



Assessment of I L-13 Cytokine Concentration in Serum of Pigeon Breeders from Al-Najaf /Iraq

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ABSTRACT:

Background: Bird Fancier's Lung (BFL) is a pathological disease identified by inflammation of Bronchiole and alveoli. It is a very common form of hypersensitivity pneumonia resulting from exposure to avian antigens. The precise disease mechanisms are unclear but both antibody and T cell responses specific for pigeon antigens are implicated. Many cytokines play a crucial role in hypersensitivity. This study was aimed to evaluate the association between increase concentration of IL-13 in serum and breeding Pigeon.

Method: This case – control study enrolled 100 people from Al-Najaf / Iraq, divided into two groups: Group (1) included 50 Pigeon breeders (as patients who were breeders of pigeons only) Group (2) included 50 non-Pigeon breeders (healthy). ELISA technique was used to measure the concentration of IL-13 cytokine in serum from Pigeon breeders and compared the results with serum from healthy volunteers. Second, depending on a questionnaire, we analyzed the results of IL-13 cytokine concentration in Pigeon breeders' group as smoking habit, age and period of breeding Pigeon.

Results: concentration of IL-13 in Pigeon breeders group were highly elevated from those of healthy non-Pigeon breeders ($P \leq 0.0001$). While data analysis through Pigeon breeders group show no significant differences of IL-13 cytokine concentration based on smoking habit, age and period of breeding.

Conclusion: IL-13 have a potential role in BFL disease resulting from pigeon breeding.

KEYWORDS: BFL: Bird Fancier's Lung, ELISA: Enzyme Linked Immuno- Sorbent Assay, Interleukin-13, Hypersensitivity Pneumonia, Pigeon breeders.

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INTRODUCTION

Bird Fancier's Lung (BFL) disease, also called bird breeder's lung, extrinsic allergic alveolitis, or hypersensitivity pneumonitis, is a pathological disease identified by inflammation of Bronchiole and alveoli (Christopoulos, K. 2023; Sánchez-Díez, S., et al 2023). BFL-mediated immunological lung disease occurs as a result of continual exposure to airborne antigens containing highly allergenic avian proteins found in dust, excretions, bird droppings, feather dander, waxy proteins that covering feathers of various birds, fomites, and mold from birds, which provoke hypersensitivity pneumonitis (HP) in susceptible individuals (Sullivan, A., et al 2020; Kuruvilla, M. E., et al 2019).

Lately the disease categorized in two different types: first the acute form belongs to cellular inflammation characterized by continual fever with Chills, cough, and myalgia. The acute form occurs as a result of discontinuous exposure to highly antigen levels. Second the chronic form that identified by lung fibrosis this occurs as a result of repetitive exposure to low antigen doses (Lanzarone, N., et al 2020; Haide, J. S., et al 2016). Continued exposure may lead to decline in lung function, and subsequently sudden death (Peiris, A. (2020).

Although the disease was firstly described in (1965) but now it considers as extremely common causes of Hypersensitivity Pneumonia (Sullivan, A., et al 2020). Until now there are no gold standard test for diagnosis, all doctors depending on the clinical signs, x-ray and different laboratory test in diagnosis of BFL (Sánchez-Díez, S., et al 2023).

Early detection and avoiding prolonged exposure to antigens are crucial to prevent the development of chronic BFL. The disease affecting the lung including interstitial, alveolar, and bronchiolar regions. It results from a non-IgE-mediated immunologic reaction

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within the lung tissue, triggered by sensitization and repeated exposure to different inhaled antigens, which can cause irreversible damage (Peiris, A. 2020; Chopra, V., et al 2017).

Sánchez-Díez et al 2023 explain the mechanism as after Leukocytes recognition (dendritic cells and macrophages) via MHC molecules which recognize by T cell these cells secrete different cytokines such as IL-13 and IL-5 that cause recruitment of eosinophil and activation of it.

Interleukin (IL)-13, a key cytokine in the interleukin family, is produced by various cells, including CD4⁺ T cells, CD8⁺ T cells, basophils, macrophages, and natural killer (NK) cells. It plays multiple roles by affecting monocytes, B lymphocytes, NK cells, and vascular endothelial cells, functioning as a regulatory mediator in immune responses. The gene for IL-13 is located on human chromosome (5q31) with many cytokine gene cluster, such as IL-3, IL-4, IL-5, and granulocyte macrophage colony stimulating factor (Jiang, J., et al 2024; Giuffrida, P., et al 2019). This study was aimed to evaluate the association between increase concentration of IL-13 in serum and breeding fencer's.

METHODS

Study population and design

This case – control study enrolled 100 man participants from Al-Najaf / Iraq divided into two group: Group (1) included 50 Pigeon breeders (as patients who were breeders of pigeons only) belong to smoking habit (24 non-smoker and 26 smoker), their ages ranged from (15 to 44) years, and they started raising birds from (1-30) years.

Group (2) included 50 non-Pigeon breeders (healthy), Non-smokers, their ages range from (15-44) years, and they do not have any acute or chronic diseases.

The study was approved by Faculty of veterinary Medicine university of Kufa and all people have informed consent.

Research plan involved two steps:

First measure the concentration of IL-13 cytokine in serum of Pigeon breeders and compared the result with serum from healthy volunteers.

Second, depending on a questionnaire, we analysed the results of IL-13 cytokine concentration in Pigeon breeders group as smoking habit, age and period of breeding Pigeon.

Sample collection

peripheral Blood samples were collected from both group by vein puncture using a sterile 5 ml syringe, then put it in serum gel tube, let it to stand at room temperature for 2h and centrifuged at 3500 rounds per minute (rpm), finally serum were isolated and transferred to a new tube and stored at -80°C (Joerns, E. K., et al 2024; Valaperti, A., et al 2019)

Enzyme Immuno-Sorbent Assay

IL-13 ELISA kit from (Solarbio life sciences, Cat. No. SEKH-0022) were used in order to measure IL-13 cytokine concentration in all serum samples according to the manufacturing procedure.

Statistical analysis

Statistical evaluation was carried out using Graph-pad Prism (version 10). Depending on test of normality Shapiro-wilk test ($p \leq 0.05$) Group comparisons of measurement data were performed using non-parametric test (mann-whitney test) were used for two group comparison while kruskal-wallis test were used for three group measurement. All data were expressed as median and P-values less than 0.05 were considered statistically significant.

Results

Serum levels of IL13 from all samples

A total of 100 male participants were enrolled in this study for measuring of IL-13 cytokine concentration, comparison occur between Pigeon breeders group (50) and control group (50) with median (395.93) & (335.38) respectively. IL-13 concentrations were increased compared with the control group, there were significant differences between these groups based on mann-whitney test ($P \leq 0.0001$), see figure 1.

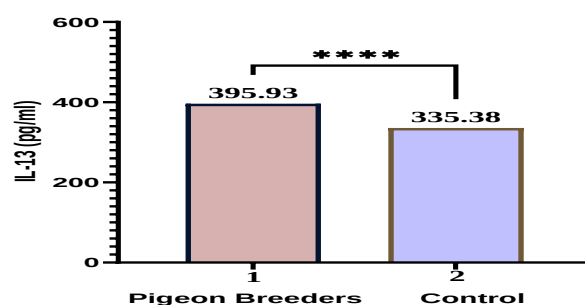


Figure 1: Comparison of IL-13 cytokine concentration between Pigeon Breeders group and control group. ****: mean significant differences ($P \leq 0.0001$).

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Serum levels of IL-13 from Pigeon breeders group

In order to further investigate Pigeon breeders group were subdivided depending on a questionnaire as smoking habit, age and period of breeding.

Smoking habit: comparison occur between smoker (n=26) and nonsmoker(n=24) pigeon breeders with median (414.56) & (377.30) respectively after analyses the results of IL-13 cytokine concentration there were no significant differences between these group based on kruskal-wallis test ($p=0.1114$), see figure 2.

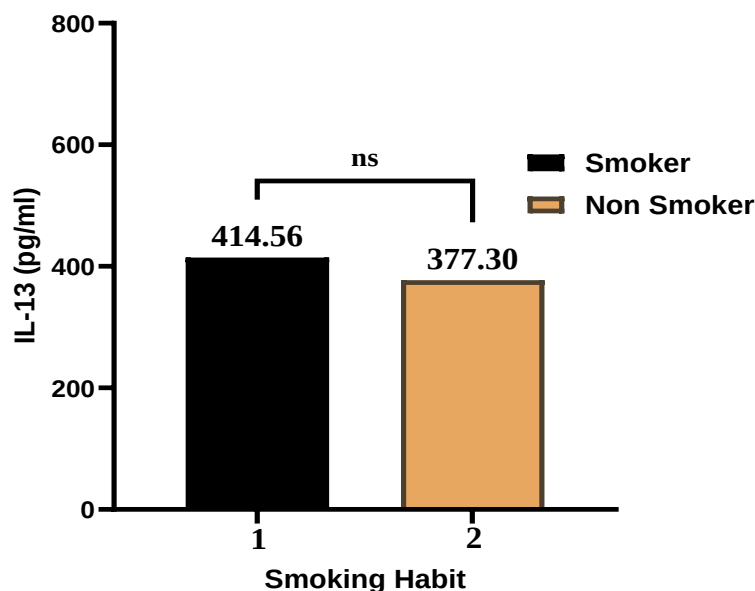


Figure 2: Comparison of IL-13 cytokine concentration among Pigeon Breeders group based on smoking habit :smoker (n=26) and nonsmoker(n=24) .ns : mean non-significant $p=0.1114$.

Pigeon breeders age were ranging from (15 to 44) years old, divided into three group: A(15-24) B(25-34) & C(35-44), median of these group were (388.17,413.01,394.38)respectively, there were no significant differences between group A and B ($p=0.979$), B and C($p=0.561$), A and C($p=0.581$) see figure 3.

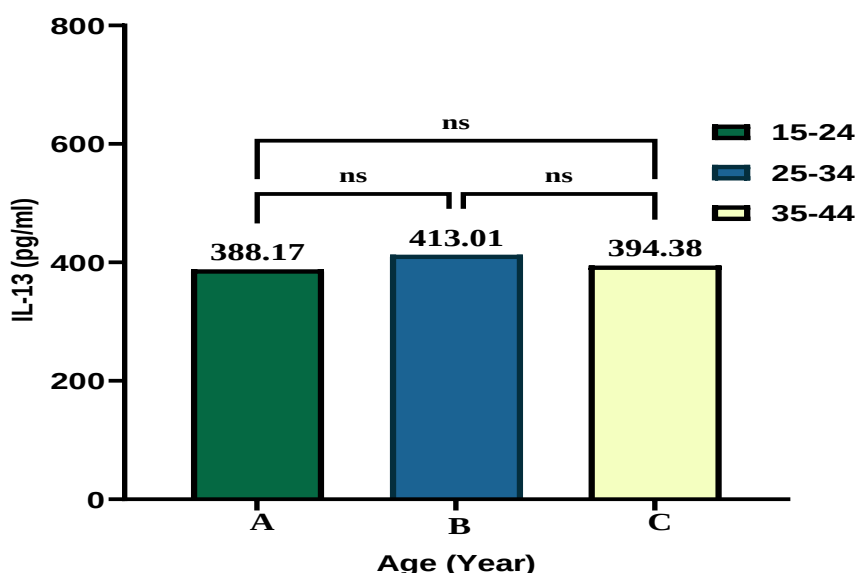


Figure 3: Comparison of IL-13 cytokine concentration among Pigeon Fanciers group based on age group. ns : mean non-significant

finally comparison were based on period of breeding, started from (1 to 30) years, divided into three interval A(1-9) B(10-19) & C(20-29) median of these group were (388.17,414.56,356.56) respectively, there were no significant differences between group A and B($p=0.082$), B and C($p=0.169$), A and C($p=0.974$), see figure 4.

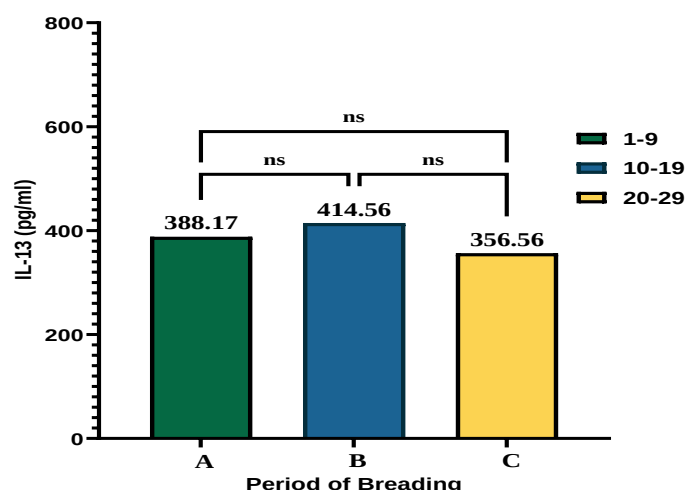


Figure 4: Comparison of IL-13 cytokine concentration among Pigeon Fanciers group based on period of breeding. ns : mean non-significant

DISCUSSION

The present study estimated the effect of exposure to different pigeon antigen on IL-13 cytokine concentration in serum of Pigeon breeders, results demonstrated that cytokine concentration in Pigeon breeders is higher and significantly different ($P \leq 0.0001$) from that of healthy non-Pigeon breeders, as shown in (Figure1) many research have the same results (Xiao, L., Li, et al 2003; Sánchez-Ortiz, M., et al 2020; Tezcan, D., et al 2021; Hussein, M. S., et al 2021), This confirmed the role of pigeon material to trigger Th2 inflammation (Gandhi, N. A., et al 2017) and also confirmed the role of IL-13 in hypersensitivity pneumonia (Alasandagutti, M. L., et al 2017; Nur Husna, S. M. N., et al 2022). (Sánchez-Díez et al 2023) explain the mechanism as after Leukocytes recognition (dendritic cells and macrophages) via MHC molecules which recognize by T cell these cells secrete different cytokines such as IL-5 and IL-13 that cause recruitment of eosinophil and activation of it.

Furthermore, Pigeon breeders group were subdivided depending on a questionnaire as smoking habit, results show no significant differences between these group, also (Feleszko, W., et al 2006) have increased levels of IL-13 in smoker, (Mertens, T. C. et al 2017) concluded that cigars smoking were induced gene signature of IL-13 production. Since Cytokines concentration reflects the severity of disease (Valaperti, A., et al 2019), both exposure to avian proteins and nicotine will induce an immunological response and form antigen-antibody immune complexes which lead to respiratory problems and then lung diseases (Kwiatka, P., et al 2025; Cui, Q., et al 2010; Cano-Jiménez, E., 2016), also continued exposure may lead to decline in lung function, irreversible pulmonary fibrosis, and subsequently sudden death (Peiris, A. 2020).

Both analysis of patients age and period of pigeon breeding in (figure 3&4) show no significant differences, the main explanation of this results are many factors effect on the disease include duration of exposure, antigen load, use or not of respiratory protection, this demonstrate why Bird fancier's lung disease infects not only Pigeon breeders but also persons who breeder 1 or more birds as well as neighbors, (Cano-Jiménez, E., 2016; Tsuyoshi Shirai, et al 2017; Morell, et al 2008)

CONCLUSION

This study demonstrates that serum concentration of IL-13 was significantly differences between Pigeon breeders' group and control group. IL-13 have a potential role in hypersensitivity resulting from pigeon breeding. until know there are no gold standard test for diagnosis, therefore in the future we anticipate to use of IL-13 cytokine as a pre-diagnostic test for Bird Fancier's lung disease.

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Legends for figures

Figure 1: Comparison of IL-13 cytokine concentration between Pigeon breeders group and control as healthy volunteer group. Data are presented in the form of median $P \leq 0.0001$. Figure

2: Comparison of IL-13 cytokine concentration between Pigeon breeder's nonsmoker group and control (no breeders & nonsmoker) group. Data are presented in the form of median $P \leq 0.01$.

Figure 3: Comparison of IL-13 cytokine concentration among Pigeon breeders group (smoker + nonsmoker). Data are presented in the form of median. Ns: mean there were no significant differences between both group $p=0.1114$.

Figure 4: Comparison of IL-13 cytokine concentration among Pigeon breeders group based on age group A (15-24), B(25-34), C(35-44) median of these group were (388.17, 413.01, 394.38) respectively. Ns: mean there were no significant differences between group A and B ($p=0.979$), B and C ($p=0.561$), A and C ($p=0.581$).