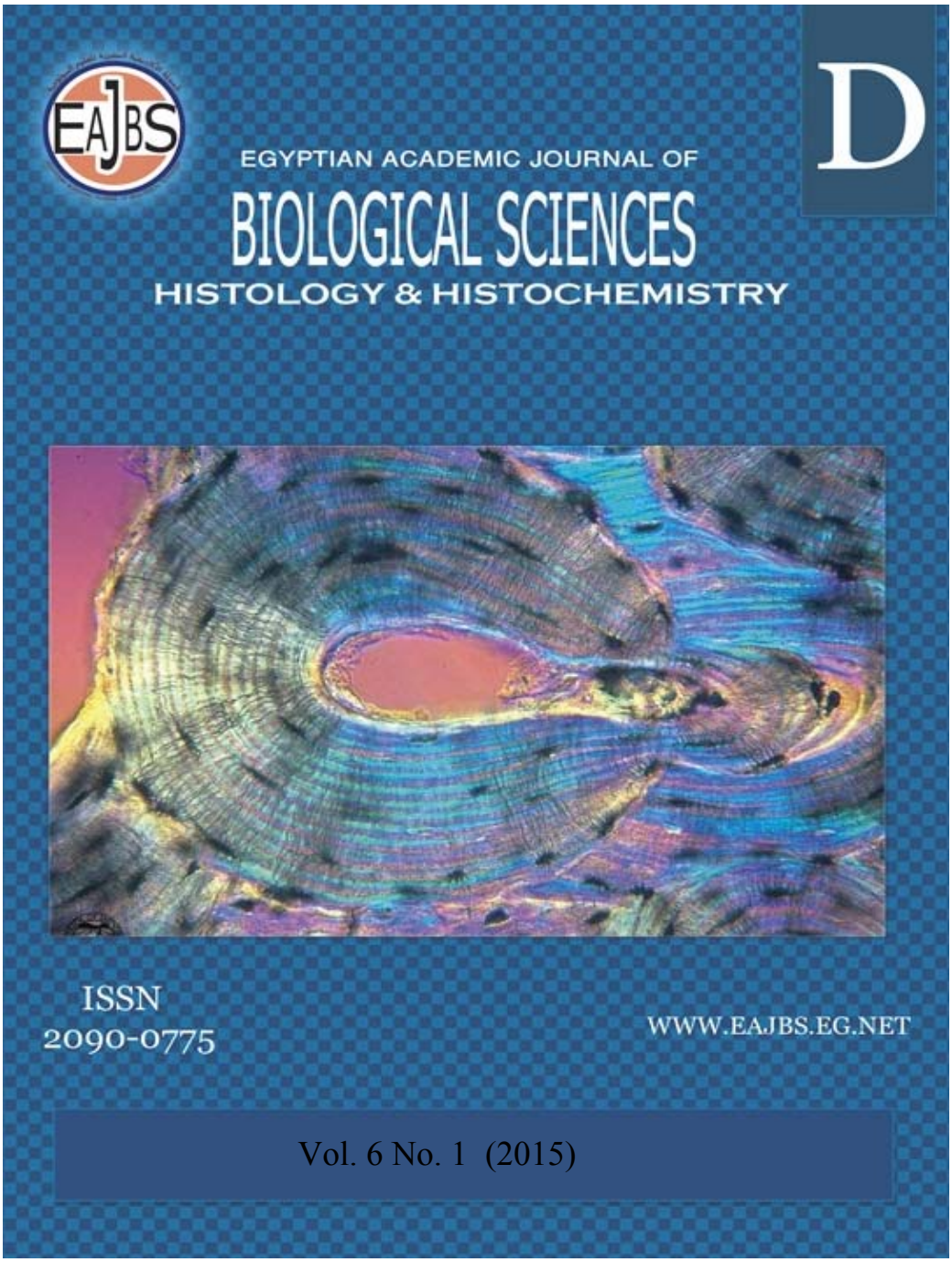

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Egypt. Acad. J. Biolog. Sci., 7(1): 53 – 57 (2015)

Egyptian Academic Journal of Biological Sciences
D. Histology & Histochemistry

2090 – 0775
www.eajbs.eg.net

Comparison of neutrophil elastase levels in obese and normal individuals

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ARTICLE INFO

Article History
Received: 3/2/2015
Accepted: 4/3/2015

Keywords:
Neutrophil
Neutrophil Elastase
PMA
Obesity

ABSTRACT

Purpose: Neutrophils being the most abundant leukocytes in humans and the first cells to arrive on the site of inflammatory immune response. These cells are classically characterized by their ability to act as phagocytic cells, to release lytic enzymes like neutrophil elastase from azurophil granules. Neutrophil elastase (NE) is known to promote inflammatory responses in several disease. The purpose of this study is to correlate neutrophil elastase expression level with obesity.

Method: Present study aims to determine the differential expression and packaging (in azurophil granules of neutrophils) of human NE using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) in normal and obese individuals.

Results: It was observed that, the obese individuals tend to have an average elevated levels neutrophil elastase in blood neutrophils as confirmed by the bands observed in SDS-PAGE. The elevated levels of neutrophil elastase in obese individuals may be attributed to chronic inflammation in fat tissue.

Conclusion: Obese individuals (Students of Hail University) have a slightly higher level of neutrophil elastase in blood leukocyte (neutrophils).

INTRODUCTION

Obesity is often defined simply as a condition of abnormal or excessive fat accumulation in adipose tissue, to the extent that health may be impaired (Drewnowski A *et al*. 1989). According to World Health Organization (WHO), obesity is classified as chronic and severe disease in developed and developing countries, affecting both adults and children (Hoyt CL *et al.* 2014) Previous data showed that obesity has reached to an epidemic proportions globally, with more than 1 billion adults overweight and at least 300 million of them clinically obese. Which is a major contributor to the global burden of chronic disease and disability. Often coexisting in developing countries with under-nutrition, obesity is a complex condition, with serious social and psychological dimensions, affecting virtually all ages and socioeconomic groups (Nathan C *et al*. 2006). Many recent studies have suggested that obesity is associated with chronic inflammation in fat tissue.

Neutrophils being the most abundant leukocytes in humans and the first cells to arrive on the site of inflammatory immune response.

Upon stimulation by pathogens or pharmacological agents such as phorbol myristate acetate (PMA), neutrophil elastase is excreted from the cell and exists either as free protein or associated with networks of extracellular traps (Brinkmann V *et al.* 2004). Neutrophils are normally found in the blood stream. During the beginning (acute) phase of inflammation, particularly as a result of bacterial infection, environmental exposure, and some cancers (Nathan C *et al.* 2006; Levinsky R *et al.* 1980). Neutrophils are one of the first-responders of inflammatory cells to migrate towards the site of inflammation. They migrate through the blood vessels, then through interstitial tissue, following chemical signals such as Interleukin-8 (IL-8), C5a, and Leukotriene B4 in a process called chemotaxis. Neutrophils are recruited to the site of injury within minutes following trauma and are the hallmark of acute inflammation (Takahashi H *et al.* 1988; Jacobs L *et al.* 2010). Together with other proteases released from activated neutrophils, neutrophil elastase plays a critical role in degrading invading pathogens and thus provides the earliest line of defense in the immune system (Boxer LA *et al.* 2010; Sato T *et al.* 2006). In addition to its expression in neutrophils, neutrophil elastase is also expressed in non-small cell lung cancer tumors and cell lines (Waugh DJJ and Wilson C 2008; De Larco JE *et al.* 2004).

Neutrophil elastase may also play a critical role in tumor invasion and metastasis (Yamashita Ji *et al.* 1997; Henriksen P A and Sallenave JM 2008), due to its ability to degrade in soluble elastin and other extracellular matrix constituents. Mutations in ELA2, the gene encoding neutrophil elastase, are the major cause of the two main forms of hereditary neutropenia (Cohen S and Burns RC 2002; Chua F and Laurent GJ 2006). NE has been found to play key role in insulin resistance in mice fed with high fat diet (Talukdar S *et al.* 2012). Neutrophil elastase has been found within atherosclerotic plaques contributing to matrix degradation and weakening of the vessel walls (Henriksen PA and Sallenave JM *et al.* 2008). These studies identify a clear role of neutrophil elastase in fat metabolism and deposition. Present study attempts to determine a possible relation with NE levels and obesity.

MATERIAL AND METHOD

Participants

Students (Number: 64) were enrolled in the study, divided into 4 groups (Table 1) Each Group included 16 students. Group 1: Non obese students and the other Group 2: Obese students. The mean age of the groups were 24±4.

All participants were subjected to anthropometric measurement and normal body composition measurement. Serum NE was measured by SDS- PAGE, while sample preparation and activation of Neutrophils was performed by Cayymann Assay kit for Neutrophil Ealsatse, Supplied by Caymann.

Sample Preparation

15 mL of whole blood samples were collected from each individual in EDTA blood collection tubes. This whole blood is then transferred to 50 ml conical tube (using sterilized disposable syringe). Blood collection tubes were then rinsed with 15 mL of filtered Assay Buffer (provided with the kit) and added to the 50 mL conical tubes containing whole blood (total diluted blood volume 30 mL). 10 ml of Cell-Based Assay Neutrophil Isolation Histopaque® (provided with the kit) was then pipetted to fresh 50 ml conical tubes. 30 ml of the diluted blood was then slowly added on the top of Cell-Based Assay Neutrophil Isolation Histopaque® in each 50 mL conical tube.

Thereafter the samples were centrifuged at 500 x g for 20-30 minutes

at 18-26°C followed by carefully aspirating the yellowish and clear top layers and leaving the reddish pellets containing neutrophils and red blood cells in the tube. 30 ml of Red Blood Cell (RBC) Lysis Buffer (provided with the kit) is then pipetted in to conical tubes containing the pellet of neutrophils and red blood cells. This mixture is then vortexed and rocked over a rocker for 10-15 minutes to lyse the red blood cells. Thereafter Centrifuging at 1,200 x rpm for 10 minutes to pellet the neutrophils followed by carefully aspirating the reddish supernatant. To this 5 ml of Roswell Park Memorial Institute medium (RPMI) Solutions containing 1% Bovine Serum Albumin (BSA) is added and mixed well. Again centrifuge the tubes at 1,200 x rpm for five minutes to pellet the neutrophils. Addition of RPMI and the centrifugation process were done twice to get more neutrophils. Thereafter the cells were resuspended in 20 ml RPMI containing 1% BSA and well mixed to ensure sufficient separation of the cells. PMA (provided with the kit) was then added at a final dilution of 1:30,000 into each culture medium. PMA at these concentrations causes a significant release of Neutrophil Elastase. Now these tubes are then centrifuged at 1200x rpm for ten minutes at 20 ^{o}C. The supernatant now contains neutrophil elastase.

Now the samples are organised for SDS PAGE as per Table 1. SDS-PAGE was performed on Biorad Tetracel. Lane 1 was loaded with biorad precision plus protein standard. Lane 2 was loaded with NE standard (caymman). Lane 3 and 4 were loaded with Group 1 and Group 2 samples respectively. Lane 5 and 6 were loaded with Group 3 and Group 4 samples respectively. SDS- PAGE was performed as per standard protocols of Biorad (not all gel images can be shown due to journals page limit).

RESULTS AND DISCUSSION

Neutrophil elastase is an important protease enzyme that when expressed apparently can cause emphysema changes; this involves the breakdown of the connective tissues. So the natural proteinase elastase is released from polymorph nuclear (PMN) leukocytes in various physiological and pathological conditions. Many researchers have found that increase in the neutrophils elastase could be an indicator to abnormal affects. Figure 1 shows a comparasion of NE from 4 different groups as classified on the basis of their Body Mass Index (Table 1). Lane 3 and 4 belongs to obese individuals and lane 5 & 6 belongs to normal individuals. It can be observed that bands in all the lanes 3,4,5,6 corrosponds to the standard NE in the lane 2.

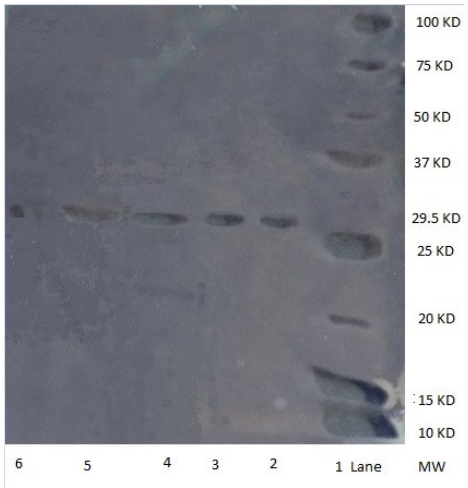

Fig. 1: SDS PAGE analysis of Neutrophil Elastase (Lane 3 and 4: Obese Individuals; Lane 5 and 6: Normal Individuals)

Table 1: Group characterization

Group Characterization	Group ID	No. Of Individuals	Body mass Range	Avg. BMI	SDS –PAGE lane No.
Obese	Group 1	16	> 30	45	Lane 3
	Group 2	16	26-30	29	Lane 4
Normal	Group 3	16	21-25	22	Lane 5
	Group 4	16	17-20	19	Lane 6

This conforms, that the protein in the bands 3, 4, 5 and 6 is NE. PMA induces the release of NE from Neutrophils without the lysis of neutrophils. This reduces the load of other proteins in the sample. It can be observed, that the bands in lane 3 & 4 are sharper and well defined as compare to the bands in lane 5 & 6 (Figure 1). The sharpness and the intensity of the bands is proportional to the concentration of NE in the sample loaded in their respective wells. Hence it can be concluded that the samples loaded in the lane 3 & 4 have a higher concentration of NE as compared to lane 5 & 6.

The sharpness of the bands in lane 3 and 4 explains the elevated levels of NE in the blood neutrophils of the Obese individuals. However this is only an indication of such behaviour and further confirmation can be done in future studies involving Western Blot and ELISA based analysis.

CONCLUSION

Serum neutrophil elastase concentration were found to be elevated in obese students comparative to normal students. This may be attributed to chronic fatty tissue inflammation, insulin resistance and sometimes undetected prehypertension in obese individuals. Many researches showed the relationship of NE with incurable diseases. There is a wide scope of research on the implications and the regulation of NE in obese individuals.

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