Mounted slides were examined and photographed using a light microscope. Mild to severe orthokeratotic parakeratosis was observed in the skin while eye revealed loss of striation in the sclera with necrosis of the layers of rods and cones in the retina of the exposed group. We conclude that sub chronic exposure to 3G cell phone radiation impaired the protective ability of the skin and also impaired accommodation.

Keywords

Histomorphology, Radiation, Mobile Phone

1. Background of the Study

Over the past several years, the rapidly increasing use of mobile phones has

raised global concerns about the biological effects of exposure to radiofrequency (RF) radiation. Numerous studies have shown that exposure to electromagnetic fields (EMFs) can be associated with effects on the nervous, endocrine, immune, cardiovascular, hematopoietic and ocular systems [1].

Concern about human exposure to radiofrequency (RF) is not new. The conveniences and satisfaction derived in the use of Global System for Mobile communications (GSM) is being threatened by claims of adverse effects on human health by radiation coming from this device. This radiation belongs to the type called non-ionizing radiation, the health hazard of which remains debatable [2]. 3G which stands for third generation is a type of mobile phone technology that allows phone calls, text messages and accessing the internet.

However, the growth in the use of cellular phone has raised the concerns about the possible interaction between the electromagnetic fields (EMF) radiation and the biological effects on human tissues, particularly the brain and the human immune system. These concerns have induced a large volume of research studies. This effect has raised concerns about the public exposure to radiation emitted from cell phone and the possible interaction between the radio frequency (RF) electromagnetic radiation and the biological effects on human tissues, particularly the brain and the human immune system. Many research works provided evidences about the possible health effects such as; brain tumor, blood brain barrier (BBB) permeability function, sleep problems, cognitive function, DNA damage, immunity system function and stress reaction [3]. GSM is a cellular network, which means that when mobile phones connect to it by searching for cells in the immediate vicinity, it operates in a number of different carrier frequency ranges that is 900 MHz or 1800 MHz bands [4].

These bands were already allocated, the 850 MHz and 1900 MHz bands are used instead Regardless of the frequency selected by an operator; it is divided into timeslots for individual phones to be used. This allows eight full-rate or sixteen half-rate speech channels per radio frequency. These eight radio timeslots (or eight burst periods) are grouped into a Time division multiple access (TDMA) frame. Half rate channels use alternate frames in the same timeslot. The transmission power in the handset is limited to a maximum of 2 watts in GSM850/900 and 1 watt in GSM1800/1900. Code division multiple access (CDMA) is a channel access method used by various radio communication technologies. One of the basic concepts in data communication is the idea that it allows several transmitters to send information simultaneously over a single communication channel. This allows several users to share a band of frequencies. This concept is called Multiple Access. CDMA employs spread-spectrum technology and a special coding scheme where each transmitter is assigned a code to allow multiple users to be multiplexed over the same physical channel. The transmission power in the handset is limited to a maximum of 6 to 7 milliwatts [4].

Frequency is the rate at which electromagnetic fields change direction, and is measured in Hertz (Hz). One megahertz (MHz) is one million cycles per second.

Analogue telephones use frequencies between 800 and 900 MHz, digital telephones use frequencies between 1800 and 1990 MHz, while microwave ovens use a frequency of 2450 MHz. Today's mobile telephones, with a total power output of 2 W, are estimated to produce insignificant local heating, and are believed to be unlikely to produce any deleterious effects.

Skin serves as a barrier for the absorption of serious hazardous materials found in the environment [5]. In skin, normal cell function depends on the presence of an intact cell membrane, which itself can serve as a target for many toxic factors.

2. Materials and Methods

The materials used during the research included:

a) 26 Wistar Rats
b) 2 Plexi cages, shown on **Plate A** and **Plate B**.
c) Plain Tube
d) EDTA Bottle
e) Digital weighing balance
f) Veterinary thermometer
g) 3G cell phone
h) Zenker's Fluids
i) 10% neutral buffered formalin

A total of 26 Wistar rats (2 weeks-old, each weighing 40 g at the time of experiment) were used. They were maintained under a control room with water and feed administered adlibitum.

The animals were divided into two experimental groups by random selection: Group A (Exposed) and Group B (Control) each with 13 Wistar Rats kept inside a plexi cage. The signal strength of the 3G was −83 dBm to −75 dBm. It was kept 4cm from the base of the flexi cage. The exposure was for 22 hour per day. Both groups were kept under a control room separated by a distance of 6 m.

All animals were generally anesthetized with Ketamine injectionas shown on **Plate C** and then decapitated. The skin tissue was excised from the dorsal area and eyes samples were taken from all the rats by enucleating of the eye balls, fixedin 10% neutral buffered formalin for a minimum of 72 hours before processing through a graded alcohol and xylene was used as a clearing agent, embedded in paraffin blocks, **Plate D**.

Tissues were sectioned at 5 μm thick and routinely stained with hematoxylin/eosin. Mounted slides were examined and photographed using a light microscope according to the method outline by Baker *et al.*, [6].

3. Results

The mean temperatures with their standard deviations of the exposed and control groups are shown on **Figure 1**. The skin of rats in the control group B, showed histomorphologically normal epidermal epithelium and hair follicle with

no observable lesion, Plate 1.

The skin of rats (Sample A) exposed to electromagnetic (EM) fields (mobile phone) radiation for 2 months revealed mild to severe orthokeratotic hyperkeratosis, Plate 2.

The skin of rats (Sample A) exposed toelectromagnetic (EM) fields radiation for 3 months, showed on Plate 3, moderate to severe orthokeratotic hyperkeratosis Haematoxylin and Eosin (H and E).

The eye of rats in the control group sample B showed no observable lesion with histopathologically normal sclera and retina (H and E). This is shown on Plate 4.

Plate 5 shows the eyes of rats (Sample A) exposed to electromagnetic (EM) fields radiation for 2 months, which revealed loss of striations in the sclera and the retina showed necrosis of the layer of rods and cones as well as the necrosis of the outer plexiform layer (H and E).

Plate 6 shows the eyes of rats (Sample A) exposed to electromagnetic (EM) fields radiation for 3-month revealing loss of striations in the sclera whereas the retina showed necrosis of the layer of rods and cones as well as the necrosis of the outer plexiform layer, (H and E).

Plate A. Specimen A (exposed).

Plate B. Specimen B (control).

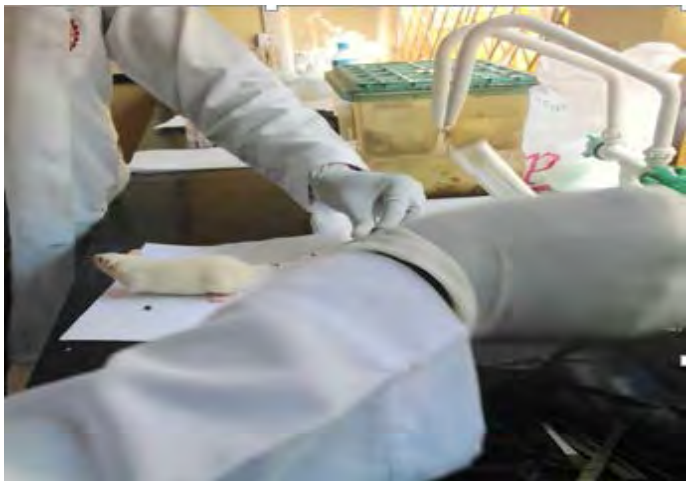

Plate C. Rat anesthetized with Ketamine injection.

Plate D. Skin and eyes samples taken from all animals, fixed in a 10% neutral buffered formalin.

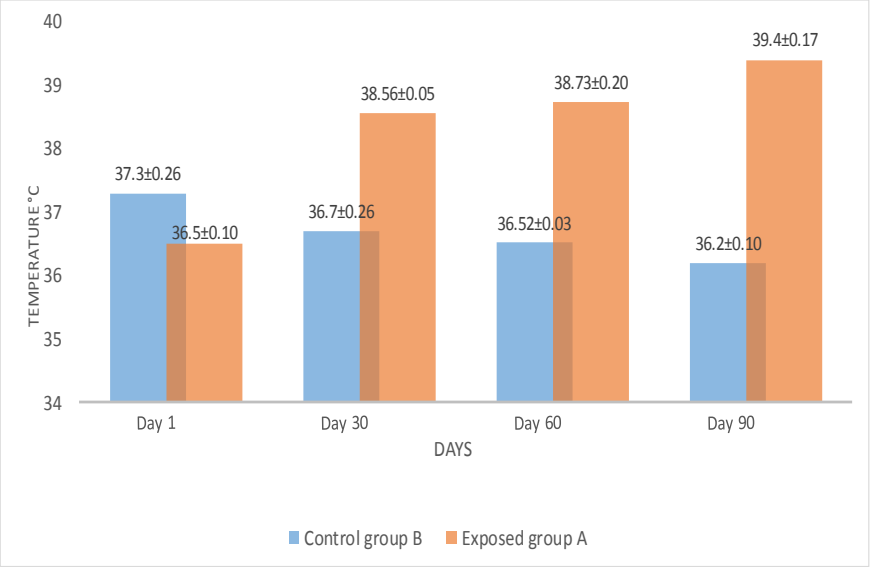

Figure 1. Temperature of exposed group A and control group B.

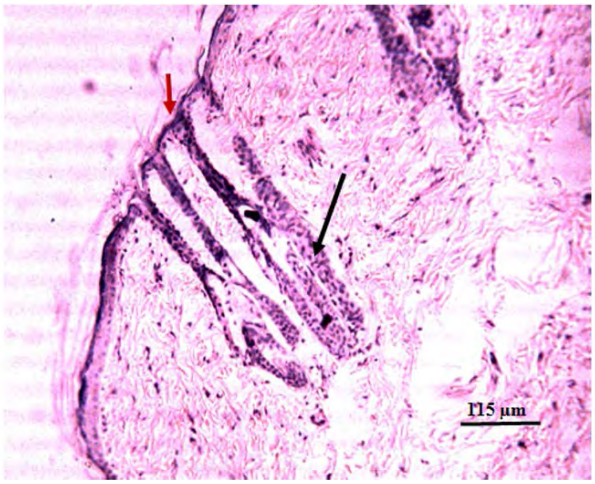

Plate 1. Photomicrograph of skin of rat showing no observable lesion, with histomorphologically normal hair follicle (↓) and normal keratin layer (↓) (H & E).

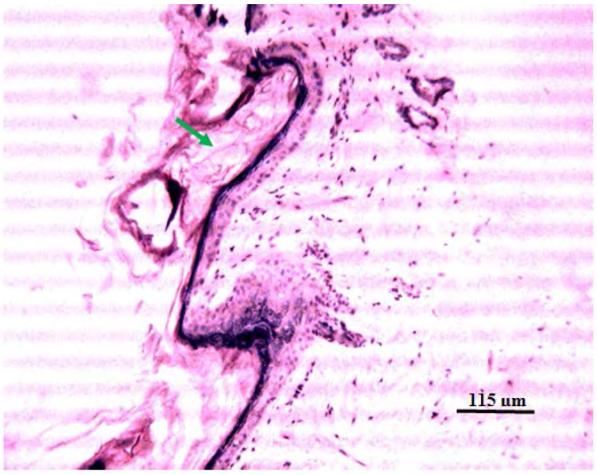

Plate 2. Photomicrograph of skin of rat exposed to 3G radiation for two months showing severe orthokeratotic hyperkeratosis (⇩) (H & E).

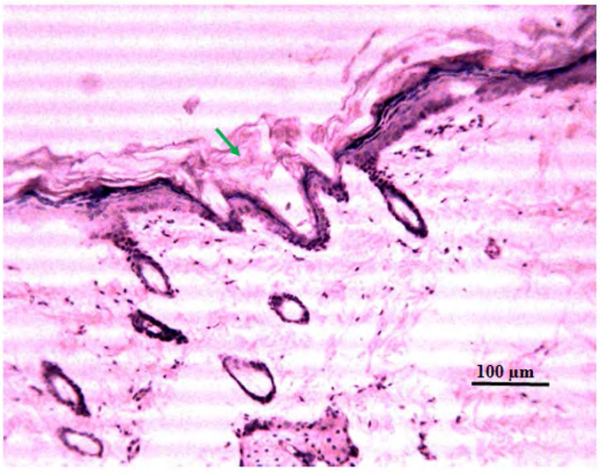

Plate 3. Photomicrograph of skin of rat exposed to 3G radiation for three months showing severe orthokeratotic hyperkeratosis (↓) (H & E).

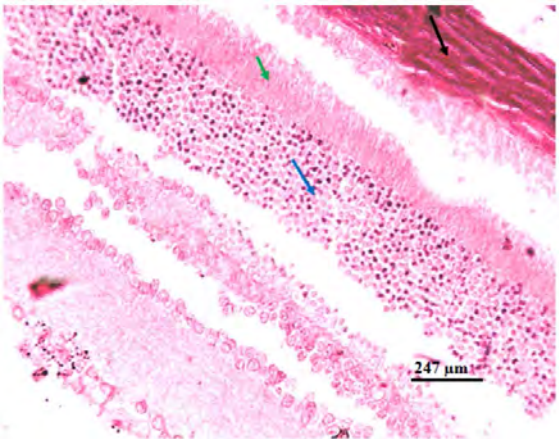

Plate 4. Photomicrograph of eye of rat showing no observable lesion, with histomorphologically normal sclera ( ), outer plexiform layer ( ) and outer nuclear layer ( ) (H & E).

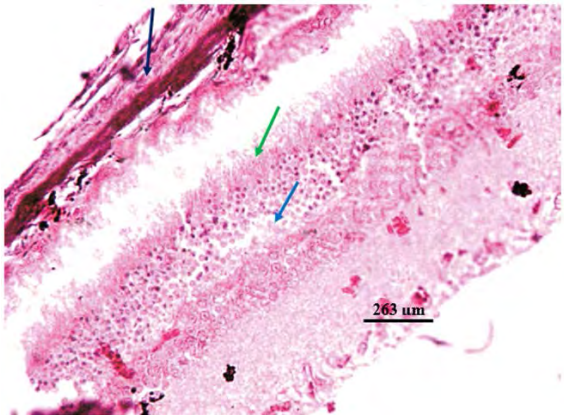

Plate 5. Photomicrograph of eye of rat exposed to 3G radiation for two months showing Zenker's necrosis of the sclera ( ), loss of striated projections at the outer plexiform layer ( ) with severe depletion of the cell of the outer nuclear layer ( ) (H & E).

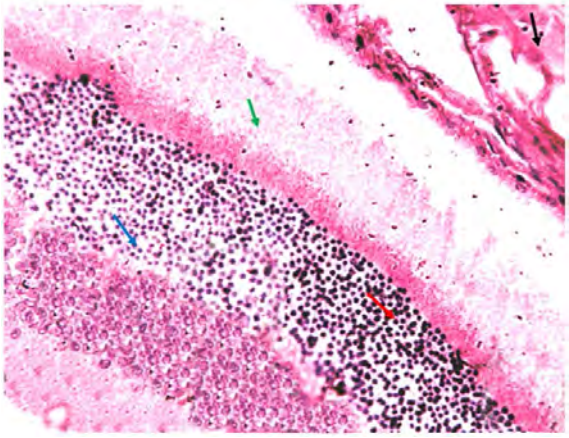

Plate 6. Photomicrograph of eye of rat exposed to 3G radiation for three months showing Zenker's necrosis of the sclera ( ), loss of striated projections at the outer plexiform layer ( )severe depletion of the cell of the outer nuclear layer ( ) with severe hyperchromatosis ( ) (H & E).

4. Discussion and Implication of Results

Following the exposure of mobile phone on the Wistar rat, when electromagnetic radiation passes from one medium to another, it can be reflected, refracted, transmitted or absorbed depending on the biological system and frequency of radiation, [7].

Skin serves as a barrier for the absorption of serious hazardous materials found in the environment [5]. In skin, normal cell function depends on the presence of an intact cell membrane, which itself can serve as a target for many toxic factors.

The differences in Photomicrograph of eyes of rats showed different layers of retina, this might be due to different cellular responses to EMF as cells might be trying to rebalance their growth and differentiation rate, [8]. On continuing the exposure, we found that the total retinal thickness in 3G group increased on 60th and 90th day. However, 3G group showed highly significant decrease in total retinal thickness on these days. It was in accordance with the findings of D'Silva *et al.* [9]. The increased intercellular spaces in the retinal layers might be due to shrinkage of cells or it might be due to cell death caused by subchronically exposure of wistar rats to RF radiation that resulted in oxidative stress rendering the cells vulnerable to damaging effects of RF radiation, [10]. The differences in Photomicrograph of eyes of rats showed the amount of damage caused by radiation [11]. Oxidative stress is an important factor in the pathophysiology of this effect, which usually causes fibrosis [12].

4.1. Implication of Temperature

Due the general principle of interaction between the cell phone radiation and dielectric (water) molecules, it is expected that the temperature should be increased. This was however, observed every irradiation session during the research. The temperature increased between ranges of 0.2˚C - 0.6˚C can caused micro thermal effect in cellular and subcellular levels. The results of this study and International Commission of Non-ionization Radiation Protection (ICNIRP) reports showed the people who spend more than 50 minutes a day using a cell phone could have early dementia or other thermal damage due to the burning of glucose in the brain [13].

4.2. Implication on Eyes

This change is probably due to natural cell death or apoptosis that normally happens in ganglion cell layer towards the end of gestation. This probably would have resulted in decreased thickness of inner plexiform layer due to loss of synaptic contact between ganglion cells and cells of inner nuclear layer. These changes show an early onset of maturation of retina in exposed groups than the control group [9] [13].

4.3. Implication on Skin

It is known that the effect and the amount of damage caused by radiation are

positively correlated with exposure time [11]. Oxidative stress is an important factor in the pathophysiology of this effect, which usually causes fibrosis [12].

The irradiated group showed an increased thickness of the stratum corneum, atrophy of the epidermis, papillomatosis, an enhanced level of basal cell proliferation, an increased thickness of the granular cell layer [14].

5. Conclusion and Recommendation

In our study, control group showed no significant in both eyes and skin of rats while there are some changes in the exposed group

Whether the reported structural changes in eye are reversible or not upon withdrawal of radiation source from 3G cell phone requires further study. The upcoming new generation phones (4G and 5G) widens the scope for future investigations to find out their possible effects on developing tissues and to compare it with other existing network systems [14].

Finally, some research studies indicated no clear association was found between the exposure to the EMF radiation and biological effects. One of the important reasons for the work presented in this paper is to answer the question of whether the use of 3G cell phone is harmful for users or not [14]. However, the research presented in this paper has not entirely answered the question. More long term studies are needed. What we could conclude is that heavy cell phone could be under high risk of negative effects due to the exposure of EMF radiation. This conclusion indicate that caution is needed when using cell phones and more research is necessary for risk assessment based on higher number of long-term users.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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