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Original Article

Advanced Electromagnetic Radiation

Pak Armed Forces Med J 2020; 70 (5): 1233-39

THERAPEUTIC EFFECTS OF HIGH FREQUENCY ELECTROMAGNETIC RADIATION AND ULTRASOUND ON INTERSTITIAL INFLAMMATION AND PROXIMAL TUBULAR DIAMETER IN KIDNEYS OF CHICK EMBRYO EXPOSED TO ADVANCED ELECTROMAGNETIC RADIATION (WI-FI)

Zubia Iftikhar, Khadija Qamar, Iram Zakria, Noreen Akhtar*, Mehwish Abaid, Aqsa Zahid

Army Medical College/National University of Medical Sciences (NUMS) Rawalpindi Pakistan, *Armed Forces Institute of Rehabilitation Medicine/National University of Medical Sciences (NUMS) Rawalpindi Pakistan

ABSTRACT

Objective: To assess the ameliorative effects of therapeutic ultrasound and high frequency electromagnetic radiation on inflammation and proximal convoluted tubular diameter, after damage induced by wireless fidelity, on kidneys of chick embryo.

Study Design: Laboratory based experimental study.

Place and Duration of Study: Department of Anatomy, Army Medical College, in collaboration with Armed Forces Institute of Rehabilitation Medicine Rawalpindi, from May to Jun 2019.

Methodology: Forty day "0" eggs of Fayomi breed weighing 48-50 grams were divided into four groups. Group A served as a control group while group B, C and D were the experimental groups exposed to wireless fidelity for 30 minutes daily for 15 days. Group C was further given therapeutic ultrasound while group D received high frequency electromagnetic radiation in the form of microwave diathermy.

Results: Therapeutic ultrasound and high frequency electromagnetic radiations both improved the interstitial inflammation (*p*-value <0.001 on intergroup comparison), and proximal tubular diameter (group A = 59.52 ± 4.55 µm, group B = 75.48 ± 1.32µm, group C, = 61.15 ± 2.71µm, group D = 56.15 ± 1.94µm; *p*-value <0.001.

Conclusion: Therapeutic ultrasound and high frequency electromagnetic radiation had ameliorative effects in reverting derangements induced by Wi-Fi in kidneys of chick embryo with HF-EMR being superior to ultrasound.

Keywords: High frequency electromagnetic radiation (HF-EMR), Therapeutic ultrasound (USG), Wireless fidelity (Wi-Fi).

INTRODUCTION

For several years researchers have devoted their time and effort to determine the possible hazardous effects of different types of radiation exposures and their outcome in terms of histological, gross and biochemical changes in tissues[1]. The possible mutagenic effect of these radiations is also studied in great detail[2,3]. Earlier when these hazardous effects of radiations were documented, studies were more focused to minimize the exposure as much as possible. With the technological boom and the widespread use of cellphones the exposure limitation has become a challenge and researchers felt the need to shift their focus from prevention to treatment. Recent studies document the possible therapeutic effects of high frequency electromagnetic radiation (EMR)[4] while targeting the beneficial outcomes of therapeutic ultrasound usage but which modality is superior remains unanswered. Tissue derangements caused by Wi-Fi have been studied previously as well and one such study was carried out to assess the impact of these radiations on testicular inflammatory pathway biomarkers in young rats. It showed that EMR caused testicular degeneration owing to oxidative stress and inflammation culminating in infertility[5]. Currently, there is evidence supporting that Wi-Fi not only causes inflammation but halts recovery from inflammation as well. A study conducted in this regard showed that Wi-Fi exposed rats having inflammation showed statistically significant

Correspondence: Dr Zubia Iftikhar, Anatomy Department, Army Medical College Rawalpindi Pakistan

Received: 28 Jan 2020; revised received: 06 Jul 2020; accepted: 08 Jul 2020

decreased morphine mediated antinociceptive effects[6]. Yet another study revealed the effects of Wi-Fi on the HEK293 cells and showed that Wi-Fi exposure caused oxidative stress and apoptotic activation in these cells[7]. While there is enormous evidence proving the hazardous effects of Wi-Fi on many organs, beneficial modalities have also been under the microscope of research. For instance, the beneficial effects of therapeutic ultrasound have been documented in studies which investigated its role in managing pain. The pain and inflammation of osteoarthritis was successfully addressed in a human sample using therapeutic ultrasound[8]. Therapeutic ultrasound also delivered promising results when its efficacy in reducing inflammation of planter fasciitis was tested in a study[9].

The other modality under the antenna of research is HF-EMR. In the form of microwave diathermy, it has proven efficacy in reducing edema, pain and inflammation. One study showed that a combined approach, including microwave diathermy showed significant improvement in managing tension type headaches[10].

This study was aimed to determine the possible reversal of radiation damage in renal tissue of developing chick embryo by using both electromagnetic radiation of high frequency and therapeutic ultrasound to provide an inter-group comparison and point out the superior modality as well.

METHODOLOGY

It was a laboratory based experiment and non-probability consecutive sampling technique was used to draw the sample[11].

This study was carried out in the department of Anatomy, Army Medical College Rawalpindi in collaboration with Armed Forces Institute of Rehabilitation Medicine (AFIRM), after seeking approval from the Ethics Review Committee, Army Medical College, and National University of Medical Sciences, Rawalpindi, from May to June 2019.

Forty fertilized eggs of Fayoumi breed were used for experiment. The eggs were obtained from Government. Post Graduate Institute for Poultry Research. Day "0" eggs were obtained and carried to the incubators placed at AFIRM. Optimum heating was ensured during the transit of eggs and minimum time delay was ensured. The eggs were then marked and grouped into 4 groups, with each group having 10 eggs. Group A served as control group. Ten eggs of group A were kept in a separate incubator. These eggs neither received any Wi-Fi radiations nor any therapeutic modality. Group B was the experimental group which was only exposed to Wi-Fi radiations alone and no therapeutic modality was offered. Group C was the experimental group which was first exposed to Wi-Fi radiations similar to group B in addition to receiving therapeutic modality in the form of therapeutic ultrasound. Group D was the experimental group which was first exposed to Wi-Fi radiations similar to group B and C, but also treatment modality in the form of microwave diathermy. The incubation period of all the groups was same i.e.; 15 days. The overall ambiance remained constant for all the groups throughout the experiment. The duration of exposure to Wi-Fi was constant for all the experimental groups. Group A was compared with group B to observe the damaging effects induced by Wi-Fi radiations. Group B was compared with group C and group D to figure out the possible therapeutic effects of the modalities implied. Group C and D were compared to technique the superior modality which had benefitted the most. Group C and D were compared with group A to assess if it was close to normal cytoarchitecture. In the experimental group, advance EMR was given by downloading an application on a mobile phone placed in the middle of 30 eggs; 15 eggs on each side, so that the eggs lie within one wave length of Wi-Fi (λ=12.5) radiations[11].

Calculated dose of therapeutic ultrasonography (USG) was given to eggs, in group C according to US dose chart 2015[12]. After application of gel on the side marked "X" of each egg probe transmitting 3 MHz was applied with intensity of

0.1-0.3W/cm² for 5 minutes daily for 15 days. Calculated dose of HF-EMR was given to eggs in group D[13]. This dose calculation was based upon the automated programming of the apparatus. HF-EMR of 100 MHz of 50 gauss was applied for 3 minutes for 15 days. At the end of each experiment, the chick embryos were dissected by opening the shell and removing the chorioallantoic and amniotic membranes and removing the yolk. The embryos were decapitated and then dissected by using the mid line ventral approach. The overlying structures were all removed carefully, to locate kidneys on the posterior abdominal wall. The tissues were fixed using 10% formalin. Hematoxylin and eosin were used for staining tissue sections. Special stains were not opted as transmission electron microscopy was done for studying ultrastructural changes. For the assessment of qualitative parameter of inflammation, all the slides were observed at 40X magnification to assess the presence and degree of parenchymal inflammation. Scoring was done as percentage of area involved[14]. For the assessment of quantitative parameter of proximal tubular diameter, three random fields of each specimen were studied at 40X and a total of 10 rounded tubules in all fields were measured. Images were taken and then opened in image J software for measurements and a multiplication factor of 2.5 was used to set the scale. The maximum tubular diameters were taken from the basement membrane of the cells on one side to the basement membrane of the cells on the other side. The mean of total 10 reading per specimen was recorded.

The data were entered in database and Statistical Package for Social Sciences (IBM-SPSS) version 21 was used for data analysis. One way ANOVA was applied for intergroup comparison of quantitative variables which were measured as means and standard deviations. A *p*-value of ≤0.05 was considered significant. Qualitative variables were presented by frequency and percentage. Chi square test was applied for comparison of qualitative variables.

RESULTS

Presence or absence of inflammation in renal tissue and the degree of severity were assessed at 40X magnification (table-I, fig-1 & 2). The control group did not exhibit inflammation in any of 10 samples. The examination of H and E stained slides of the experimental group B revealed that there was disruption of cellular architecture in renal tissue. There was inflammation noted both

Table-I: Comparison of inflammation between control group A and experimental groups B, C and D with intergroup comparison.

Groups	Inflammation Absent	Inflammation Mild	Inflammation Moderate	Inflammation Severe	Inflammation Very Severe	*p*-value
A	100%	-	-	-	-	<0.001
B	-	10%	50%	30%	10%	
C	30%	70%	-	-	-	
D	80%	20%	-	-	-	
A	100%	-	-	-	-	0.003
C	30%	70%	-	-	-	

Table-II: Mean tubular diameters of PCT in control group A and experimental groups B, C and D.

Groups	A	B	C	D	*p*-value
Mean tubular diameter (µm)	59.52 ± 4.55	75.48 ± 1.32	61.15 ± 2.71	56.15 ± 1.94	<0.001

Table-III: Intergroup comparison of mean tubular diameters of PCT in control group A and experimental groups B, C and D.

Groups	Comparison	*p*-value
A	B	<0.001
	C	0.596
	D	0.62
B	C	<0.001
	D	<0.001
C	D	0.002

in the glomeruli and in the interstitial tissue. Inflammatory foci were seen upon microscopic examination. Group C showed fewer inflammatory foci and increased cellularity. While group D showed minimal inflammatory foci, but the cellularity remained increased in this group as well. Shrunken glomeruli and increased urinary space were among the striking histological findings among specimen of group B. Group C and D showed glomeruli which tend to revert back to the diameters closer to those of group A, although group D showed maximum improvement in this aspect. In control group A, no inflammation was seen in any of the specimens (grade 0). Group B showed <25% interstitial inflammation in one specimen (grade I), 25-50% in five specimens (grade II), >50% in three specimens

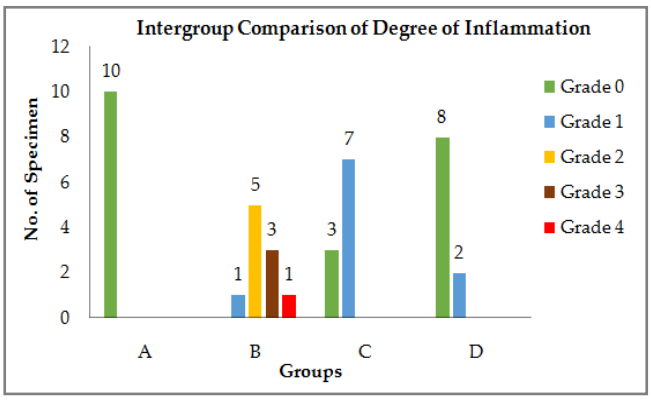

Figure-1: Fequency of inflammatory infiltrates in control group A and experimental groups B, C, and D.

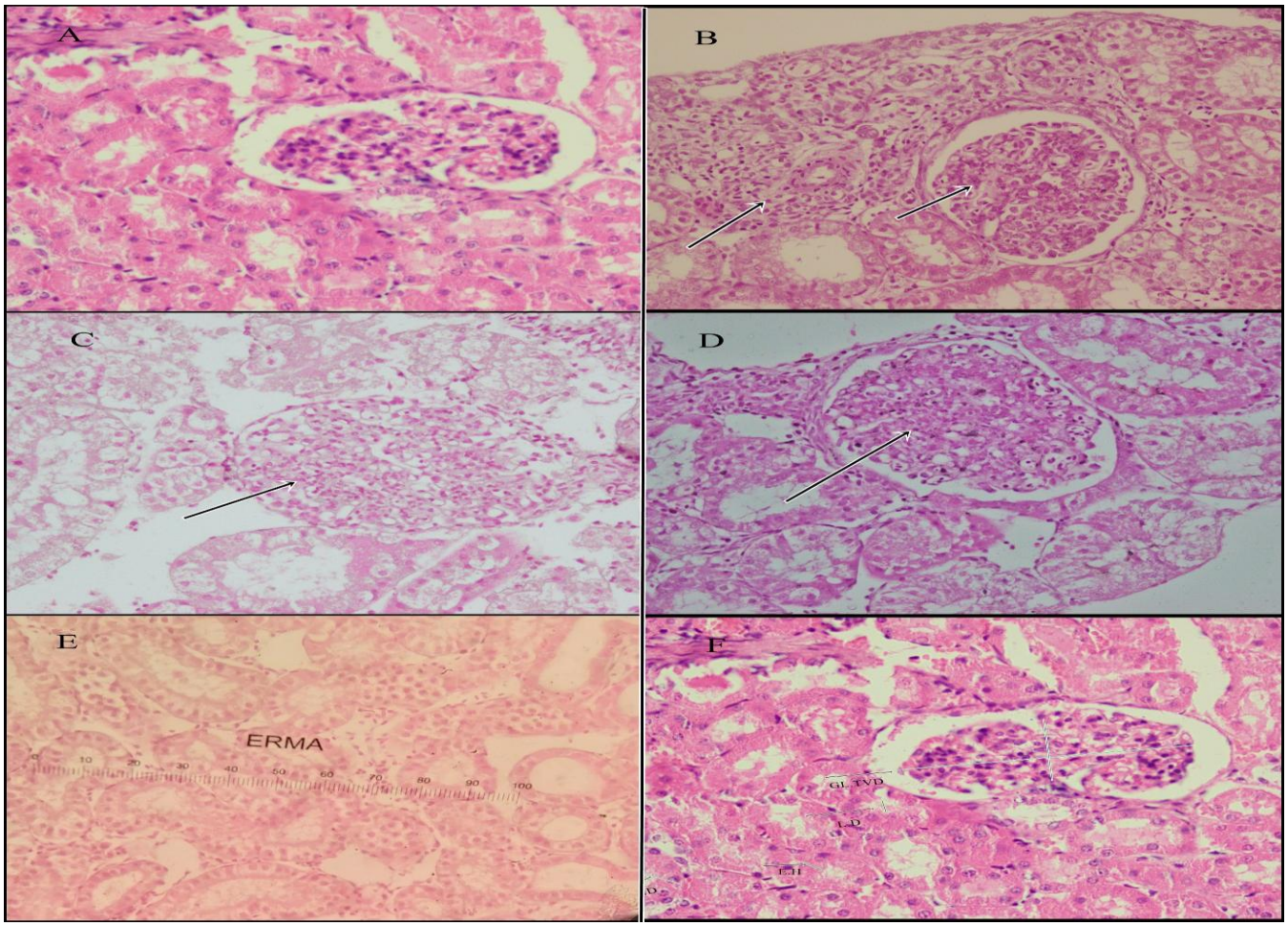

Figure-2(A-F) A, B, C and D from respective groups showing grade III interstitial inflammation (black arrows) in group Band grade I interstitial Inflammation (black arrow) in group C and D respectively. Inflammatory foci are visible. (40X, H & E). Photomicrograph E represents the scale bar at 40X and photomicrograph F depicts the method of taking microscopic measurements at 40X.

(grade III), and entire interstitium was found to be involved in one specimen (grade-IV). Group C showed absence of inflammation in three specimens (grade 0) while seven specimens exhibited <25% area involved in inflammatory changes (grade-I). Group D showed absence of inflammation in eight specimens and two specimens showing involvement of <25% area (grade-I).

Mean tubular diameter of proximal tubules in the renal tissue of the control group came out to be 59.52 ± 4.55µm while group B showed a

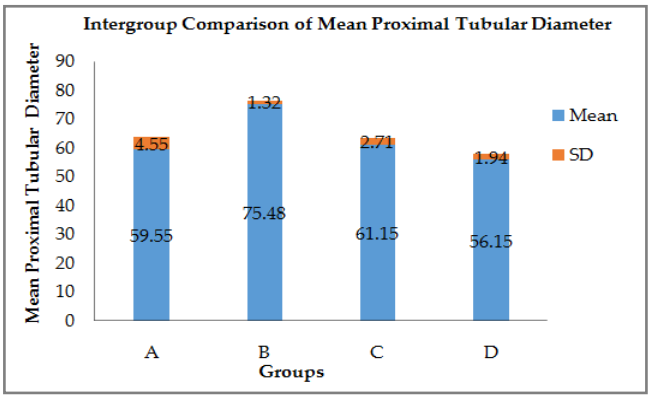

Figure-3: Comparison of mean proximal tubular diameter between control group A and experimental groups B, C and D.

mean tubular diameter of 75.48 ± 1.32µm, group C had a mean tubular diameter of 61.15 ± 2.71µm, while group D had a mean set at 56.15 ± 1.94µm respectively (table-II & III, fig-3).

DISCUSSION

The major function of kidneys is to remove all waste metabolites by the ultra filtration of blood into forming what is termed as urine. So, it is obvious that kidneys receive more of an insult casused by harmful metabolites by virtue of the nature of their function, making renal tissue impairment and metabolic derangements a common issue clinicians come across in their professional practices. From simple dehydration culminating into hypovolemic shock and acute renal shutdown causing acute tubular necrosis[15] to nonsteriodal anti-inflammatroy drugs (NSAIDs) and other pain killer induced oxidative stress, it is the renal tissue which is rendered vulnerable[16].

Apart from this, radiation induced damage in renal tissue has become a growing concern for clinicians as technology in terms of telecom has progressed by leaps and bounds with nothing to keep a check on the amount of an individual's exposure to these radiations[17]. In 2018, M Berkoz in Turkey found a highly significant association between the exposure to Wi-Fi and their effect on renal tissue, in terms of oxidative stress when melondialdehyde (MDA) and nitric oxide (NO) levels in kidneys of experimental groups in rats were higher than control group while the levels of glutathione reductase (GSH) and catalase were significantly lower in experimental group than control group (p-<0.001) it[18]. Bedir in 2018 also got highly significant results (p<0.001) when he established acute renal injury in rats exposed to electromagnetic radiation[19].

Upon examination of the routinely stained hematoxylin and eosin histological slides, the remarkable observations included inflammatory changes. No inflammation was recorded in control group A while group B showed grade I inflammatory changes in one specimen, grade II inflammatory changes in five specimens grade III inflammation was present in three specimens and one specimen showed grade IV inflammation. Group C showed absence of any inflammatory changes in three specimens and grade I inflammation was observed in seven specimens. Group D showed absence of inflammatory changed in eight specimens and two specimen showed grade I inflammation. The grading of inflammation was done at 40X using modified criteria[14].

This result was in accordance with the result obtained by Kalaivani[20] and Rehman[21] who obtained similar findings after damage were induced in the renal tissue by NSAIDs and Wi-Fi respectively.

While observing the quantitative parameters, our findings concluded that there was a highly significant difference observed between control group A and experimental group B with p-value of <0.001. It was observed that the mean tubular diameter of experimental groups was increased.

This was suggestive of the swelling of the cells of the tubules and thickening of the basement membranes. Upon inspection of slides lytic changes and vacuolar degeneration could be evident. These changes contribute in the deterioration of renal function on the whole. When the tubular diameter, increases out of proportion to the luminal diameter then the filtrate starts accumulating in the Bowman's capsule, thereby increasing the capsular hydrostatic pressure. Rise in the hydrostatic pressure hampers the net filtration by lowering the net filtration pressure. Thus, glomerular filtration rate (GFR) is reduced markedly and renal impairment ensues[22].

While experimental group B showed derangement of architecture, the experimental groups C and D both showed improvement in a way that there was significant difference seen in intergroup comparison of group B with group C and D with *p*-value <0.001 in both cases. From this observation it can be concluded that both interventional modalities played a role in normalizing the damage induced by Wi-Fi as the intergroup comparison of both groups C and D with group A yielded no significant results. Also, the results were able to help point out that HF-EMR brought more improvement in terms of normalizing proximal convoluted tubular diameters as compared to ultrasound.

Similar results regarding tubular diameters were reported in a study conducted in Pakistan by Rahman in 2015, using chick embryo model, where after exposure to Wi-Fi, significant difference in tubular diameters was noted (*p*-value <0.005)[21]. Another study, conducted by Dasdag, in 2015, to figure out effects on reproductive system of rats exposed to Wi-Fi, showed derangements in seminiferous tubules where the tubular diameter and thickness of tunica albuginea were significantly reduced (*p*-value <0.001)[23]. A study conducted by Asghari in 2016, also revealed similar damaging effects on reproductive tissue as documented by many others[24]. Yet another study, done in Turkey by Topal in 2015, using pregnant rat model exposed to Wi-Fi showed severe hydropic degeneration in liver parenchyma of the newborn rats, thereby, demonstrating the wide spectrum of damage caused by Wi-Fi in various organs[25].

ACKNOWLEDGEMENT

The authors are highly thankful to the officers and staff at AFIRM for their facilitation and would like to extend their gratitude to the officers and staff at CREAM Lab, AMC, for their support as well.

Disclosure

We are highly indebted to National University of Science and Technology for providing grant for this project.

CONCLUSION

Therapeutic ultrasound and high frequency electromagnetic radiation had ameliorative effects in reverting derangements induced by Wi-Fi in kidneys of chick embryo with HF-EMR being superior to ultrasound.

CONFLICT OF INTEREST

The study has no conflict of interest to be declared by any author.

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