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Effect of Static Magnetic Field on Bone Marrow Cellular Density

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***Abstract*—This study was undertaken to investigate the influence of static magnetic field (SMF) on bone marrow cellular density (BMCD) variation proportionally to bone trabeculae. Female albino Wistar rats exposed with 2.4 ± 0.2 millitesla for 1–4 weeks duration continuously versus 1 h, 2 h, 6 h, and 8 h/day. Trephine biopsy of femurs bone was examined under optical microscope. Data analyzed with ImageJ software. Results showed that short time exposure per day did not enhance the BMCD compare to high exposure period/day. Six hours/day exposure during 1 week increased the marrow cellular density (hypercellularity) significantly ($P \leq 0.05$) compares to bone trabeculae. Contrarily, 8 h/day exposure reduced the BMCD slightly and significantly (hypocellularity, about 50% reduction) due to 1 week and 4 weeks exposure duration, respectively. The SMF has associated bone marrow cellularity tendency of rat's femur.**

Index Terms—Bone marrow, Optical microscope, Static magnetic field, Trephine biopsy

I. Introduction

Bone marrow examination can be aspiration or biopsy depending on clinical condition (Lee, et al., 2008). Bone marrow specimen can provide a complementary evaluation of hematology-related diseases such as anemia. Bone marrow trephine biopsies (BMTs) are used to diagnose pathological diseases such as hematopoietic disorder, cancers, and infections (Yong, 1992). The procedure of bone marrow biopsy in human can be uncomfortable and rarely hemorrhage or excessive bleeding associated. Therefore, the obtained biopsy information is outstanding to make the final clinical decision (Pedersen, et al., 1993). Bone marrow examination is mostly asked by a specialist since cytometric and microscope examinations of peripheral blood require further investigation.

The effect of pulsed magnetic field (PMF) and static magnetic field (SMF) on biological system is still under investigation (Pirkhider Yaba and Ismail, 2019). Researches have been conducted to investigate the influence of magnetic field on hematology *in vitro* and *in vivo* (Vergallo and Dini, 2018; Mustafa, Yab and Ismail, 2020d). Previously, the influence of PMF on main blood cells was examined. For instance, exposure with 0.97 millitesla (mT) during 50 and 100 days (Cakir, et al., 2009), 5 mT during 1–4 weeks, 3 μT during 15–120 days (Cetin, Bilgili and Eraslan, 2006), and 0.5 and 1.5 mT for 5 selective days (Ciejka, et al., 2011) altered blood parameters in peripheral blood. Regarding SMF exposure, 128 mT:1 h/day for 5 days (Amara, et al., 2006), 10 days (Sihem, et al., 2006), 13 days (Chater, et al., 2006), and 16 mT during 28 days (Djordjevich, et al., 2012) changed peripheral blood cells counts significantly. Since blood cells are originated from bone marrow in animals, the effect of SMF on bone marrow cellular density (BMCD) requires an investigation.

A few studies demonstrated the bone marrow activity-related magnetic field. Human mesenchymal stem cells (HMSCs) metabolism and growth were inhabited under the influence of 20 mT (50 Hz) during 23 days exposure (Yan, et al., 2010). SMF exposure (15 mT) for 5 h exhibited no effect on rats bone marrow stem cells (Sarvestani, et al., 2010). Femurs bone formation was studied under the influence of SMF (Yan, Tomita and Ikada, 1998). Bone calcium content and bone mineral density have increased significantly after 12 weeks exposure. Moderate SMF (3, 15, and 50 mT) effect on biophysical stimulator of osteoblastic differentiation and proliferation studied in human bone marrow (Kim, et al., 2015). Results revealed an increase of calcium release, proliferation, ALP activity, and mineralized nodule formation. Chondrogenic differentiation in human BMSC was stimulated with 0.4 T SMF exposure during 14 days (Amin, et al., 2014). The rate of BMSC viability and proliferation of rats has decreased under the influence of 15 mT SMF (Jouni, Abdolmaleki and Movahedin, 2013). The effect of SMF on BMCD and bone trabeculae density has not been studied previously using trephine biopsy. Here, we investigate the effect of 2.4 mT SMF on BMCD variations in rats' femurs.

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II. Procedure

A. Housing Animals

In this study, 80 female albino Wistar rats weigh 190 ± 10 g participated and divided into four main groups (Groups 1–4). Each main group of 20 rats was split into five subgroups (A, B, C, D, and E), subgroups of four rats, kept in a plastic cage having about 40 cm of length. The subgroups "A" considered as the control groups and "B–E" exposed with SMF for 1–4 weeks, respectively. The animal house provided within biology department/Salahaddin University-Erbil, Kurdistan Region-Iraq. The standard living atmosphere was 12:12 h darkness/light, temperature of 22 ± 2°C. Rats were housed in bedded plastic cages and received a standard rat chow and drink (tap water).

B. Experimental Design

To generate SMF, a set-up designed with 6 Helmholtz coils connected in series, Fig. 1. Copper wires connected the coils and a 20 V/12 Amp-DC power supply fed current to the set-up. A digital Teslameter measured the generated SMF. A highly stable and uniform SMF of 2.4 ± 0.2 mT generated in a wide area between the two ends of the set-up. Temperature and magnetic field stability observed and calibrated against operating duration. The set-up was free of sound, vibration and temperature increase during exposure operation (Mustafa, Yab and Ismail, 2020a; Mustafa, Yab and Ismail, 2020b).

C. Exposure Method

The four main groups of 1–4 exposed to the SMF for a period of 1 h/day, 2 h/day, 6 h/day, and 8 h/day, respectively. In addition, from each main group, subgroups of B, C, D, and E exposed for 1–4 weeks duration, respectively. SMF (2.4 ± 0.2 mT) exposure was applied 7 days a week, between 8:00 AM and 4:00 PM. Control groups maintained at the same room (3 meters away from exposure source) where other groups of rats exposed to SMF.

D. Bone Collection

Rats were anesthetized with a mixture of xylazine and ketamine hydrochloride. Next, they sacrificed to collect femur bone. The left side femurs was collected from each rat with a blade and cleaned. They kept in a 10% neutral-buffered formalin and taken to perform trephine biopsy (BMT) examination.

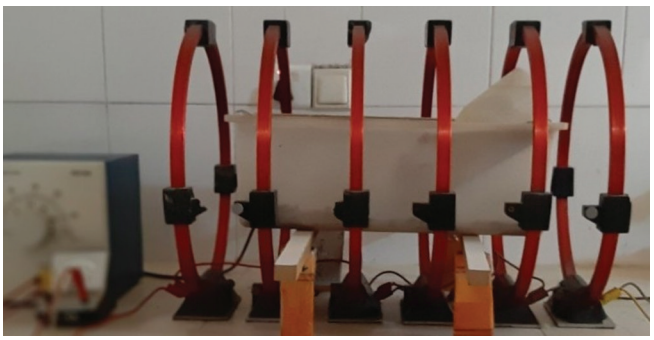

Fig. 1. Coil set-up. Six similar coils were used to generate a uniform static magnetic field. The coils were originally bought from PHYWE. Each coil consists of 154 turns and a radius of 20 cm. The length of the set-up was 70 cm.

E. Bone Marrow Biopsy and Histological Slide Preparation

The methodology of BMT is explained by John D. Bancroft (Suvarna, Layton and Bancroft, 2018). In pathology laboratory, the tissue processing of the bone marrow started as the specimens fixed in formalin and 10% acid hydrochloride for 10 h. Afterward, they inserted into formalin for 14 h. In decalcification process, the specimen placed into ethanol (96%) for 4 h. Later, the ethanol was replaced by xylol 100% for 2 h. Next, the specimen was embedded in paraffin wax overnight. During tissue sectioning process, the paraffin wax blocks were cut with 1–5 μm thickness. The sectioned specimen stained with hematoxylin and eosin, to provide excellent cytomorphology. They were deparaffinized in xylene for 10 min and rosined in distilled water (DW) for 1 min. Then, the samples left in hematoxylin for 40 min, ethanol 5 min, and finally eosin for 10 min. The slides were mounted and monitored by optical microscope device, power of 400× and 600× magnifications.

F. Data Quantification and Analyses

ImageJ software was used to analyze the microscopic images quantitatively. Particles area were determined of a minimum 10 pixels and larger. The original microscopic images converted and filtered with color threshold with a dark background (Figs. 2 and 3). Manual manipulation used to refine the converted images and qualifying trabeculae networks and the bone marrow distribution. Desired sections of trabeculae bones examined separately. Student's t-test used to determine the statistical significance of variation.

III. Results of ImageJ analysis

Quantification data of ImageJ is compared with a direct microscopic observation. The hypercellular and hypocellular cellularity of bone marrow were examined in portion of bone trabeculae. Table 1 shows the SMF effect on bone marrow cellularity due to 1 h/day, 2 h/day, 6 h/day, and 8 h/day exposure. Analyzed microscopic images are illustrated in Figs. 2 and 3. Accordingly, the SMF influenced the existence of bone marrow between trabecular networks at high exposure time per day (6 h and 8 h). Eight hours exposure per day during 1 week reduced the BM cellularity slightly. However, 4 weeks exposure decreased BMCD significantly ($P \leq 0.05$). Two weeks and 3 weeks exposure did not alter the bone marrow distribution. The 6 h/day exposure during 1 week declined the density of bone marrow significantly. Other exposure durations did not stimulate the marrow cellularity of the femurs bone.

Trabeculae bone to bone marrow ratio variation is analyzed. Microscopic images were taken of normal and variant portions and examined directly (with eyes) and indirectly using ImageJ software. Decision was made

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Table I
Cellular Tendency Response Versus 2.4 mT

1 h/day exposure	Cellular effect	2 h/day exposure	Cellular effect	6 h/day exposure	Cellular effect	8 h/day exposure	Cellular effect
Control	Normal	Control	Normal	Control	Normal	Control	Normal
1 week	Normal	1 week	Normal	1 week	Significant increase ($P \leq 0.05$)	1 week	Decrease insignificantly
2 weeks	Normal	2 weeks	Normal	2 weeks	Normal	2 weeks	Normal
3 weeks	Normal	3 weeks	Normal	3 weeks	Normal	3 weeks	Normal
4 weeks	Normal	4 weeks	Normal	4 weeks	Normal	4 weeks	Hypocellular ($P \leq 0.05$)

The bolded area demonstrates the BMCD variations under the influence of SMF

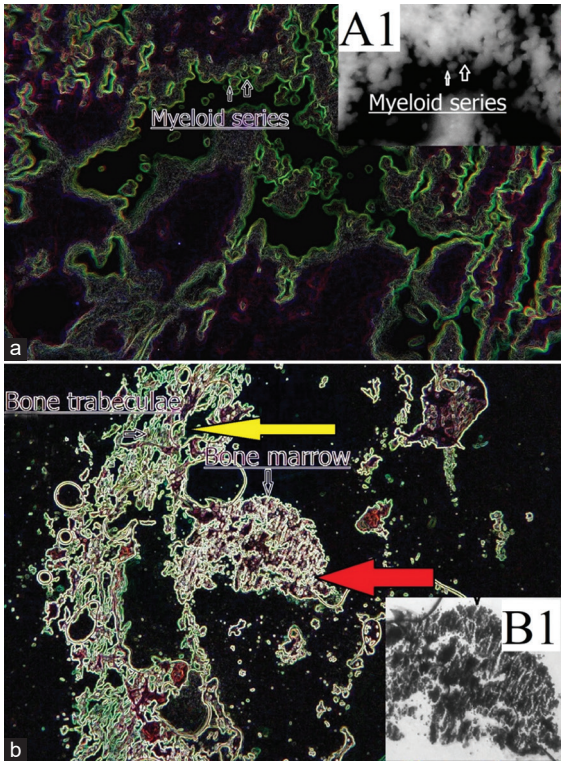

Fig. 2. Bone marrow distribution is illustrated. (a) Control image and (b) distribution of bone marrow in two different regions unequally.

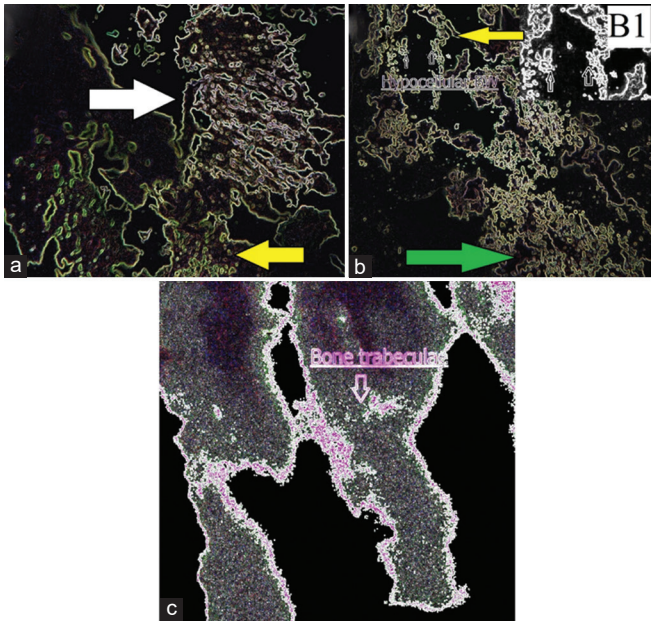

Fig. 3. Marrow cells and trabeculae bone distribution are shown with different exposure time per day. (a) Eight hours/day exposure for 1 week (bone marrow reduced insignificantly), (b) 8 h/day for 4 weeks (bone marrow reduced significantly), and (c) 6 h per day exposure (bone trabeculae increased and less marrow found).

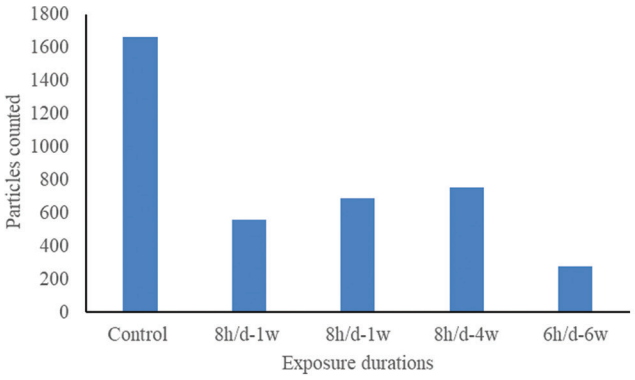

Fig. 4. Particle counted with ImageJ software. A minimum size of particles of 10 pixels is taken. Particles with a smaller than that value did not counted.

based on both analyzing methods. Since the ratio of bone trabeculae to marrow should be between 75% and 95%, a direct microscopic image can predict the change. Figs. 2 and 3 show the microscopic observation of bone marrow. Quantitative changes shown in Fig. 4. Fig. 5 represents the particle size variation and distribution under the influence of SMF. Networks of bone trabeculae found in Fig. 2. Image A is a normal distribution of the networks with a soft distribution of bone marrow uniformly. Myeloid cells highlighted as a sample of marrow cells among trabeculae. Image A1 is the filtrated portion of myeloid cells. Image B highlights two regions of bone trabeculae (yellow arrow) and marrow cells (red arrow). Bone marrow reduced slightly in the first region (yellow arrow) in respect to the second region (red arrow). Quantitative data illustrated a cell number decline compare to the control image (Fig. 4). A similar trend appears in image A of Fig. 3 (white and yellow arrows). Therefore, a highly reduction of bone marrow cellularity observed in image B (yellow arrow compares to the green arrow). Quantitatively, the particle numbers are almost half in compare to the control image (1600–800 particles). Image E is a large network of bone trabeculae with a fewer distribution of particles in between. As seen, the marrow cells reduced in ratio to trabeculae. We believe that the cellularity is varied based on a direct analyses of the microscopic images. The statistical analysis revealed a significant variation under the 6 h/day exposure.

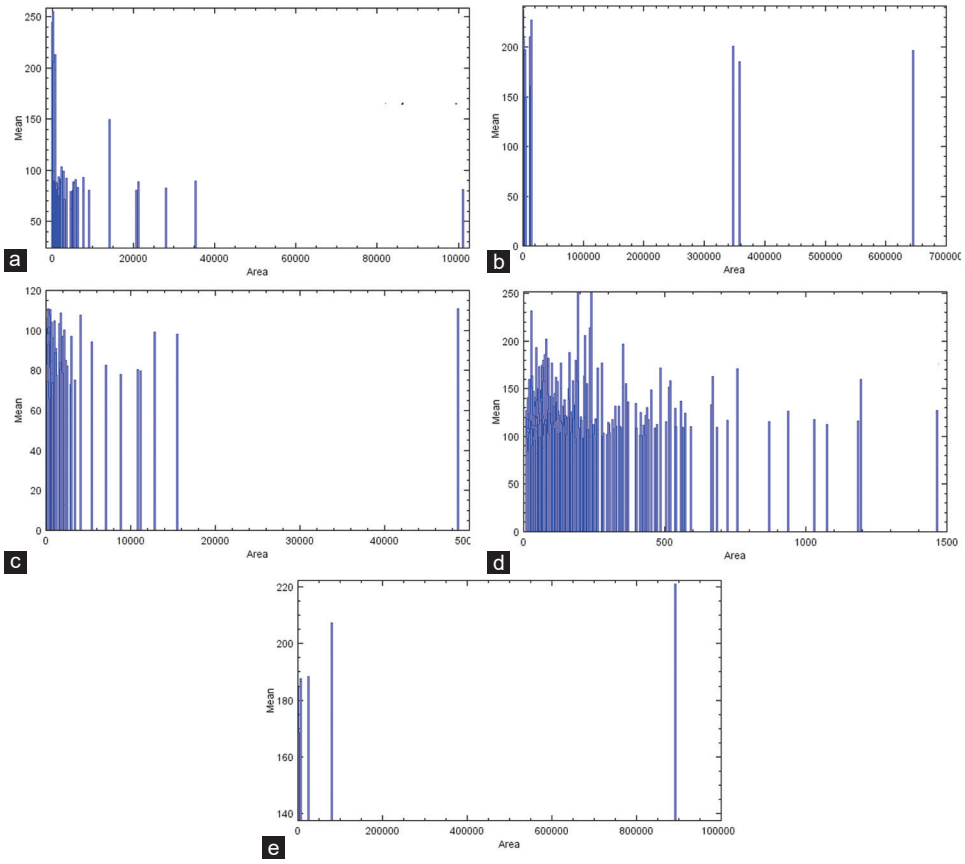

Fig. 5. Particles distribution with area. (a) Control, (b-d) 6 h/day exposure and (e) 8 h/day exposure.

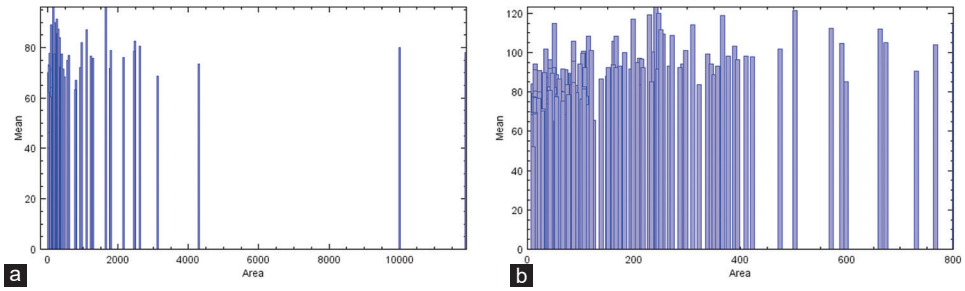

Fig. 6. Particle size distribution in two region of image B of Fig. 3. (a) Less dense and smaller size and (b) higher dense and larger size of particles.

Fig. 4 is the quantitative illustration of particles counted with ImageJ software. High exposure time per day reduced the cellular density in respect of control image. Quantitative data of images A and B of Fig. 3 show almost the same marrow density. Therefore, the BMCD is hypocellular (significant reduction) in image B in contrast to an insignificant reduction within image A.

Particles' area viability is shown in Figs. 5 and 6. Quantitative analysis indicated the presences of SMF influences on the area distribution. The most particle distribution found in control image was <10K pixels (image A). Eight hours exposure per day (1 week) reduced considerably since most of particles measured possessing <10K pixels (images B and C). Significant changes are shown of 4 weeks exposure (image D), analyzed results found the particles with area of <1000 pixels. There is no evidence to prove the area variation related, if only, to the applied magnetic field. However, a correlation can exist since the tendency of the marrow cells was affected with the applied magnetic field.

IV. Discussion

Our previous studies indicated the *in vivo* influence of 2.4 mT SMF on blood cells (red blood cells (RBCs), white blood cells (WBCs), and platelets-PLTs (Mustafa, Yab and Ismail, 2020d; Mustafa, Yab and Ismail, 2020e; Mustafa, Yab and Ismail, 2020c; Mustafa, Yab and Ismail, 2020b). Rats were exposed with 2.4 mT SMF for short to long time per day for a period of 1–4 weeks. A 6 h/day exposure during 1 week raised WBCs with about 30%, PLTs with 13%, and RBCs reduced with 10%. WBCs declined tremendously under the influence of 8 h/day (4 weeks). RBCs and PLTs counts fluctuated. The present study of bone marrow shows

that our previous results of blood main cells are the response of bone marrow variation under the influence of SMF. We have expected the stimulation or destimulation effect of bone marrow for the influence of the magnetic field, whereas blood main cells are formed within bone marrow during a complex division of hematopoietic stem cells. It is not clear why some of the exposure periods of SMF did not influence the bone marrow. However, we believe that the biological system responses differently even at the same condition and highly controlled external factors.

Stimulating the bone marrow activities can induce bone marrow cell differentiation and stem cells growth rate. Exposed HMSCs with extremely low-frequency magnetic field (20 mT) during 23 days inhabited its growth and metabolism, although not affected the cell differentiation (Yan, et al., 2010). Other studies demonstrated that pulsed electromagnetic field enhanced the bone marrow mesenchymal stem cells proliferation in human and resulted an increase of marrow cell densities (Sun, et al., 2009). A 180 mT SMF effect on bone mineral density examined. No significant variation was found in bone mineral density of femurs among exposed groups of animals. Whereas, the 180 mT has reduced the bone mineral densities significantly in distal regions (Xu, et al., 2010). Osteoporosis also examined under the influence of moderate 0.2–0.6 mT magnetic field (Chen, et al., 2020). It was found that the magnetic field inhibits the adipogenic differentiation of bone marrow-derived mesenchymal stem cells depends on the intensity of exposure.

The marrow and trabeculae bone portions are naturally varied dependently to age. Bone marrow consists of hemopoietic stem cells and fats. The ratio between them is conservative in adults. The normal distribution and density of hematopoietic stem cells are vital and abnormality of it can cause chronic diseases such as cancer (Berg, et al., 1998). Several factors are contributing. Regarding magnetic field, the primitive factor of how bone marrow responds to magnetic field is controversial (Berg, et al., 1998). One anticipation is the chemical characteristics of hematopoietic cells containing protons. Protons interact with magnetic field. The outcome effect is a function of the strength of the field, period of exposure, and the nature of the biological cell. Mustafa, Yab and Ismail (2019) reviewed the influence of various intensities (low, intermediate, and high) of SMF on blood parameters. Several intermediate exposure sources changed blood counts significantly, of which other low and high exposure intensities showed an insignificant effect (Pirkhider Yaba and Ismail, 2019). Despite several studies concerns biological effect of magnetic field, there are studies which claim the safety of magnetic field. Regarding to non-linearity effect of SMF, we showed a significant effect due to high exposure time per day due to 4 weeks treatment, yet no promotion of change found <3 weeks of exposure.

The area distribution of the cells is through charge attraction or repulsion among the cells. The magnetic field produces a magnetic force which rotates the cells into a certain direction (Mustafa, Yab and Ismail, 2020d). If the applied magnetics force overcomes the repulsion force between the cells, they intense in a smaller space or vice versa. Therefore, other factors can contribute such as physiological and psychological effects since the applied magnetic field may not overcome the natural forces in biological system. Other than SMF, external factors were controlled.

V. Conclusion

We investigated the proportional variation of BMCD to bone trabeculae associated with SMF in femurs albino rats. The short time exposure/day did not change the cellular density of bone marrow. The microscope distribution of the marrow showed in normal pattern. However, the 6 h/day exposure assisted an increase of BMCD (marrow to trabeculae bone) due to 1 week of exposure. Eight hours/day exposure led to reduce cellular density (hypocellularity of trabeculae portion). ImageJ analysis revealed the changes for particles existence under applied magnetic field. We believe that the marrow density variation attributes cell activities and differentiation. This investigation supports the hematological study shows the SMF-related blood components variations.

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