Changes in Molecular Structure of Hemoglobin in Exposure to 50 Hz Magnetic Fields

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Abstract: The rapid development of new technological applications of static magnetic fields such as security systems, power plants and in medical practice as magnetic resonance imaging –MRI affects the human population. In certain occupations, clinical patients are exposed to over magnetic field strengths. The aim of this work is to elucidate the biological effect of short duration exposure, (one hour / day for a period of four consecutive days) to moderate and intense static magnetic field in the range of (0.5, 1.0 and 1.5T) which equivalent to that emitted from common sources of static magnetic field as (MRI) systems on the absorption spectra of hemoglobin (Hb) molecules and on its electric conductivity. In addition, the protein content and the electrophoresis mobility were measured for the plasma from the control and exposed albino rats, about 2 months age. The results indicated that, exposure of the animals to moderate and strong static magnetic fields resulted in changing in the absorption spectra and conductivity measurements of Hb molecules. Moreover, the total protein of the plasma increased and the electrophoretic mobilities also changed. It was concluded from the results that exposure to either diagnostic or interventional (MRI) may be hazardous for patients and more hazardous for machine operators. [Nature and Science 2010;8(8):236-243]. (ISSN: 1545-0740).

Keywords: Magnetic field; Hemoglobin; Plasma; Conductivity; Electrophoresis

1. Introduction:

During the past decade, considerable evidence has been accumulated with regard to the biological effects, both *in vivo* and *in vitro*, of magnetic field specially focused on sources of exposure and interaction mechanisms(Rongen, 2005; Straume, 2008 and Kundi, 2009) . Feychting (2005), listed the sources of human exposure to static magnetic fields. (1) In certain occupation settings e.g. in the aluminum and chloralkali industries, in arc welding processes, certain railways systems and (MRI) operators. (2) Non occupational settings (MRI) for medical diagnosis are another source. She summarized the epidemiological evidences of static magnetic field exposure and long term health effects, and the majority of these have focused on cancer risks, and reproduction (spontaneous abortions, infertility, gender ratio, developmental effects).

In an attempt to explain the biological effects of static magnetic fields (SMFs), Dini and Abbro (2005), classified SMFs as weak (< 1mT), moderate (1mT to 1T), strong (1-5T) and ultra strong (> 5T).

There is substantial evidence indicating that moderate intensity SMFs are capable of influencing a number of biological systems, particularly those whose function is closely linked to properties of membrane channels (Rosen, 2003) and ionic composition (Amara et al., 2004). Moderate intensity (SMFs) induced modifications of cell shape, cell surface and cytoskeleton, progressively achieved during the entire period of exposure, the cell had a less flat shape due to partial detachment from the culture dishes (Dini and Abbro, 2005). Henrykowska et al. (2009), indicated that exposure to 50 Hz magnetic field of 10 mT induced oxidative stress and free radicals generation in human blood platelets producing a number of adverse effects within the cell and thus may lead to systemic disturbances in the human body.

Some results indicated that working near a 1.5T (MRI) system or while seated close to the bore of the field (where the field strength was less than 0.7T) may lead to neurobehavioral effects (De Vocht et al., 2003) and neuro- cognitive function adverse effect (Chakeres and de Vocht, 2005). Clements et al. (2000), followed 20 children exposed to MRI at four times within 20 weeks, and 35 unexposed children from birth until 9 month of age .The results showed a small decrease in lengths of exposed children, and an increase in gross motor function.

Ali et al.(2003), studied the effect of 50 Hz, 0.2 mT magnetic fields on red blood cells properties and heart functions of albino rats. The result indicated decrease of RBCs membrane elasticity, permeability, and changes in molecular structure of hemoglobin. The injuries of the heart of the animals were attributed to the loss of some physiological functions

of the RBCs because of exposures of the rats to the magnetic field. Recent studies found a high relation between exposure to non ionizing radiation and acute childhood leukemia (Calvente et al., 2010). Feizi and Arabi (2007), concluded that the presence of high-voltage overhead power lines within 500 m of residential areas should be considered a risk factor for acute childhood leukemia. Exploring the biological effects of static magnetic fields has therefore become important. Investigation of the mechanisms and determination of the threshold field levels for biological effects are also essential to estimate safety of magnetic fields.

The present research work aimed to investigate the effect of short time exposure, one hour / day (represent four consecutive sessions of clinical patients exposed to moderate and strong static magnetic field of a magnetic resonance imaging system, MRI) on the molecular structure of hemoglobin and plasma total protein of albino rats.

2. Materials and Methods:

Experimental animals

Twenty-four albino rats 2-2.5 month's age were used in this experiment and equally divided to four groups housed in small cages made of non magnetic material (plastic cages) 6 rats per cage. Group 1 as control and groups 2, 3, 4 were exposed to moderate and strong magnetic fields at field strengths of 0.5, 1.0 and 1.5 Tesla respectively, for exposure rate of 1 hr / day for a period of 4 days.

Field exposure apparatus

The 50 Hz uniform magnetic field was produced using 150 MM electromagnet with two coils model 3473 E 70 manufactured in Germany, the temperature was regulated at 23±2°C through water cooling system. The magnetic flux density at its gap centre between the two poles ranged from zero to 3.1 Tesla. The magnetic field intensities were measured by using a digital teslameter (Phywe, 13610.93, Göttingen, Germany) with an axial hall probe which determines both static and electromagnetic field in the frequency range of DC – 5 KHz and in the magnetic field intensity range, 10μT- 2.5 T.

At the end of exposure periods the rats were anesthetized, and the blood sample were collected from the ventricle of the heart of the rats using heparinized syringes. The heparinized blood was then centrifuged at 4000 r.p.m. for 15 min. The supernatant were removed for the determination of plasma total protein according to the method of Lowry et al.,(1951) and electrophoretic separation of plasma proteins according to the method of laemmli, (1970).

Extraction of hemoglobin

The packed RBCs were washed three times with 5 volumes saline then recentrifuged. The packed RBCs were lysed with two volumes of deionized water. The hemolysate was centrifuged at10, 000 r.p.m for 20 min at 4°C to remove erythrocyte ghosts according to the method of Trivelli et al.(1971) and the following measurements were carried out.

Electrical conductivity measurement

The electrical conductivity was determined using a conductivity meter type digimeter L21/L21C aqualytic autotemprature (Rosenberg and Postow, 1973). Measurements were performed at constant frequency (1500 Hz in the range of 0 to 200 μ Siemen /cm). The conductivity meter was calibrated before measurements using a standard solution.

Absorption spectrum of hemoglobin

Absorption spectra of the Hemoglobin molecule for control and exposed rats were measured at wave length 200 to 700 nm by a double beam UV/Vis spectrophotometer model-240 manufactured by shimadzue -Japan.

Statistical evaluation

All results are presented as mean ± standard error of the mean. Statistical significances of the differences between the mean of the two groups of samples were assessed using Student's t test. Differences were considered to be statistically significant at $p < 0.05$, high significant at $p < 0.01$ and very high significant at $p < 0.001$.

3. Results and Discussion:

Plasma total protein

Figure 1 illustrated the protein concentration of the plasma of all groups exposed to moderate and strong magnetic field. The protein concentration of normal plasma protein was 64.8±1.9 mg/ml. After exposure of the rats to a magnetic field of strength 0.5, 1.0 Tesla per day for 4 days, the total protein high significantly increased to 75.5±2.3 mg/ml ($p<0.01$) and 80.5±4.3 mg/ml ($p<0.01$), respectively. Moreover, the protein concentration for the group exposed to 1.5 Tesla increased to 92.3±3.5 which was a very high significant ($p<0.001$).

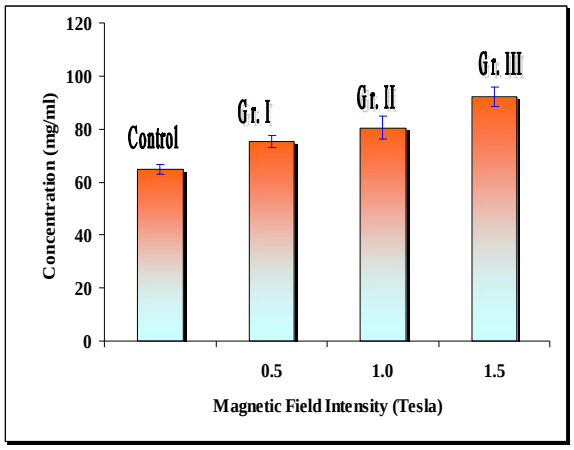

Figure 1. Protein concentration of rats' plasma after exposure to different intensities of magnetic fields.

Plasma electrophoresis

The SDS poly acrylamide gel electrophoresis profile (Figure 2) of the control plasma protein characterized by the presence of 15 bands which varies in their molecular weight and intensities. After exposure to moderate and strong magnetic field the mobility of the bands were significantly changed.

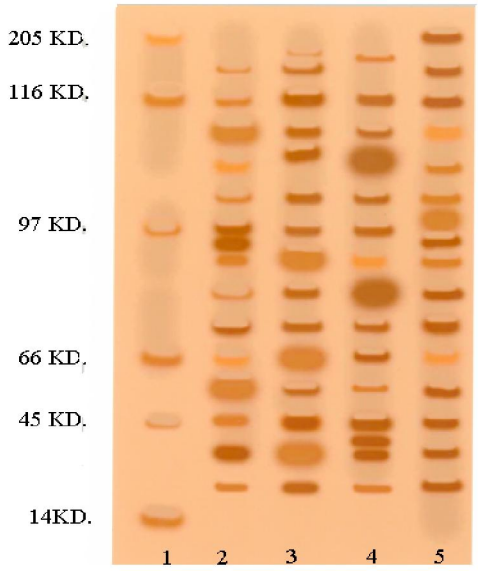

Figure 2. SDS polyacrylamide gel electrophortic separation; lane (1) represents standard protein, lane (2) represents control group, lane (3) represents group "I" exposed to 0.5 T, lane (4) represents group "II" exposed to 1.0 T, lane (5) represents group "III" exposed to 1.5 T, respectively.

The pattern showed that, there was a pronounced change in the molecular weight towards high molecular weight region. For group I and group II which exposed to 0.5 and 1.0 Tesla the molecular weight increased from 154 KD for the control group to 179 KD and 181 KD, respectively (Figures 3 and 4). Also, for group III which exposed to 1.5 Tesla the molecular weight shifted towards high molecular weight with the appearance of new two bands at 212, 163 KD (Figure 5). Moreover, there was a pronounced change in the molecular weight region of 98 KD with the appearance of new protein fractions at 92 KD for group I, 90 KD for group II . In addition, group III showed a disappearance of the band at 98 KD and appearance of new protein fractions at 90, 96, and 100 KD which illustrated a clear fractionation of the low mobile protein. The intensities of the peaks and their mobilities changed as compared with the control group.

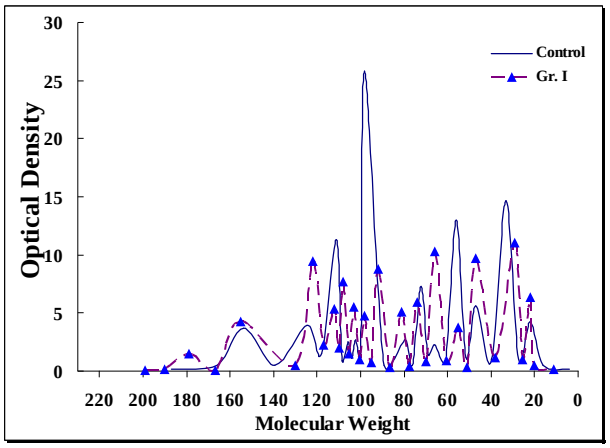

Figure 3. Electrophoretic pattern of rats' plasma protein after 1.0 hr exposure to 0.5 Tesla magnetic field.

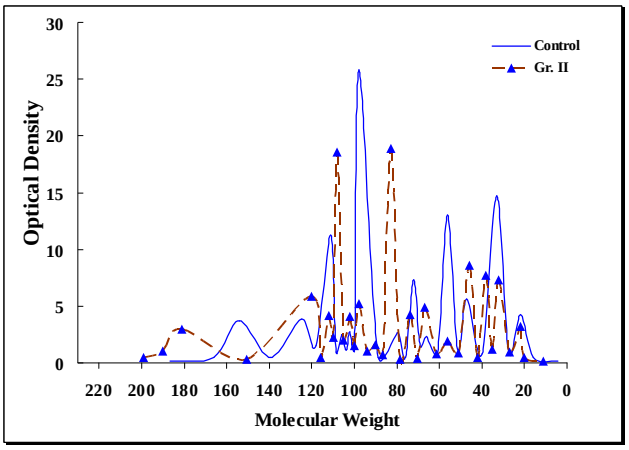

Figure 4. Electrophoretic pattern of rats' plasma protein after 1.0 hr exposure to 1.0 Tesla magnetic field.

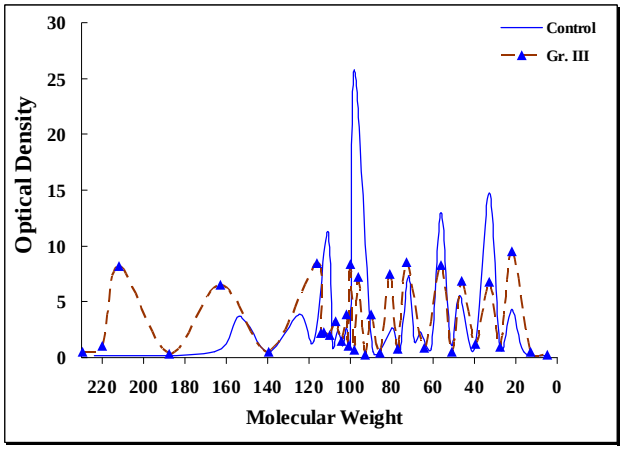

Figure 5. Electrophoretic pattern of rats' plasma protein after 1.0 hr exposure to 1.5 Tesla magnetic field..

Electrical conductivity of hemoglobin

The mean values of electrical conductivity of hemoglobin of exposed rats as compared to control group were illustrated in Figure 6. The data showed that, the electrical conductivity of control was 68.27±0.96 µS /cm and for group I exposed 0.5 Tesla showed significant increase (P< 0.05) in electrical conductivity (72.52±0.64 µS /cm). In addition, there were a very high significant increase in the electrical conductivity (P<0.001) for groups 2 and 3 exposed to 1.0 and 1.5 Tesla with a mean values of 101.85±0.74 and 108.15±0.59 µS /cm, respectively.

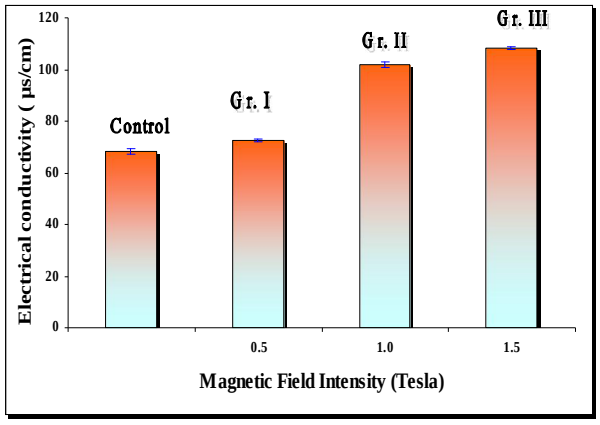

Figure 6. Electrical conductivity of rats' hemoglobin for control and after exposure to different intensties of magnetic fields.

Absorption spectrum of hemoglobin

The absorption spectra of hemoglobin were illustrated in Figure 7 at wave length range 250-700 nm. The bands were named, at 280nm (protein band), at 340 nm (globin heme interaction band) and refers to non covalent bond between globin`s histidine and heme iron, at 420 nm (soret band), at 542 nm (Fe-N in porphyrine) and at 578 nm (heme-heme interaction band). Great differences were detected in heme parts at visible wave lengths, with the appearance of a new band at 630 nm. The relatively disappearance of globin band at 270 nm and globin- heme interaction band at 340 nm when whole body exposed to moderate and strong magnetic field. Moreover, there was an increase in the width at half the maximum of the soret band besides the disappearance in the heme-heme interaction band and decrease in A_{578}/A_{542} ratio.

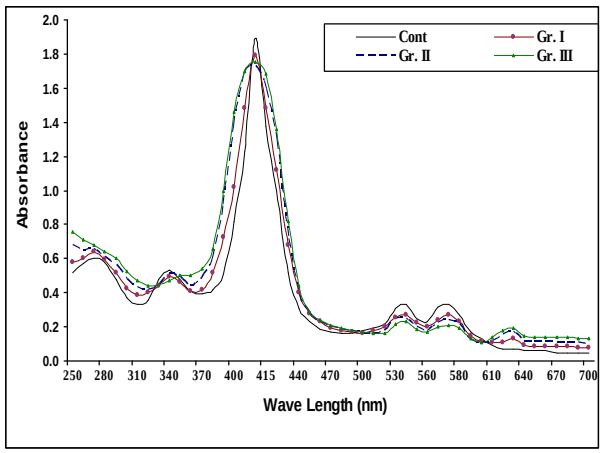

Figure 7.Absorption spectra of oxy hemoglobin for control and after exposure to different intensties of magnetic fields.

4. Discussions

The present study is an attempt to investigate the effect of strong and moderate magnetic fields with different intensities (0.5, 1.0, 1.5 Tesla) referred to different flux densities emitted from different types of scanners of MRI systems (Formica and Silvestre, 2004) , and to evaluate the degree of risk from exposure to such magnetic fields. It provides guidance for the assessment of the occupational and public health significance of magnetic fields and to indicate areas that may be hazardous.

The obtained results of the plasma protein separated by SDS poly acrylamide gel electrophoresis techniques in the presented work demonstrated a change in the protein as a result of magnetic field exposure, which in turn may result in an increase in total plasma protein. This results was in agreement with other findings reported by some authors that *in vivo* exposure to magnetic field at 1.5 mT caused significant changes in plasma protein levels of rats (Ubeda et al., 1997).This observation was supported by another work (Ibrahim et al., 2005). They concluded the same results of increasing total plasma protein induced by different intensities of 50 Hz magnetic field. In addition, Hayman et al (1991), found increase in total protein concentration caused by clinical MR images of hematomas at law and intermediate field strengths. This increase in the total protein content may give an interpretation about the appearance of high molecular weight protein as an aggregate which illustrated in Fig. 3, 4 and 5. The hypothesis of the increase of plasma protein may result in formation of new protein molecules which give us a conclusion that there is a new protein which resulted from the magnetic field exposure. After exposure to different intensities of magnetic fields, this new protein caries different charges and molecular weights that led to combination or recombination of the different plasma protein. This concluded proposal might be the main factor that plays an important role in the electrophoretic separation of the plasma proteins. As a result; the proteins occupying this wide range may represent the high molecular weight proteins and may possibly having also different charge density. The pattern indicates the formation of such larger protein structures, which completely differ from the native protein of the control plasma.

It was documented that, magnetic field induced deteriorating effect on antioxidant defensive system is contributed by other reactive oxygen species, other than superoxide radicals (Lee et al., 2004 and Henrykowska, 2009). This promoted oxy hemoglobin oxidation to Met hemoglobin. The exposure to constant magnetic field of different intensities could promote the autoxidation rate of oxy hemoglobin to met hemoglobin (Attia et al., 1995 and Sallam and Awad, 2008). This leads to broken of hydrogen bonds between hydrophobic non polar groups leading to the unfolding of globular protein .The intermolecular charge repulsion is a driving force for unfolding. These findings are in accordance with our observations of exposure to moderate and high intensity constant magnetic field in the range of 0.5-1.5 T, caused unfolding of globular protein with formation of a new groups exposed to the surface besides the polar hydrophilic groups leading to increasing in electrical conductivity.

When whole body exposed to moderate and strong static magnetic field there are different degrees of globin unfolding which termed as a sign of molecular destabilization. The magnitude of destabilization increases with increasing intensity of magnetic field, this is concomitant with increase in the methemoglobin band at 630 nm (Fig.7) indicate the increase in the number of autoxidized (ferric) hemoglobin sub units and conversion of a large amount of hemoglobin to met hemoglobin and this is easily degradated to globin and free heme . In addition, the increase in the width at half maximum of the soret band besides the disappearance in the heme-heme interaction band and decrease in A_{578}/A_{542} ratio, draw a line of evidences about the low stabilization of hemoglobin macro-molecule as a result of magnetic field effect. This effect reflects the function of hemoglobin which converted from oxy hemoglobin to non functional met hemoglobin with decreasing oxygen affinity .This was confirmed by Mawatari et al,(1983, 1987).They demonstrated the dependence of HbO_2 affinity on the spin state of ferric heme-iron and the absorbance intensity of heme-heme interaction band .All these findings makes it possible to suggest that, the hemoglobin exist in a new conformational state with low stabilization.

5. Conclusion

The available evidence from epidemiological studies on blood is not sufficient to draw decisive conclusion about potential health effects of moderate and strong static magnetic field exposure at the levels encountered in the environment or at work places.

An increasing exposure to static magnetic fields in the general public is likely, *e.g.* with the development of new medical applications using strong fields , and when magnetically levitated trains are taken into operation .

Further research in this area is warranted, *e.g.* a cohort study of MRI workers or of workers in industries where MRI systems are manufactured. A

cohort approach would allow studies of different types of outcomes and limitation.
Another exposed group is patients undergoing MRI scans. In new studies, improvement of the exposure assessments and adequate confounding control is crucial.

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