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Tumor Necrosis Factor-Alpha (TNF- a) Expression in Mice Infected with Aspergillus Fumigatus Using Immunohistochemichal Technique

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Abstract

Expression of TNF-α in the lung, liver kidney were shown in the nuclei of the tissue cells and detected by IHC technique. Depending on the scoring system used for the TNF-a, two parameters were dependent; the intensity of the staining of the nuclei and the percentage of the cells Giving positive expression. The intensity of the nuclei of the stained cells was negative if there is no expression. Expression was found positive in all the studied organs ,but different in score and intensity, in lung over expression(score 3+) in 7, 14 and 21 days post infection with moderate staining at 7 day and intense staining at 14, 21 day respectively, in liver expression was (score 2+, score 2+,and 3+) with moderate staining at 7 and 14 day post infection while there is intense staining at 21 post infection. The expression in the spleen was (score1+) with light staining at the period between 7 to 14 day then increase at21 day reach to score 2+with moderate staining. TNF-α expression in all studied sample compared with the positive control. Results show significant difference (*P* value >0.01) in the expression between the studied organs.

***Keywords**:* TNF-α; A.fumigatus; scoring system.

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1. Introduction

TNF-α is a cytokine produced by a large variety of cells, including macrophages, DCs, $CD4^+$ and $CD8^+$ T cells, and B cells [1] this cytokine plays a role in acute inflammation ,immunological reaction and tissue injury. It activates neutrophils enhance cytolytic activity of macrophages, augment NK-cell activity, promote T- and B-cell proliferation, and modulate endothelial cell surface antigens [2].

TH-1 type cells induced by the infection may traverse the fetal interface or may produce pro or anti-inflammatory cytokine that effect the trophoblast .the production of these cytokines are partly under genetic control and the level of the cytokines production particularly TNF alpha and IFN gamma ,have been found associated with genetic polymorphisms ,there is a relationship between TNF-a gene polymorphism and the risk of occurrence of abortion [3].

Ampel [4] reported that autoclaved spherules and arthroconidia of A.fumigatus induced the production of TNF-α by adherent mononuclear cells from healthy human donors.The role of humoral immune response against coccidioidomycosis is not clear

Kirkland and Cole, [5] showed that passive transfer of serum from mice vaccine Formalin-killed spherules (FKS)did not protect recipients, also Beaman and his colleagues [6]recorded that neither serum nor B cells from immune mice transferred protection against challenge in mice.

2. Materials and Methods

Immunohistochemical analysis for the detection of TNF alpha antigen in paraffin embedded sections paraffin- embedded sections from each specimen were cut at 5 Ìm, mounted on glass and dried overnight at 37˚C . The tissue sections were deparaffinized in xylene (2×10 min) and dehydrated through graded alcohol (100 % ,2× 5 min)and 95 % and 70 % for 5 min). Endogenous peroxidase activity was blocked using (1-1.5) % H2O2 for 5-10 mintus. .Tissue samples were heated to retrival antigens in citric buffer (pH 9.0) at 100 ˚C for 10 min The sections were incubated with mouse monoclonal antibody against TNF alpha (diluted 1:50 over night at 37 C. Then sections were washed in buffer solution and covered with Biotinylated secondary antibody for 2 hours at 37 C, AB enzyme was applied for 30 min at 37 C .

Further processing of the sections for detection was performed using the dextran-polymer method , and diaminobenzidine (DAB; Sigma). After being washed, the sections were counterstained with Mayer's hematoxylin, washed in water,and successively immersed in graded ethanol solutions and xylene before cover slipping. All samples were processed under the same conditions. When counting the number of positive cells in the staining tissues samples, at least 10 high-power fields were chosen randomly on each section. Additionally, the number of macrophage was counted in the fields.

All immunostained sections were examined by the same two observers with a ×400 objective under the light microscope (Olympus Bx50;Olympus Optical Co, Ltd, Tokyo, Japan) for evaluation of TNF alpha expressions. In the mice infected with A.fumigatus and control specimens TNF alpha expression in macrophages were

evaluated by counting 1000 cells of each section. TNF alpha expression was quantitatively assessed as 0 (no stained cells), score 1 (from 1-25 postive cells), score 2 (from 26-50 positive cells), score3(from 51-75 positive cells)and score 4(from 75 and over). The intensity was scored as 0 (absence), (low), (moderate), or (high). The pattern and intensity of staining in the different cell types of samples was evaluated by two independent observers using a light microscope at a magnification of 200 x (20 x objective and 10 x ocular). The degree of staining in each placental cell type was graduated as described by (12). The presence of lesions in the tissue samples was investigated in formalin fixed tissue samples embedded in paraffin .

In cases where serial sections had been used for H&E and IHC section comparisons could be made between lesion sites and IHC labeling. Histological evaluation of the tissues of organs was compromised occasional necrosis of endothelial cells. However, immunohistochemical labeling allowed the identification of TNF alpha antigen in all cases studied. Lesions were visible in all samples, after the likely lesion sites had been identified by the IHC labeling AS IN TABLE 1.

Table 1: Primary antibody used in this study

No.	Antibody	Company	Code	Clone	Dilution
1	TNF alpha	SANTAKRUZE	00-9978-0	DAB	150

3. Results and Discussion

Result of immunohistochemistry; Expression of TNF-a in the three organs were shown in the nuclei of the tissue cells and detected by IHC technique. Depending on the scoring system used for the

TNF-a, two parameters were dependent; the intensity of the staining of the nuclei and the percentage of the cells giving positive expression. The intensity of the nuclei of the stained cells was negative if there is no expression.

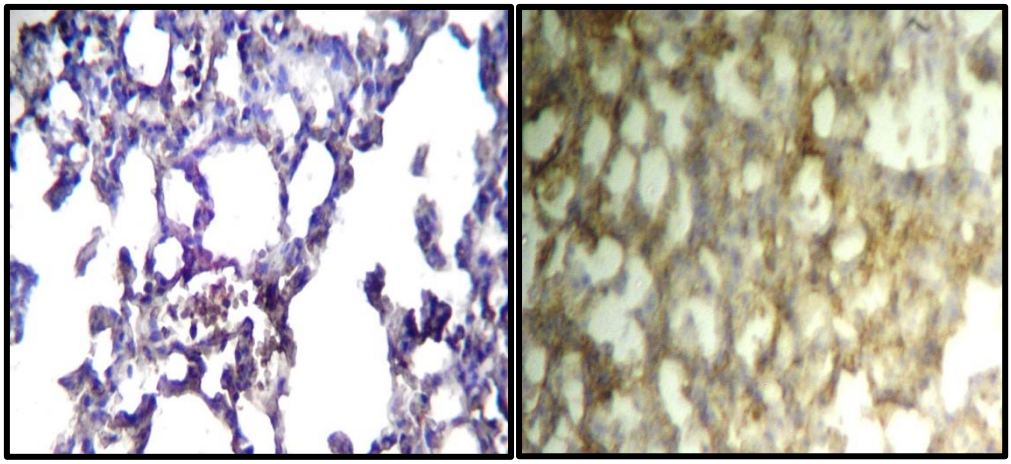
Expression was found positive in all the studied organs, but different in score and intensity, in lung over expression (score 3+) in 7, 14 and 21 days post infection with moderate staining at 7 day and intense staining at 14, 21 day respectively, in liver expression was (score 2+, score 2+,and 3+) with moderate staining at 7 and 14 day post infection while there is intense staining at 21 post infection.

The expression in the spleen was (score1+) with light staining at the period between 7 to 14 day then increase at 21 day reach to score 2+ with moderate staining. TNF-a expression in all studied sample compared with the positive

control. Results show significant difference (P value >0.01) in the expression between the studied organs. Correlation between the TNF-a expression and number and size of lesion in the infected tissues were studied statistically

and the study revealed that there was a significant relationship between the immunoreactive cell and its intensity with number and size of lesion in the same tissue (P value >0.01).

Immunopostivityof TNF alpha protein was high among the necrotic cells in the lung (Figure 1). Whereas, in the control placental tissues from normal delivered lung,TNF alpha immunopositive cells were markedly few (Figure 2) ,and there was significant differences between the control tissue and infected animal tissue. In addition, we recorded the presence severe pathological lesion in the lung, liver and spleen that associated with high intensity of TNF-alpha ,which were recorded by Immunohistochemistry , However, ,we found high score and intensity of TNF-alpha protein that were recorded by immunohistochemistry in the studied organs in which Aspergillus fumigatus low score of TNF-alpha protein by IHC ,which were reported infections as in (Figure 1,2,3).



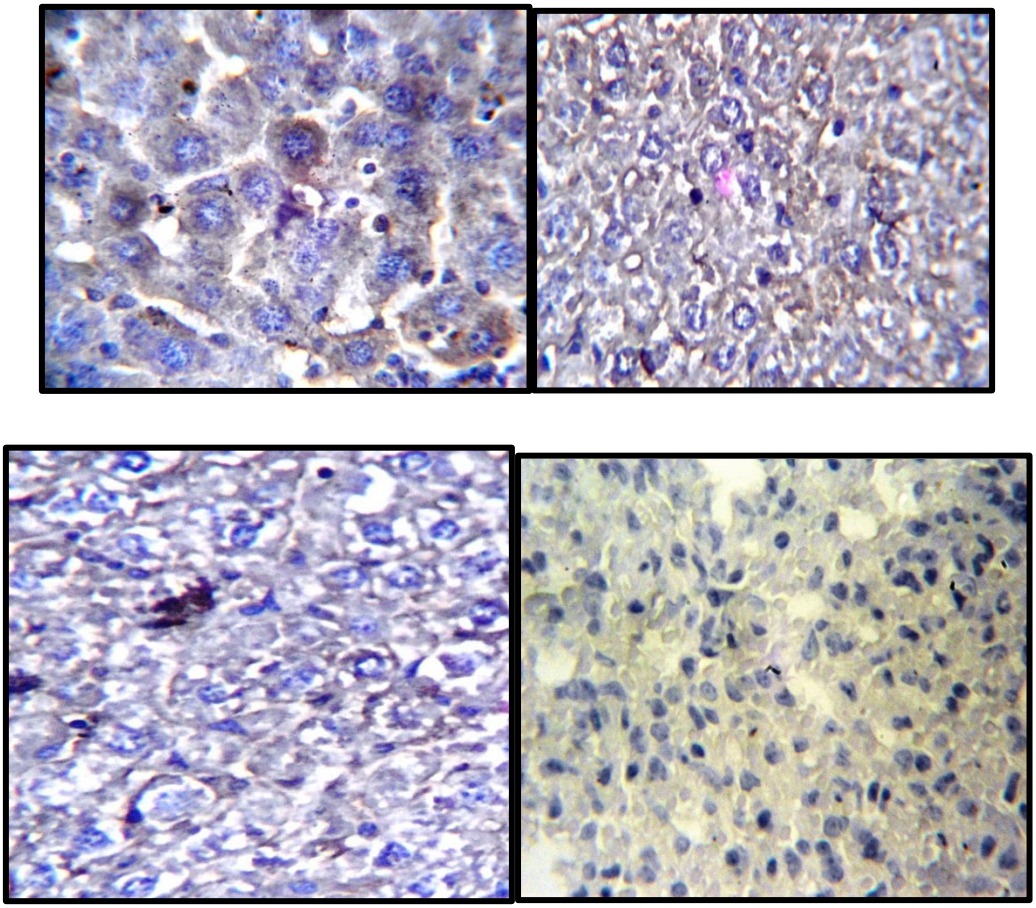
IHC lung

Figure 1: Section of different lung tissue showing TNF-alpha expression (cytoplasmic staining) of Macrophage .Score 3, strong Intensity.(IHC).Stained by DAB-chromogen (Brown) and counter by Hematoxyline(Blue).40X.

Calhoun and his colleagues [7]explained that recovering from infection with chronic or progressive aspergillosis is associated with a polyclonal B-lymphocyte activation, as evidenced by elevated levels of IgG, IgA, and IgE in serum . The serum IgA level is elevated in approximately 20% of patients, being manifested most often in patients with chronic pulmonary disease [8].

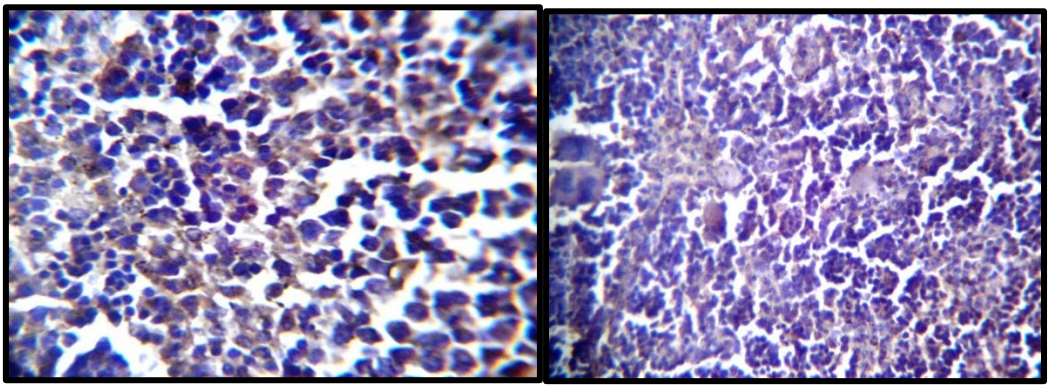
There are many studies on the function of the TNF polymorphisms showing the influence of the different alleles on the in vitro and in vivo levels of TNF production. [9]. However, recent studies suggest that not only polymorphisms within the TNF cluster are important in the regulation of TNF production but also the receptors as well (TNF R). This suggests that investigating polymorphisms within the TNF cluster and TNF receptors will be important in understanding the role of TNF regulation in a given disease[10]**.** Morse and his colleagues [11] indicated direct association between polymorphism in cytokine gene promoter sequences and levels of the

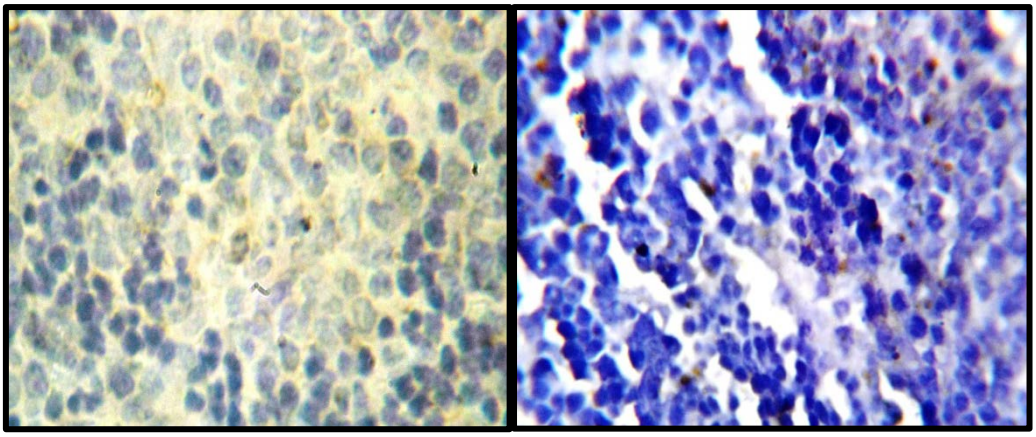
mRNA or protein production. A G to A substitution at position -308 in the promoter of the tumor necrosis factor alpha (TNF –alpha) gene ,increases in vitro transcription of TNF –alpha by approximately 6 to 9 – fold [12] .



IHC liver

Figure 2: Section of different liver tissue showing TNF-alpha expression (cytoplasmic staining) of Macrophage .Score 2, moderate Intensity.(IHC).Stained by DAB-chromogen (Brown) and counter by Hematoxyline(Blue).40X.





IHC spleen

Figure 3: Section of different spleen tissue showing TNF-alpha expression (cytoplasmic staining) of Macrophage .Score 3, strong Intensity.(IHC).Stained by DAB-chromogen (Brown) and counter by Hematoxyline(Blue).40X.

Morse and his colleagues [10] described a further polymorphism in the promoter region of TNF alpha at -238, in a G to A substitution. Although some studies have assessed the association of polymorphisms in CTLA-4 and the fungal illness. Polymorphisms in other genes that play a role in the immune response, such as cytokines, have been correlated with Paracoccidioidomycosis (PCM). These include polymorphisms in IL-10 (-1082 G/A) and TNF-α (-308 G/A) in PCM patients It was found that the IL-10-1082G allele, when homozygous, could be associated with an increased risk of contracting the illness [13].

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