ORIGINAL RESEARCH PAPER

Plastic Surgery

IMPACT OF INTERLEUKIN 6 IN THE MANAGEMENT OF LYMPHEDEMA

KEY WORDS: Lymphedema, Il6, Serum Marker.

Ravikumar Gopalakrishnan* Department Of Plastic And Reconstructive Surgery, Thanjavur Medical College,TN.DR.MGR Medical University ,Tamilnadu, India.
*Corresponding Author

Sivakami Thiagarajan Department Of Anatomy,Thanjavur Medical College,Tamilnadu, India.

Sugapradha GR Multidisciplinary Research Unit, Department Of Health Research, Newdelhi, India.

Jenifer P Multidisciplinary Research Unit, Department Of Health Research, Newdelhi, India.

ABSTRACT

BACKGROUND: Lymphedema is a progressive disease characterized by chronic limb swelling and adipose deposition resulting from congenital abnormalities, obstruction, injury, or infection of the lymphatic system. Current diagnostic options are limited and rely primarily on limb circumference or volume measurements. Identification of a serum marker for lymphedema may facilitate diagnosis and response to treatment. Lymphedema is characterized clinically by adipose tissue deposition and inflammation; therefore, we investigated serum level of interleukin-6 (IL-6), an important physiologic regulator of these processes in various grades of lymphedema.

METHODS: The study was conducted in the lymphedema unit under the Department of Plastic and Reconstructive Surgery, Thanjavur Medical College from January 2018 to December 2018. Patient demographics including body mass index (BMI) and lymphedema grade were collected. The lymphedema patients were classified into seven stages as per Fifth WHO Expert Committee on Filarial lymphedema and endorsed by the American Society of Lymph ology. Sixty six patients of various stages of lymphedema were evaluated for IL 6 expression in their serum.

RESULTS: IL6 is significantly increased in Grade III lymphedema patients. IL-6 is a known regulator of adipose homeostasis in obesity and has been shown to be increased in primary and secondary models of lymphedema. In Grade III lymphedema there is more fat deposition and lipodermatosclerosis.

CONCLUSIONS: Although it is evident that inflammatory changes are necessary for adipose deposition in lymphedema, it remains unclear which inflammatory pathways regulate adipose homeostasis in lymphedematous tissues. Our study proves that there is a definite correlation between serum levels of IL6 in various stages of lymphedema. Identification of a drug modulating the IL6 level may prevent the progression of lymphedema to higher grades. IL-6 may be a useful serum marker that may aid in the diagnosis and treatment of lymphedema.

INTRODUCTION

Lymphedema is a progressive disease characterized by chronic limb swelling, adipose deposition and fibrosis of the affected region resulting in functional problems, decreased quality of life, and recurrent infections.[1] Lymphedema develops in a delayed fashion, often years or even decades, after the inciting lymphatic injury.[2, 3] Although several risk factors such as radiation, infection, extensive surgical dissection, and obesity have been identified, it remains difficult to predict which patients will display the lymphedema phenotype and how severe their manifestations may be.[4,5]These findings suggest that lymphatic injury alone is not sufficient for the development of lymphedema.

Over the years, animal studies, especially those utilizing mice, have provided significant insight into the cellular and molecular mechanisms of inflammation, much of which has been supported by the characterization of changes in human lymphedematous tissues.[6, 7, 8] IL-6 is a known regulator of adipose homeostasis in obesity and has been shown to be increased in primary and secondary models of lymphedema.[9] Therefore, the purpose of this study was to determine the expression of IL-6 in various grades of lymphedema.

METHODS

An observational study was conducted in the lymphedema unit under the Department of Plastic and Reconstructive Surgery, Thanjavur Medical College, Tamilnadu, India between January 2018 and December 2018 to evaluate the expression of IL6 in the serum of patients with various grades of lymphedema. Sixty six patients of various grades of lower limb lymphedema were taken up for the study (Figure 1).

Inclusion criteria: Males and females between 27 years and 65 years of age, body weight within the appropriate range, patients on no regular medical treatment for other diseases and able to communicate effectively with study personnel were taken up for the study.

EXCLUSION CRITERIA:

Any disease or condition which might compromise the haematopoietic, renal, endocrine, pulmonary, central nervous, cardiovascular, immunological, dermatological, gastrointestinal or any other body system, history of allergic conditions – asthma, urticaria, eczema, history of autoimmune disorders, history of psychiatric disorders and recent history of alcoholism (<2 years) and smokers were excluded from the study.

Ethics committee approval was obtained. After getting informed consent the patients were thoroughly investigated to rule out other causes of oedema of legs (renal, cardiac, endocrine and hepatic). Patient demographics including body mass index (BMI) were collected. Doppler examination was done for all the patients. The lymphedema patients were classified into seven grades as per Fifth WHO Expert Committee on Filarial lymphedema and endorsed by the American Society of Lymphology. Blood samples were collected from all the patients. Serum from all the 66 patients were separated and labelled according to the grading of lymphedema.

REAGENT PREPARATION:

1.Preparation of standard:IL-6 Standard (200pgm/ml) +1.09ml standard diluent,2.Preparation of Control: IL-6 control+1.0 ml Standard diluent,3.Biotinylated antibody diluent-7ml-(Ready to use),4. Preparation of Biotinylated anti IL-6(0.4ml), 5.Standard diluent serum -7ml-(Ready to use), 6.Streptavidin HRP diluents -23ml-(ready to use), 7.Preparation of Streptavidin HRP-5microlitre, 8.H2SO4 Reagent –Ready to use, 9.Wash Buffer 1ml content +200ml distilled water, 10 ml content+2000ml distilled water, 10.TMB substrate 11ml –Ready to use and 11.Standard diluents buffer (for cell culture supernatants).

PROCEDURE:

100µl of sample and diluted standard/ controls and 50µl Biotinylated anti-IL-6 were added in all wells, followed by incubation for 1 hour at room temperature and washed for three times. 100µl 0f streptavidin –HRP was added, incubated 30 min at room temperature and again washed for three times. 100µl of ready to use TMB was added and protected from light. When the colour developed for 12-15 min, 100µl H2SO4 was added and absorbance was read at 450nm (Figure 2).

RESULTS

Il6 was significantly increased in Grade III lymphedema patients. 7 out of 22 Grade III lymphedema patients showed marked increase in IL6 levels. In 4 Grade III lymphedema patients the IL6 level was above 230 pg/ml (Figure 3). 4 out of 17 Grade II lymphedema patients showed IL6 levels more than 200 pg/ml. 3 out of 10 Grade IV lymphedema patients showed IL6 levels more than 200pg/ml. There was not much increase in serum IL6 levels in Grade I, V and VI patients (Figure 4). These findings suggested that both adipose deposition and chronic inflammation were associated with IL-6 expression in lymphedema. Loss of IL-6 function resulted in increased adipose deposition in Grades IV, V and VI lymphedema patients. Drugs that promote increased IL-6 expression may act to decrease adipose deposition and progression of lymphedema.

DISCUSSION

Lymphedema is a chronic debilitating disease that occurs as a result of lymphatic injury, obstruction, infection, or developmental anomaly.[10] Abnormal lymphatic function in patients who suffer from lymphedema initially leads to accumulation of protein rich interstitial fluid that is treated with massage and compression garments. However, in its late stages, lymphedema results in pathologic fibro adipose tissue deposition, making the disease less likely to respond to these treatments.[11] Understanding the mechanisms that regulate the progression of lymphedema is therefore important for development of novel treatment strategies.

Daniel Cuzzone et al…used a variety of mouse models to test the hypothesis that lymphatic fluid stasis increases the expression of IL-6, including microsurgical disruption of the superficial and deep lymphatics of the tail and axillary lymph node dissection (ALND).They analyzed IL-6 expression in serum samples obtained from breast cancer survivors with or without lymphedema. They analyzed tissue expression of IL-6 in matched biopsy samples obtained from lymphedematous and contralateral normal limbs of patients with lymphedema. A significant elevation of IL-6 was noted locally in regions of lymphatic fluid stasis in both the mouse tail model as well as the mouse ALND model. Serum levels of IL-6 were elevated in mice with tail lymphedema and after ALND as compared to sham surgical controls.[12]

Wallenius et al. found that mice that lacked IL-6 went on to develop adult onset obesity and that supplementation with IL-6 partially reversed this outcome.[13] Karlsen et al. found that genetically engineered Chy mice, a model for primary congenital lymphedema, had elevated levels of IL-6 at later stages of the disease.[14] We did this study because we have more number of lymphedema patients and the exact aetiology is not known in many of these patients. Our study showed a definite correlation between serum level of IL6 and grading of lymphedema.

IL-6 may be a useful serum marker that may aid in the diagnosis or treatment of lymphedema. Serial estimation of serum IL-6 levels may be used to follow a patient over time as means of following response to surgical or medical treatments for lymphedema. This is important since current means of following lymphedema patients rely almost exclusively on volume or circumference measurements. These measurements are often cumbersome to perform and fraught with technical difficulties, making them less reliable for following lymphedema.

CONCLUSION

IL6 is significantly increased in Grade III lymphedema patients. Therapeutic regimes modulating the IL6 may prevent the patients from progressing to higher Grades. IL6 may act as a marker to screen the population at risk of developing lymphedema.

ACKNOWLEDGEMENTS

Authors would like to thank Department of Plastic Surgery and Multidisciplinary Research Unit of Thanjavur Medical College,Tamilnadu, India.

DECLARATIONS

Funding: No funding sources

Conflict of interest: Nil

Ethical approval: The study was approved by the Institutional Ethics Committee

Figure 1: Lymphedema leg of different grades

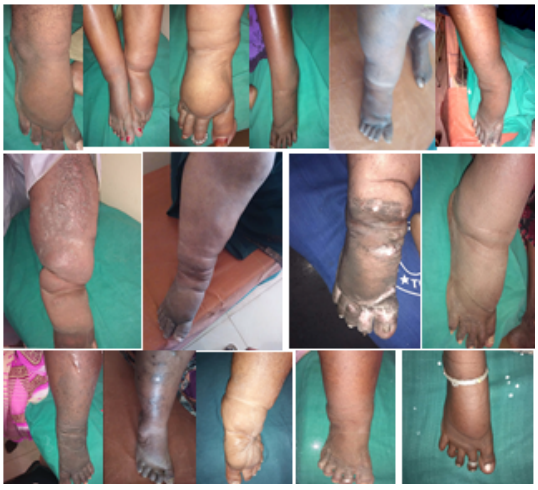


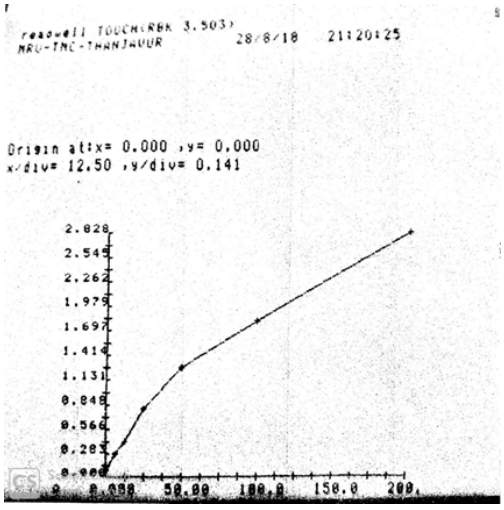
Figure 2: IL 6 Plate loading chart of various grades of lymphedema.

Figure 3: Serum IL 6 levels in different grades of lymphedema

NO	Sample	ABS	CONC	Grade
A1	C1	2.828	200.0	Standard
G2	S8	3.034	220.0	Gr-II
F3	S15	2.866	203.7	Gr-II
B4	S19	3.155	231.8	Gr-II
H4	S25	3.069	223.5	Gr-II
A6	S34	3.258	241.8	Gr-III
B6	S35	3.126	229.0	Gr-III
C6	S36	3.033	220.0	Gr-III
D6	S37	3.156	231.9	Gr-III
E6	S38	3.380	253.7	Gr-III
F6	S39	3.150	231.3	Gr-III
G6	S40	2.859	203.1	Gr-III
E8	S54	3.026	219.3	Gr-IV
F9	S63	3.009	217.6	Gr-IV
G9	S64	2.925	209.5	Gr-IV

ABS-Absorbance

Figure 4: Serum IL 6 levels in different grades of lymphedema



REFERENCES

1. Warren, A.G.; Brorson, H.; Borud, L.J.; Slavin, S.A. Lymphedema: A comprehensive review. Ann. Plast. Surg 2007; 59:464–472. [CrossRef] [PubMed]
2. Cemal, Y.; Pusic, A.; Mehrara, B.J. Preventative measures for lymphedema: Separating fact from fiction.J. Am. Coll. Surg. 2011; 213: 543–551. [CrossRef] [PubMed]
3. Petrek, J.A.; Senie, R.T.; Peters, M.; Rosen, P.P. Lymphedema in a cohort of breast carcinoma survivors 20 years after diagnosis. Cancer 2001; 92: 1368–1377. [CrossRef]
4. Fu, M.R.; Conley, Y.P.; Axelrod, D.; Guth, A.A.; Yu, G.; Fletcher, J.; Zagzag, D. Precision assessment of heterogeneity of lymphedema phenotype, genotypes and risk prediction. Breast 2016; 29: 231–240. [CrossRef] [PubMed]
5. Kilbreath, S.L.; Refshauge, K.M.; Beith, J.M.; Ward, L.C.; Ung, O.A.; Dylke, E.S.; French, J.R.;Yee, J.;Koelmeyer, L.; Gaitatzis, K. Risk factors for lymphoedema in women with breast cancer: A large prospective cohort. Breast (Edinburgh, Scotland) 2016; 28: 29–36. [CrossRef] [PubMed]
6. Frueh, F.S.; Gousopoulos, E.; Rezaeian, F.; Menger, M.D.; Lindenblatt, N.; Giovanoli, P. Animal models in surgical lymphedema research—A systematic review. J. Surg. Res. 2016; 200: 208–220. [CrossRef] [PubMed]
7. Shin, W.S.; Rockson, S.G. Animal models for the molecular and mechanistic study of lymphatic biology and disease. Ann. N.Y. Acad Sci. 2008; 1131: 50–74. [CrossRef] [PubMed]
8. Catherine L. Ly, Raghu P. Kataru and Babak J. Mehrara. Inflammatory manifestations of lymphedema. Int. J. Mol. Sci. 2017; 18: 171; doi:10.3390/ijms18010171
9. Rockson, S.G. The lymphatics and the inflammatory response: Lessons learned from human lymphedema.Lymphat. Res. Biol. 2013; 11: 117–120. [CrossRef] [PubMed]
10. Tabibiazar, R., Cheung, L., Han, J., Swanson, J., Beilhack, A., et al. Inflammatory manifestations of experimental lymphatic insufficiency. PLoS Med. 2006; 3: e254. [CrossRef] [PubMed]
11. Torrisi, J.S. Hespe, G.E. Cuzzone, D.A. Savetsky, I.L. Nitti, M.D.; Gardenier, J.C.; et al. Inhibition of inflammation and inos improves lymphatic function in obesity. Sci. Rep. 2016; 6: 19817. [CrossRef] [PubMed]
12. Daniel A. Cuzzone, Evan S. Weitman, Nicholas J. Albano, Swapna Ghanta, Ira L. Savetsky, Jason C. Gardenier et al. IL-6 regulates adipose deposition and homeostasis in lymphedema. Am J Physiol Heart Circ Physiol 2014;306: H1426–H1434.
13. Wallenius V, Wallenius K, Ahren B, Rudling M, Carlsten H, Dickson SL, Ohlsson C, Jansson JO. Interleukin-6-deficient mice develop mature-onset obesity. Nat Med 2002; 8: 75–79.
14. Karlsen, T.V., Karkkainen, M.J., Alitalo, K., Wiig, H. Transcapillary fluid balance consequences of missing initial lymphatics studied in a mouse model of primary lymphoedema. J. Physiol. 2006; 574: 583–596. [CrossRef] [PubMed]