Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2019 16(2): 1132-1142. **OPEN ACCESS**

Association of high-altitude and smoking on the levels and phenotype of αβ-T-cells and γδ-T-cells

Mazen Almehmadi[1,4], Alaa Shafie[1,4], Abdullah Bashiri[1], Rayan Babukayr[1], Mutaz Alknani[1], Nawaf Alqurashi[1], Aedh Alghamdi[1], Abdulaziz Al-hajjiahmed[1], Eid Althobaiti[1], Mustafa Halawi[2], Abdurhman Hazzazi[3] and Amal Alhamad [3]

[1]Collage of Applied Medical Sciences, Taif University, Taif city, **Saudi Arabia**
[2]Collage of Applied Medical Sciences, Jazan University, Jazan city, **Saudi Arabia**
[3] Regional Laboratory and Blood bank, Dammam, **Saudi Arabia**.
[4]High altitude research Centre, Taif University, Taif city, **Saudi Arabia**

***Correspondence:** Dr.mazen.ma@gmail.com **Accepted: 09** Mar. 2019 **Published online:** 17Apr 2019

This study objective was to investigate the effect of smoking on the percentages of αβ-T-cells and γδ-T-cells in peripheral blood of healthy subjects. Samples were collected from 32 participants from Taif city and 24 from Makkah. Taif city is a high-altitude city 1879 meters above sea level and naturally oxygen levels are lower than in Makkah. A total of 15 cigarette-smokers, 25 shisha-smokers and 16 non-smokers participated in this study from Taif city and Makkah. Peripheral blood mononuclear cells (PBMCs) were collected from donors and separated by density gradient. They were then stained by anti-αβ-TCR, anti-γδ-TCR, anti-CD4 and anti-CD8 antibodies. Proportions of αβ-T-cells, CD4+ αβ-T-cells and CD8+ γδ-T-cells were expanded in both groups of smokers. However, CD4+ αβ-T-cells, γδ-T-cells and double-negative γδ-T-cells declined in shisha-smokers in Taif. High-altitude can affect the percentage of αβ-T-cells, and γδ-T-cells especially in shisha-smokers.

Keywords: γδ TCR, αβ TCR, high-altitude, shisha, Cigarette-smokers

INTRODUCTION

Smoking is a habit that is practiced worldwide and it was reported that a third of the adult population of the world practice this habit and the associated mortality rate is over one million per year (Pfeifer et al., 2002; Sander L, Gilman, 2004). Smoking has been reported to affect several biological mediators of inflammation leading to immunosuppressant effects (Meuronen et al., 2008; Gonçalves et al., 2011), moreover, smoking suppresses these inflammatory immune defense processes via inhibiting the inflammatory mediators expression in the airway (Meuronen *et al.*, 2008). Smoking is connected to Alzheimer's disease (Cataldo, Prochaska and Glantz, 2010), cardiovascular diseases (Mainali et al., 2015) and several types of cancer including lung cancer, bladder cancer, pancreatic cancer, breast cancer (Warren, Sobus and Gritz, 2014; Alexandrov et al., 2016; Michaud, 2016). Studies showed increases in the levels of CD8+ cells in bronchoalveolar lavage in smokers and declines in the levels of CD4+ T-cells in the non-smoker group (Forsslund et al., 2014), and second-hand-smoking led to increased levels of CD4+CD45RA+ cells in adolescents (Vardavas *et al.*, 2010). In people whose lungs were damaged by smoking, Th1 cells were found to induce emphysema via the effect of CXCR3 ligands, Interferon-induced protein-10 (CXCL-10) and monokine induced by interferon (CXCL9) (Grumelli et al., 2004). Th17 cells, on other hand, were increased in chronic obstructive pulmonary disease (COPD) patients (Vargas-Rojas et al.,

2011).

Human T cells are comprised of 95% conventional T-cells expressing αβ-TCR heterodimers and another minor subset of unconventional T-cells which express γδ-TCR heterodimers (Wang *et al.*, 1992; Glusman *et al.*, 2001). T-cell activation triggered by the engagement of TCR with MHC on the surface of antigen presenting cells (APC), and the function of T-cells are achieved by continued TCR signaling and attachments (Goldsmith and Weiss, 1988; Germain, 1994; Alcover, Alarcón and Di Bartolo, 2018). Following this activation of T-cells, cytokines are released to modulate the immune response (Reinherz, 2015). Both αβ-T-cells and γδ-T-cells are derived from the same precursor, while αβ-T-cells migrate to the thymus as a naïve cell the other small subtype γδ-T-cells acquire their specific function in the thymus and provide limited plasticity (Fahl, Coffey and Wiest, 2014). These cells show significant differences from each other, γδ-T-cells prefer epithelial surfaces and are infrequent in secondary lymphoid organs (Hayday, 2000; Carding and Egan, 2002; Fahl, Coffey and Wiest, 2014). γδ-T-cells are not MHC restricted and their roles in the immune system are not well recognized as for the other conventional αβ-T-cells (Salerno et al., 2001; Fahl, Coffey and Wiest, 2014).

Taif city is a high-altitude city with an elevation equal to 1879 m above the sea level. Oxygen levels are reduced with high-altitude, plasma volume in blood decrease due to the increase in haemoglobin concentration due to dehydration and hypoxia, thus with the increased viscosity of the blood the rise of the danger of stroke and thromboembolism (Peacock, 1998). This study has aimed to evaluate the effect of smoking regular cigarettes and shisha on the levels of both αβ-T-cells and γδ-T-cells between Taif and Makkah residents. In both populations, levels of CD4+ and CD8+ T-cells will be evaluated and compared. Our recent findings showed High-altitude have effect on the levels of ISG-15 (Alhazmi et al., 2018; Almehmadi, 2019).

MATERIALS AND METHODS

PBMC collection:

Peripheral blood mononuclear cell (PBMC) samples were obtained from a panel of 56 healthy male volunteers aged between 18 and 54 years, following written consent. Participants must have been smokers for at least 5 years and during this period they must have been living in Taif or Makkah, all the smokers consumed at least 1 pack per two days, and shisha smokers consumed at least 1 per two days. The usual habit of shisha smokers was to smoke conventional cigarettes as well, but cigarette smokers did not smoke shisha most of the time or never at all. About 3 ml of blood was drawn into EDTA tubes. Ethical approval for this study was obtained from Taif university committee of research. PBMCs were prepared by density gradient centrifugation using Ficoll-Paque ™ Plus (GE Healthcare, Little Chalfont, Bucks, UK). PBS was used to wash the cells after they were removed from the interface after centrifuging for 25 minutes with brake off at 4°C. Participants were divided into three groups: non-smokers, cigarette smokers and shisha smokers.

Flow cytometric analysis:

About $1x10^6$ cells were placed in 50 ul of PBS for staining by conjugated monoclonal antibodies (mAb) purchased from BD Biosciences. FITC Mouse Anti-Human TCR αβ (561673), FITC Mouse IgM, κ Isotype Control (555583), PE-Cy™7 Mouse Anti-Human TCR γ/δ-1 Clone 11F2 (655410), isotype Mouse IgG_1, κ PE-CY7 (557872), anti-CD8 PE (sk1) (345773), isotype Mouse IgG1 k (550617), anti-CD8 FITC (sk1) (345772), Isotype Mouse IgG1 k (550616) and anti-CD4 FITC (561840), Isotype Mouse IgG1, k (554679).

A FACSCanto was used for the detection of the stained cells and FACs diva application for the analysis. Doublet discrimination was applied after lymphocytes were gated to remove doublets to count only single cells. For statistical analysis Graph-Pad prism v5.03 was used by applying a Paired-t-test.

RESULTS

During the staining process cells were stained in two tubes for both types of T-cells; each tube had three colors with anti-αβ-TCRs or anti-γδ-TCRs, CD4+ mAb and CD8+ mAb and cells were counted as percentage of the total lymphocyte population. The results of a demographic study are shown in Table 2.1 and after comparing age groups no significant differences were found between groups.

Table 2.1. **Number of participants and average age are illustrated.**

		Taif	Makkah
Subject n		32	24
Average Age	Non-smoker	29.1	25.6
	Smoker	37.5	32.28
	Shisha-S	32	32.4

a- αβ-T-cells

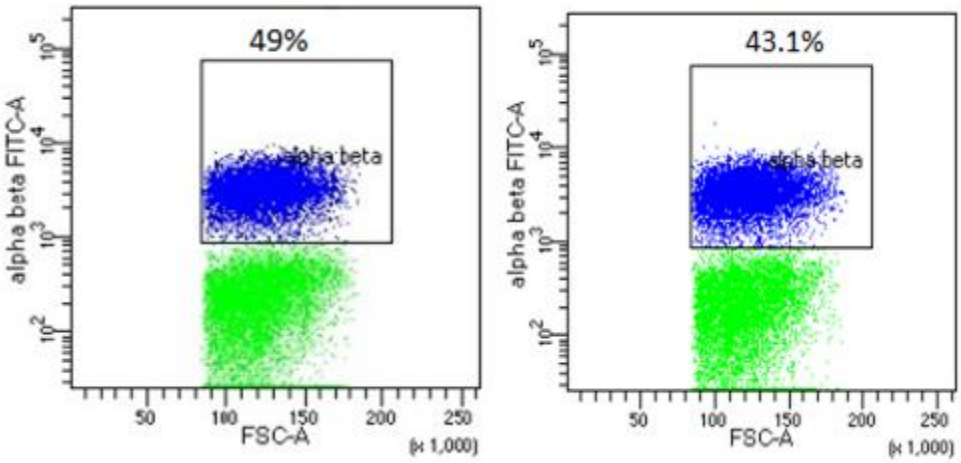

Figure 1. Representative staining results for αβ-T-cells. The blue colored population is αβ-T-cells; the left scatter-plot represents a shisha-smoker from Taif and the right scatter-blot represents a shisha-smoker from Makkah.

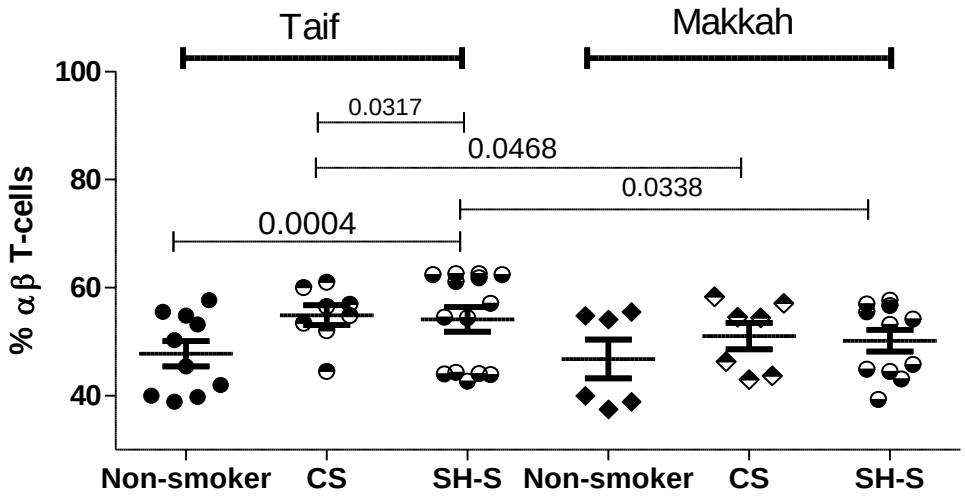

Figure 2. αβ-T-cell percentages as part of total lymphocytes were compared between non-smokers, cigarette smokers (CS) and Shisha smokers (SH-S).

To study the percentage of αβ-T-cells as part of total lymphocytes between the three groups in two different cities at different altitudes, representative staining results are shown in Figure 1; PBMCs were stained by anti- αβ-TCR-FITC and the results are illustrated in Figure 2. Significant differences ($P < 0.05$) were found between shisha-smokers and both non-smokers and cigarette smokers in Taif ($P = 0.0004$ and 0.0317 respectively). Moreover, shisha-smokers had significantly higher proportions of αβ-T-cells in Taif than those from Makkah ($P = 0.0255$). Cigarette smokers from Taif also had significantly higher proportions of αβ-T-cells than those from Makkah ($P = 0.0468$).

CD4+ and CD8+ αβ-T-cells:

To study the percentage of CD4+ and CD8+ αβ-T-cells as part of αβ-T-cells between the three groups in two different cities with different altitude, PBMCs were stained by three-color staining using a combination of anti-αβ-TCR-FITC, anti-CD8-PE and anti-CD4-APC; the results for CD8+ αβ-T-cells are illustrated in Figure 3 and CD4+αβ-T-cells are illustrated in Figure 4.

CD8+αβ-T-cell percentages as part of total αβ-T-cells were compared between non-smokers, cigarette smokers and Shisha smokers. Proportions of CD8+αβ-T-cells were significantly higher in shisha-smokers than both non-smokers and cigarette-smokers in Taif (0.0060 and 0.0140 respectively), also cigarette smokers showed significant higher proportions of CD8+αβ-T-cells than non-smokers in Makkah ($P = 0.0287$). Shisha-smokers had significantly higher proportions of CD8+αβ-T-cells than cigarette smokers in Makkah ($P = 0.0338$). No significant differences were detected when Taif groups were compared to Makkah groups.

CD4+αβ-T-cell percentages as part of total αβ-T-cells were compared between non-smokers, cigarette smokers and Shisha smokers as illustrated in figure 4. Proportions of CD4+αβ-T-cells were significantly reduced in shisha-smokers than non-smokers and cigarettes-smokers in Taif, CD4+αβ-T-cells were significantly reduced in shisha-smokers than non-smokers and the latter had significantly more CD4+αβ-T-cells than cigarette-smokers.

CD4+αβ-T-cells percentage as part of total αβ-T-cells were compared between non-smokers, cigarette smokers and Shisha smokers. 5 significant results were detected, CD4+αβ-T-cells were significantly reduced in shisha-smokers than non-smokers and cigarette-smokers in Taif, CD4+αβ-T-cells were significantly reduced in shisha-smokers than non-smokers and the latter has significantly more CD4+αβ-T-cells than cigarette-smokers.

To study the percentages of γδ-T-cells as part of total lymphocytes between the three groups in two different cities at different altitudes, PBMCs were stained by anti-γδ-TCR-PE-CY7, representative staining results are shown in Figure 4, and the results are illustrated in Figure 5. Proportions of γδ-T-cells were significantly lower in shisha-smokers than non-smokers in Taif ($P = 0.0062$). Proportions in shisha-smokers from Taif were significantly lower compared with those from Makkah. No other significant results were detected. The average percentage as part of total lymphocytes of γδ-T-cells in non-smokers from Taif was 4.65% while in shisha-smokers it was 3.3%.

CD4+ and CD8+ γδ-T-cells

To study the percentage of CD4+ and CD8+ γδ-T-cells as part of γδ-T-cells between the three groups in two different cities at different altitudes, PBMCs were stained by three-color using a combination of anti-γδ-TCR-PE-CY7, anti-CD8-FITC and anti-CD4-APC, the results for CD8+ αβ-T-cells are illustrated in Figure 6. Shisha-smokers from Taif and Makkah showed significantly higher proportions of CD8+γδ-T-cells than non-smokers ($P = 0.0182$ and 0.0033 respectively). Regarding CD4+ γδ-T-cells, PBMCs were collected from 56 participants and only very few showed between 1% to 2.1% CD4+ γδ-T-cells which are not sufficient to perform statistical analysis. However, double negative γδ-T-cells were compared between those groups.Double negative (DN) γδ-T-cells in Shisha-smokers from Taif and Makkah showed significantly lower proportions of DN +γδ-T-cells than non-smokers (Figure 7).

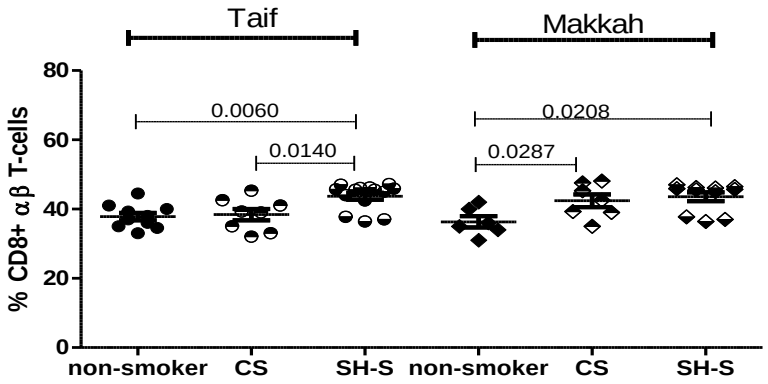

Figure 3. CD8+αβ-T-cell percentages as part of total αβ-T-cells were compared between non-smokers, cigarette smokers (CS) and Shisha smokers (SH-S).

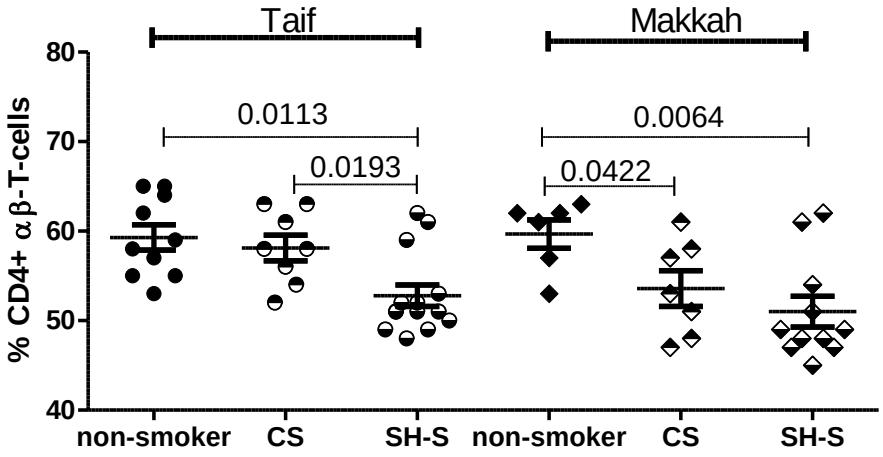

Figure 4. CD4+αβ-T-cell percentages as part of total αβ-T-cells were compared between non-smokers, cigarette smokers (CS) and Shisha smokers (SH-S).

b-γδ-T-cells

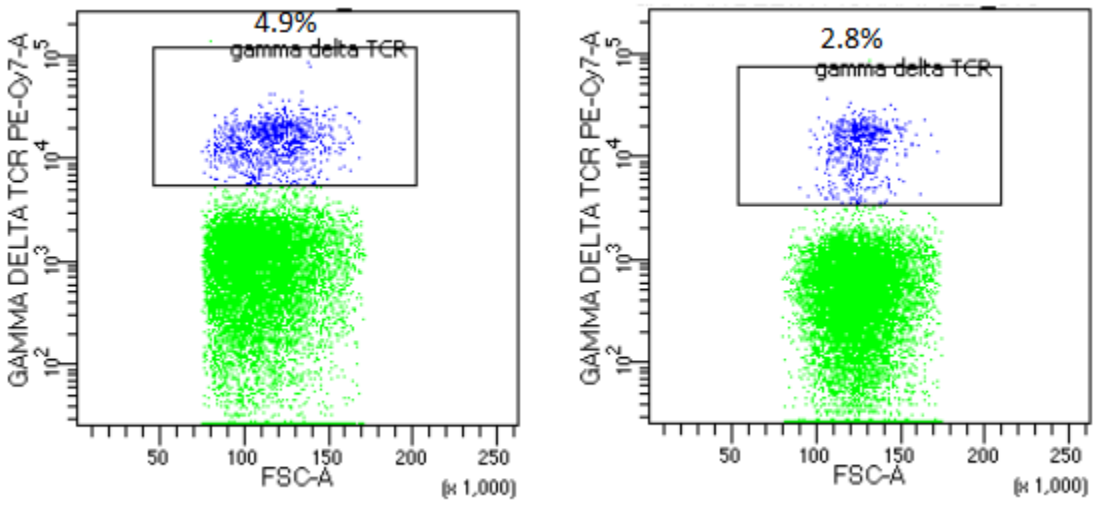

Figure 4. Representative staining results for γδ-T-cells. The blue colored population is γδ-T-cells. The left scatter-blot is from a shisha-smoker from Makkah while the right scatter-blot is from a shisha-smoker from Taif.

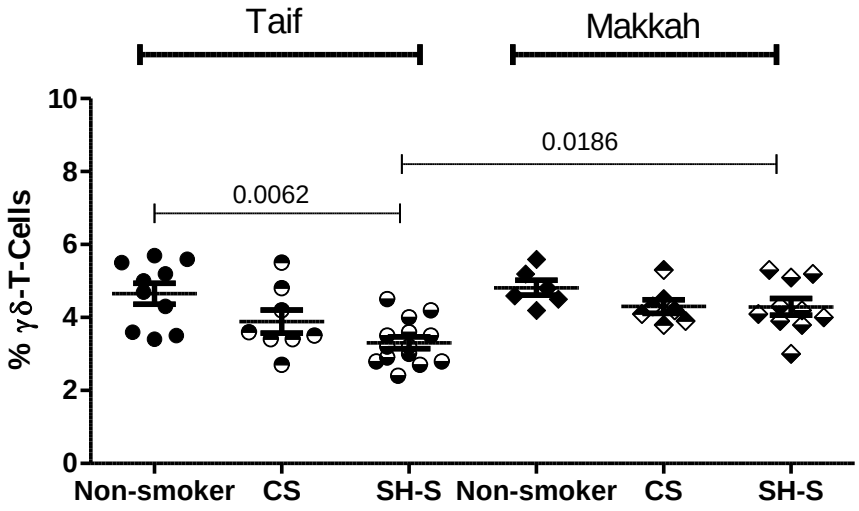

Figure 5. γδ-T-cell percentages as part of total lymphocytes were compared between non-smokers, cigarette smokers (CS) and Shisha smokers (SH-S).

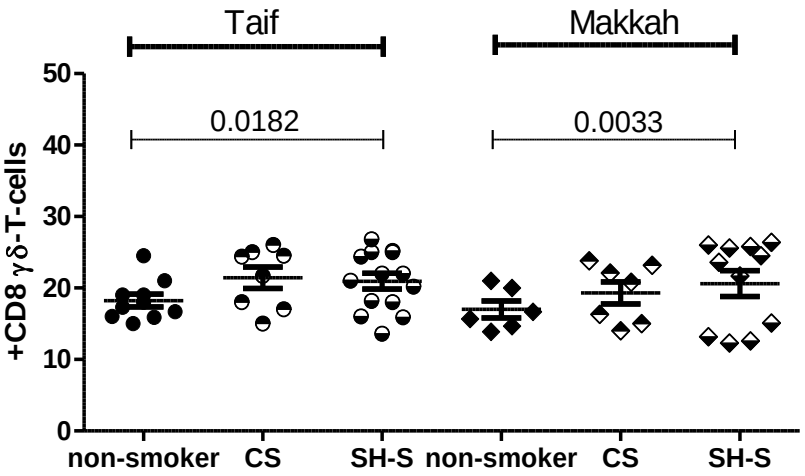

Figure 6. CD8+γδ-T-cell percentages as part of total γδ-T-cells were compared between non-smokers, cigarette smokers (CS) and Shisha smokers (SH-S) in Taif and Makkah.

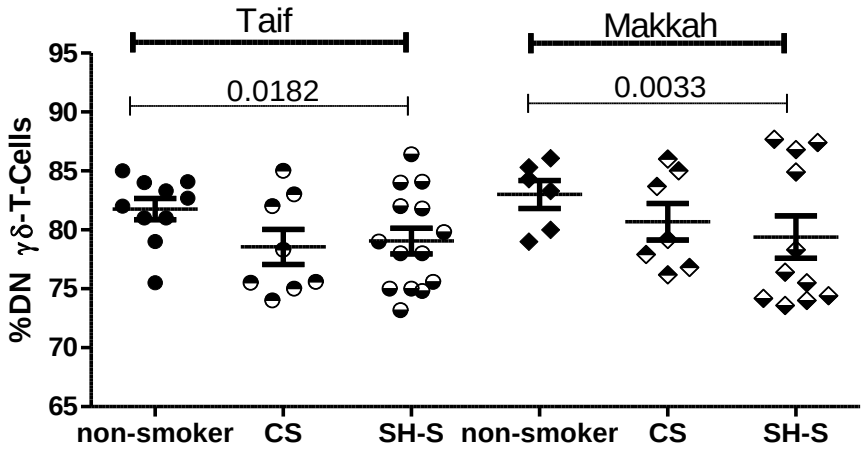

Figure 7. DN-γδ-T-cell percentages as part of total γδ-T-cells were compared between non-smokers, cigarette smokers (CS) and Shisha smokers (SH-S) in Taif and Makkah.

DISCUSSION

This study has aimed to evaluate the relative numbers of αβ-T-cells and γδ-T-cells in non-smokers, cigarette smokers and shisha smokers between two different cities at different altitudes. CD4+ and CD8+ T-cells from both populations have been studied to evaluate if there are any differences due to the altitude and smoking status. Shisha or hookah usage expose the individuals to high amounts of carbon monoxide, extremely high levels of carcinogenic polycyclic aromatic hydrocarbons, tar, nicotine more than the amount in cigarette (Pfeifer et al., 2002; Shihadeh and Saleh, 2005; Neergaard et al., 2007; Barnett et al., 2011; Choe et al., 2018), and it is associated with cardiovascular diseases and cancer (Barnett et al., 2011; Barnett, Lorenzo and Soule, 2017; Choe et al., 2018). This study has confirmed that high-altitude can affect the T-cell levels between shisha-smokers, cigarette smokers and non-

smokers. Therefore, samples were collected from two different cities with about 1600 m difference in altitudes. Overall, proportions of αβ-T-cells were found to be significantly higher in shisha smokers than cigarette smokers and non-smokers in Taif. Shisha also led to significant expansion in the number of αβ-T-cells compared to cigarette smokers in Taif as well suggesting smoking shisha and cigarettes in a high-altitude city can lead to this expansion. This was also detected when levels of αβ-T-cells were found to be significantly higher in shisha-smokers from Taif than shisha-smokers from Makkah. Proportions of CD8+ αβ-T-cells were found to be higher in shisha-smokers than non-smokers in both Taif and Makkah; this result is consistent with other studies showing smoking can increase the numbers of CD8+ T-cells (Forsslund et al., 2014). Furthermore, another study performed in mice models showed that CD8+ T-cells increased by about 1.48 times compared to controls in nicotine treated mice (Wasén et al., 2017). CD8+ T-cells also increased in asthmatic-smokers and the ratio of CD4+ to CD8+ T-cells was lower in smokers than non-smokers (Ravensberg et al., 2013). However, another study performed in COPD patients showed that CD8+ T-cells were reduced in COPD patients who smoked but to a lesser extent than in non-smokers and smoker without COPD; this study also found that smokers have fewer CD8+ T-cells than non-smokers. Moreover, this study is inconsistent with that of Ravensberg et al (Ravensberg et al., 2013) and found that the ratio of CD4+ to CD8+ T-cells was increased in smokers compared to non-smokers (Koch et al., 2007).

Regarding CD4+ αβ-T-cells, the percentage was calculated as part of total αβ-T-cells and compared between non-smokers, cigarette smokers and Shisha smokers. CD4+αβ-T-cells were significantly reduced in shisha-smokers compared to non-smokers and cigarette-smokers in Taif and non-smokers has significantly more CD4+αβ-T-cells than cigarette-smokers. Regarding CD4+ αβ-T-cells, this study is consistent with studies showing insignificant declines in CD4+ αβ-T-cells in asthmatic-smokers than non-smokers (31) and the same was found in smoker with occupational asthma (Sjaheim et al., 2006). However, our findings contrast with other studies showing expansion of CD4+T-cells in smokers in whom naïve cells were induced to shift to memory CD4 T-cells due to repeated exposure to tobacco, leading to exhaustion of T-cells and immune aging (Nakata et al., 2007). Gastroesophageal reflux is a common disease related to smoking, and CD4+-T-cells were found to be reduced in smokers with gastroesophageal reflux disease after treatment with proton-pump inhibitors (Ahmed Osman et al., 2018). This study has evaluated CD4+ αβ-T-cell proportions of total αβ-T-cells at high-altitude and low-altitude as well suggesting shisha-smoking and cigarette smoking can have adverse effects on total numbers of CD4+ αβ-T-cells.

According to a recent study, hypoxia can reduce the density of γδ-T-cells (Siegers et al., 2018). Our study shows that γδ-T-cell percentages were affected by shisha-smoking. Taif shisha-smokers displayed significantly lower percentages than Taif-non-smokers and Makkah shisha-smokers. This reduction occurred while αβ-T-cells were expanded in the same group tested, especially CD8+ αβ-T-cells. Generally, the unconventional γδ-T-cells have not been thoroughly studied. The present results are inconsistent with other findings which showed increases in γδ-T-cells in smokers with healthy lungs (Pons et al., 2005). Other studies detected an expansion of γδ-T-cell numbers in the lungs of smokers (Richmond et al., 1993; Majo, Ghezzo and Cosio, 2001). CD8+ γδ-T-cells were significantly expanded in shisha-smokers in Taif and Makkah, which is consistent with the findings of Pones et al (Pons et al., 2005). The observed expansion of CD8+ γδ-T-cells was at the expense of DN γδ-T-cells which were found to decline in numbers in Shisha-smokers from both cities.

CONCLUSION

Our study has shown that living in high-altitude cities and smoking shisha can affect the levels if both αβ-T-cells and γδ-T-cells. The difference in levels is due to hypoxia and ambient air pollution between both cities.

CONFLICT OF INTEREST

The author declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

This work was done in Taif University and we would thank the deanship of scientific research, high-altitude centre and everyone who participated in this study

AUTHOR CONTRIBUTIONS

The study design and analysis done by Dr MA, Dr AA and Dr. MH, Samples collection and analysis were done by Mr. AB, Mr. MA, Mr. NA,

Mr. AA, Mr. AH, Mr., EA. Some reagents were provided by Mrs., AA and Mr. AH.

REFERENCES

Ahmed Osman, H. et al., (2018) 'Effect of Acid Suppression on Peripheral T-Lymphocyte Subsets and Immunohistochemical Esophageal Mucosal Changes in Patients With Gastroesophageal Reflux Disease', *Journal of Clinical Gastroenterology*, p. 1. doi: 10.1097/MCG.0000000000001098.

Alcover, A., Alarcón, B. and Di Bartolo, V. (2018) 'Cell Biology of T Cell Receptor Expression and Regulation', *Annual Review of Immunology*. doi: 10.1146/annurev-immunol-042617-053429.

Alexandrov, L. B. et al., (2016) 'Mutational signatures associated with tobacco smoking in human cancer', *Science*. doi: 10.1126/science.aag0299.

Alhazmi, A. S. et al., (2018) *Effect of High Altitude on Thyroid and Thyroid Stimulating Hormones Levels in Taif City*. Available at: https://actascientific.com/ASNH/pdf/ASNH-02-0142.pdf (Accessed: 4 February 2019).

Almehmadi, M. (2019) 'Effect of High-Altitude on Serum-ISG-15 Levels in Influenza Patients', 2, pp. 2581–3226. Available at: https://actascientific.com/ASMI/pdf/ASMI-02-0137.pdf (Accessed: 10 January 2019).

Barnett, T. E. *et al.* (2011) 'Carbon Monoxide Levels Among Patrons of Hookah Cafes', *American Journal of Preventive Medicine*. Elsevier, 40(3), pp. 324–328. doi: 10.1016/j.amepre.2010.11.004.

Barnett, T. E., Lorenzo, F. E. and Soule, E. K. (2017) 'Hookah Smoking Outcome Expectations Among Young Adults.', *Substance use & misuse*. NIH Public Access, 52(1), pp. 63–70. doi: 10.1080/10826084.2016.1214152.

Carding, S. R. and Egan, P. J. (2002) 'γδ T cells: functional plasticity and heterogeneity', *Nature Reviews Immunology*. Nature Publishing Group, 2(5), pp. 336–345. doi: 10.1038/nri797.

Cataldo, J. K., Prochaska, J. J. and Glantz, S. A. (2010) 'Cigarette smoking is a risk factor for Alzheimer's disease: An analysis controlling for tobacco industry affiliation', *Journal of Alzheimer's Disease*. doi: 10.3233/JAD-2010-1240.

Choe, E. H. *et al.* (2018) 'Shisha smoking as a possible cause of bilateral granulomatous lung lesions', *Respirology Case Reports*, 6(9), p. e00374. doi: 10.1002/rcr2.374.

Fahl, S. P., Coffey, F. and Wiest, D. L. (2014) 'Origins of γδ T Cell Effector Subsets: A Riddle Wrapped in an Enigma', *The Journal of Immunology*. doi: 10.4049/jimmunol.1401813.

Forsslund, H. *et al.* (2014) 'Distribution of T-cell subsets in BAL fluid of patients with mild to moderate COPD depends on current smoking status and not airway obstruction', *Chest*. doi: 10.1378/chest.13-0873.

Germain, R. N. (1994) 'MHC-dependent antigen processing and peptide presentation: providing ligands for T lymphocyte activation.', *Cell*, 76(2), pp. 287–99. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8293464 (Accessed: 26 October 2018).

Glusman, G. *et al.* (2001) 'Comparative genomics of the human and mouse T cell receptor loci', *Immunity*. doi: 10.1016/S1074-7613(01)00200-X.

Goldsmith, M. A. and Weiss, A. (1988) 'Early signal transduction by the antigen receptor without commitment to T cell activation.', *Science (New York, N.Y.)*, 240(4855), pp. 1029–31. Available at: http://www.ncbi.nlm.nih.gov/pubmed/3259335 (Accessed: 26 October 2018).

Gonçalves, R. B. *et al.* (2011) 'Impact of smoking on inflammation: Overview of molecular mechanisms', *Inflammation Research*. doi: 10.1007/s00011-011-0308-7.

Grumelli, S. *et al.* (2004) 'An immune basis for lung parenchymal destruction in chronic obstructive pulmonary disease and emphysema', *PLoS Medicine*. doi: 10.1371/journal.pmed.0010008.

Hayday, A. C. (2000) 'γδ Cells: A Right Time and a Right Place for a Conserved Third Way of Protection', *Annual Review of Immunology*, 18(1), pp. 975–1026. doi:

10.1146/annurev.immunol.18.1.975.

Koch, A. *et al.* (2007) 'Modification of surface antigens in blood CD8+ T-lymphocytes in COPD: effects of smoking.', *The European respiratory journal*. European Respiratory Society, 29(1), pp. 42–50. doi: 10.1183/09031936.00133205.

Mainali, P. *et al.* (2015) 'Tobacco and Cardiovascular Health', *Cardiovascular Toxicology*. doi: 10.1007/s12012-014-9280-0.

Majo, J., Ghezzo, H. and Cosio, M. G. (2001) 'Lymphocyte population and apoptosis in the lungs of smokers and their relation to emphysema.', *The European respiratory journal*, 17(5), pp. 946–53. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11488331 (Accessed: 28 October 2018).

Meuronen, A. *et al.* (2008) 'Decreased cytokine and chemokine mRNA expression in bronchoalveolar lavage in asymptomatic smoking subjects.', *Respiration; international review of thoracic diseases*. Karger Publishers, 75(4), pp. 450–8. doi: 10.1159/000114855.

Michaud, D. S. (2016) 'Pancreatic Cancer', in *International Encyclopedia of Public Health*. doi: 10.1016/B978-0-12-803678-5.00322-2.

Nakata, A. *et al.* (2007) 'Relationship between cumulative effects of smoking and memory CD4+ T lymphocyte subpopulations', *Addictive Behaviors*. doi: 10.1016/j.addbeh.2006.11.007.

Neergaard, J. *et al.* (2007) 'Waterpipe smoking and nicotine exposure: A review of the current evidence', *Nicotine & Tobacco Research*, 9(10), pp. 987–994. doi: 10.1080/14622200701591591.

Peacock, A. J. (1998) 'ABC of oxygen: oxygen at high altitude.', *BMJ (Clinical research ed.)*. BMJ Publishing Group, 317(7165), pp. 1063–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9774298 (Accessed: 26 October 2018).

Pfeifer, G. P. *et al.* (2002) 'Tobacco smoke carcinogens, DNA damage and p53 mutations in smoking-associated cancers', *Oncogene*. doi: 10.1038/sj.onc.1205803.

Pons, J. *et al.* (2005) 'Blunted T-lymphocyte response in chronic obstructive pulmonary disease', *European Respiratory Journal*, 25(3), pp. 441–446. doi: 10.1183/09031936.05.00069304.

Ravensberg, A. J. *et al.* (2013) 'CD8+ T cells characterize early smoking-related airway pathology in patients with asthma', *Respiratory Medicine*, 107(7), pp. 959–966. doi: 10.1016/j.rmed.2013.03.018.

Reinherz, E. L. (2015) 'αβ TCR-mediated recognition: relevance to tumor-antigen discovery and cancer immunotherapy.', *Cancer immunology research*. NIH Public Access, 3(4), pp. 305–12. doi: 10.1158/2326-6066.CIR-15-0042.

Richmond, I. *et al.* (1993) 'Distribution of gamma delta T-cells in the bronchial tree of smokers and non-smokers.', *Journal of clinical pathology*, 46(10), pp. 926–30. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8227410 (Accessed: 26 October 2018).

Salerno, A. *et al.* (2001) 'Role of Gamma-Delta T Cells in Cutaneous Hypersensitivity Reactions', in *Gamma-Delta T Cells*. Basel: KARGER, pp. 87–98. doi: 10.1159/000058834.

Sander L, Gilman, X. Z. (2004) *Smoke: A Global History of Smoking* -. London. Available at: https://books.google.com.sa/books?id=mM5bYb_uVcwC&printsec=frontcover&hl=ar&source=gbs_ge_summary_r&cad=0#v=onepage&q&f=false (Accessed: 26 October 2018).

Shihadeh, A. and Saleh, R. (2005) 'Polycyclic aromatic hydrocarbons, carbon monoxide, "tar", and nicotine in the mainstream smoke aerosol of the narghile water pipe', *Food and Chemical Toxicology*, 43(5), pp. 655–661. doi: 10.1016/j.fct.2004.12.013.

Siegers, G. M. *et al.* (2018) 'Functional Plasticity of Gamma Delta T Cells and Breast Tumor Targets in Hypoxia.', *Frontiers in immunology*. Frontiers Media SA, 9, p. 1367. doi: 10.3389/fimmu.2018.01367.

Sjaheim, T. *et al.* (2006) 'Reduced bronchial CD4+ T-cell density in smokers with occupational asthma', *European Respiratory Journal*, 28(6), pp. 1138–1144. doi: 10.1183/09031936.06.00032906.

Vardavas, C. I. *et al.* (2010) 'Passive smoking alters circulating naïve/memory lymphocyte T-cell subpopulations in children', *Pediatric Allergy and Immunology*, 21(8), pp. 1171–1178. doi: 10.1111/j.1399-3038.2010.01039.x.

Vargas-Rojas, M. I. *et al.* (2011) 'Increase of Th17 cells in peripheral blood of patients with chronic obstructive pulmonary disease', *Respiratory Medicine*, 105(11), pp. 1648–1654. doi: 10.1016/j.rmed.2011.05.017.

Wang, J. C. *et al.* (1992) 'Physical mapping of loci in the distal half of the short arm of the

human X chromosome: Implications for the spreading of X-chromosome inactivation', *Somatic Cell and Molecular Genetics*. doi: 10.1016/j.puhe.2012.12.006.

Warren, G. W., Sobus, S. and Gritz, E. R. (2014) 'The biological and clinical effects of smoking by patients with cancer and strategies to implement evidence-based tobacco cessation support', *The Lancet Oncology*. doi: 10.1016/S1470-2045(14)70266-9.

Wasén, C. *et al.* (2017) 'Smoking activates cytotoxic CD8+ T cells and causes survivin release in rheumatoid arthritis', *Journal of Autoimmunity*, 78, pp. 101–110. doi: 10.1016/j.jaut.2016.12.009.