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Differences of plasma IL-1 and TNF-α in healthy Chinese Population

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Abstract: Pleiotropic proinflammatory cytokines, interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α), involved in the regulations of various immune responses, inflammatory processes and hematopoiesis. In the present study, the expression levels of IL-1 and TNF-α were detected by enzyme-linked immunosorbent assay (ELISA). Following the cytokine blockade as a successful clinical therapy for autoimmune diseases such as rheumatoid arthritis, the patients are more susceptible to a variety of opportunistic infections. IL-1 and TNF-α may be useful predictive biomarkers of diseases and offer potential targets for therapeutic intervention of inflammatory diseases. However, our results showed that the plasma IL-1 level was significantly higher in women compared to men (69.5 ± 19.8 pg/ml in men and 80.1 ± 19.5 pg/ml in women, respectively); the plasma levels of TNF-α were higher in men than women (20.8 ± 4.9 pg/ml and 18.7 ± 7.1 pg/ml, respectively). The significant gender difference of plasma interleukin-1 (IL-1) and TNF-α levels present in healthy adults in Jiangsu Province, China (P=0.002 and P=0.015, respectively), and may be as a hint for sex differences of susceptibility to many diseases and elementary immune response.

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1 Introduction

Cytokines, with 8 to 40,000 Da molecular weight, are a group of small soluble or membrane-bound precursor proteins or glycoprotein messenger molecules [1]. They could be divided into two categories, proinflammatory (e.g. IL-1, IL-6, TNF-α, TGF-β) and antiinflammatory (e.g. IL-1Ra, IL-4, IL-10, IL-13) cytokines. The balance between proinflammatory and antiinflammatory cytokines has been noticed in a number of diseases [2]. Interleukin-1 (IL-1) (including α and β subtype) is the prototypic multifunctional cytokine, its various biological role can be listed as endogenous pyrogen, leukocyte endogenous mediator, epidermal cell derived thymocyte activating factor and so on [3, 4]. The cell signaling pathways with IL-1 involved can regulate inflammation, angiogenesis, hematopoiesis and cognition [5, 6]. IL-1 and other analogous cytokines play a key role in cell growth, tissue repair and many acute or chronic inflammatory diseases [3, 7-12]. Tumour necrosis factor-α (TNF-α), secreted by macrophages, can make active responses to infection, inflammation and cancer [13]. It plays a major role in tissue inflammation [14] and remodeling [15] by stimulating the production of collagenase. Particularly, TNF-α can activate the production of other proinflammatory cytokines, up-regulate some transcription factors and promote keratinocytes proliferation, which may lead to pathological inflammation in several organs, such as skin and joints [16, 17]. IL-1β and TNF-α, as the well-known proinflammatory cytokines, could be treated as the mediators of shock [18-21], also present at high concentrations in bronchoalveolar lavage fluid of patients with sustained ARDS [20]. Studies have indicated that TNF-α and IL-1β can directly alter vascular tone [22] and increase microvascular permeabilities in endothelial monolayer [23-25].

Gender difference, as an important factor in many diseases, has become increasingly noticeable in recent years. Meanwhile, more and more clinical case reports were recorded with respect to gender difference in cytokine expression levels. The levels of IL-1β and IL-6 in colorectal cancer patients were significantly higher in men than in women. Followed by acute psychological stress, men showed a significant decrease in the production of IL-1β and IL-6 and TNF-α, whereas women displayed no change during this period. When infected with influenza virus, men had statistically significant higher specific secretion of TNF-α. However, few reports could be found about the possible gender difference in cytokine expression levels in healthy population. Therefore, we would like to take IL-1 and TNF-α for instance, exploring possible gender difference in cytokine levels in Chinese healthy adults.

In this study, we enrolled 158 healthy young adults aged between 20 and 45 years old, employing ELISA approach to identify the plasma level of IL-1 and TNF-α in these volunteers. Relevant statistical analysis was carried out, with an expectation to find the possible gender difference in expression of these two proinflammatory cytokines.

2 Patients and methods

2.1 Ethics Statement

The present study protocol was approved by Danyang People's Hospital's Institutional Review Committee on Human Research. All healthy adult volunteers provided written informed consent before any study-related procedure was performed.

2.2 Study population

A total of 158 healthy individuals (70 females and 88 males) in Jiangsu Province, China were enrolled for our study. All subjects were young adult: the ages (20~45 years) between females and males were comparable. This study has been approved by Ethics Review Board of Danyang People's Hospital and all volunteers have signed an informed consent form.

Information on health status of all participants was obtained from the medical examination center of Danyang People's Hospital of Jiangsu Province. No acute pathological condition and other inflammatory disorder was found in the subjects enrolled for the study. All subjects who had the history of chronic diseases, hormone sensitive diseases or autoimmune diseases were excluded from the present study.

2.3 Study design

We designed a population-based examination of gender difference of plasma pleiotropic proinflammatory cytokines in Chinese healthy adults, selected the subjects with appropriate gender proportion and age ranging from 20 to 45 year old.

The concentrations of cytokine were determined in a total of 158 plasma samples from the study subjects. After reference to normal concentrations, more than 150pg/ml in IL-1 and 65pg/ml in TNF-α were excluded from the subjects respectively. Some additional subjects were also excluded from the study because of missing data for some of the analysis parameters. Therefore, the final study population size for the analysis was 144 and the mean ± SD age was 29.3 ± 0.5 years (64 females and 80 males, with mean ± SD ages being 29.4 ± 0.5 and 29.2 ± 0.8 years, respectively).

2.4 Plasma separated

The blood samples were taken in the non-fasting state before 7:30 a.m. and collected into ethylenediamine tetraacetic acid (EDTA)-anticoagulated tubes, then centrifuged immediately at 1500 rpm for 10min, with plasma and blood cells separated. The plasma was collected and stored at -70°C until analysis.

2.5 Quantification for plasma level of cytokines by enzyme-linked immunosorbent assay (ELISA)

158 plasma samples from subjects were available as described previously and were pre-stored at -20°C until the assay. Plasma levels of IL-1 and TNF-α were determined using a commercial ELISA kit (rat-anti human IL-1 and rat-anti human TNF-α; Adilitteram Diagnostic Laboratories, Inc., USA) strictly according to the manufacturer's instructions. The detection limit was 1pg/ml. All ELISA measurements were conducted in duplicate and the mean concentrations were calculated.

2.6 Statistical analysis

All data were showed as the mean ± SD (Table 1). Normality was checked by Kolmogorov-Smimov test or Shapiro-Wilk test. In order to determine the correlation between variables, Spearman rank correlations were performed. We grouped the results of analysis on the basis of gender, compared the variables between men and women group used the independent-samples t test or Mann-Whiteny test to evaluate the mean difference.

The SPSS for windows statistical package was used to perform all statistical evaluation (SPSS Inc., Chicago, IL, USA). The level of significance used for all of the above analysis was two tailed, P values less than 0.05.

3 Results

In our study, the term "baseline" is defined as the circulating levels of the cytokines in young subjects (the range from 20 to 45 years) at healthy status.

The descriptive data stratified by gender are presented in Table 1 and 2, which can be intuitively understood in Figure 1. Specifically, the plasma levels of IL-1 displayed in Table 2 did not show any detectable age-associated trend (correlation coefficient r=-0.106, P=0.205) at the healthy status. However, the plasma levels of TNF-α showed the significant age-associated trend (correlation coefficient r=0.504, P less than 0.001). Baseline plasma levels of IL-1 (P=0.002) were significant higher among the young women than among young men. In contrast, the baseline plasma levels of TNF-α were lower among women group than among men group, and these difference was statistically significant (P=0.015). Those results can be shown in detail in Figure 2a and 2b. By the way, there is a negative correlation showed in Table 3 between IL-1 and TNF-α to all subjects (P=0.025). No significant correlation can be found between IL-1 and TNF-α at grouping levels (grouped by gender, men and women, respectively).

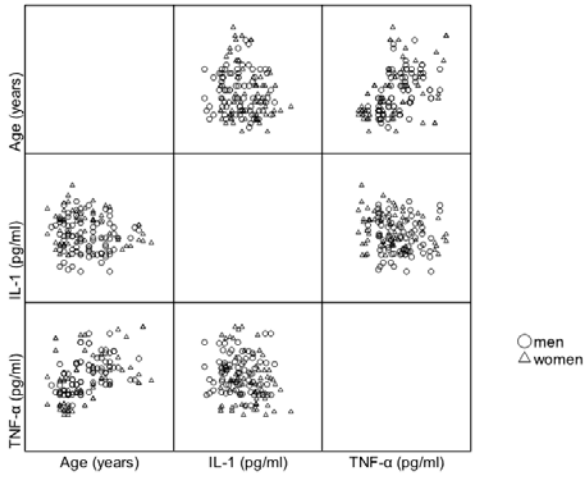

Figure 1: Matrix scatter show the correlation between age, IL-1 and TNF-α, respectively.

Table 1: Descriptive data stratified by gender

	Study population		
Study parameters (Variable)	**Men** n **=80**	**Women** n **=64**	P **value**
Age(years)	29.4±4.8	29.2±6.5	NS
IL-1 (pg/ml)	69.5±19.8	80.1±19.5	0.002[a]
TNF-α (pg/ml)	20.8±4.9	18.7±7.1	0.015[b]

Plus-minus values are means±SD. For normally distributed variables, P values were computed with t-test (0.002[a]). For non-normally distributed variables, P values were computed with the Mann-Whiteny test (0.015[b] & NS). NS, P more than 0.05.

Table 2: The age-related trends of plasma cytokines levels in our study

		n	Correlation coefficient	Statistical significance
IL-1	All subjects	144	-0.106	0.205
	Men	80	-0.131	0.245
	Women	64	-0.017	0.895
TNF-α	All subjects	144	0.504	<0.001*
	Men	80	0.573	<0.001*
	Women	64	0.432	<0.001*

Correlation between the age and plamsa levels of IL-1(upper part) and TNF-α(lower part) among all study subjects and groups stratified by gender, respectively. All P values were calculated by Spearman's rank test. n, number of subjects. *, the significant difference.

Table 3: The correlation between IL-1 and TNF-α in study subjects

	n	Correlation coefficient	Statistical significance
All subjects	144	-0.187	0.025*
Men	80	-0.148	0.190
Women	64	-0.123	0.334

The negative correlation between IL-1 and TNF-α to all subjects (P=0.025). But, no significantly correlation between IL-1 and TNF-α at grouping levels (grouped by gender, men and women, respectively). *, it's significantly.

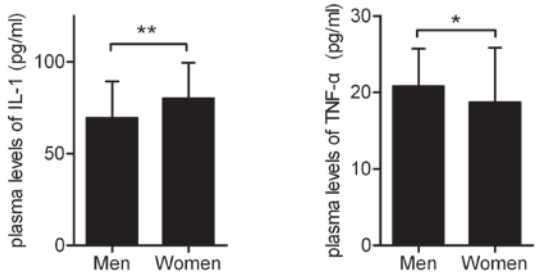

Figure 2a: Bar graph showed plasma levels of IL-1 (left) and TNF-α (right) of each gender, respectively. **P=0.002 VS men group for IL-1; *P=0.015 VS women group for TNF-α.

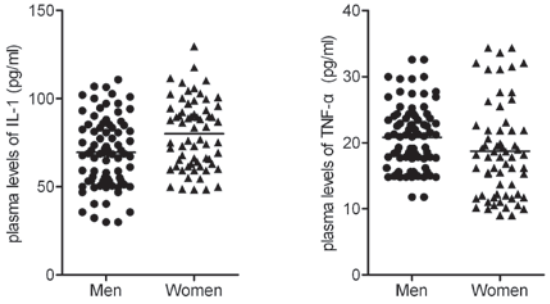

Figure 2b: Columnar scatter graph showed plasma levels of IL-1 (left) and TNF-α (right) of each gender, respectively. Round represent males and triangles represent females. Horizontal line represents the average of each group.

4 Discussion

The most important finding of the present study can be summarized in a word: there is a gender difference in plasma level of proinflammatory cytokine IL-1 and TNF-α in healthy adults in Jiangsu, China. Specifically, the plasma level of IL-1 is significantly higher in females than in males, in contrast, the plasma level of TNF-α is higher in men than in women.

So far, most attentions have been focused on the gender difference of cytokine levels and disease susceptibility in patients or stimulated model animals. For instance, there was increasing evidence indicating that a gender difference exists in cytokine production in inflammatory responses. Males are associated with excessive IL-1β and IL-6 production and organ failures after traumatic injury [26]. In female mouse model of aortic aneurysm, reduced expressions of multiple chemokines and inflammatory cytokines finally results in smaller aneurysms [27]. In addition, women have been exhibited to produce significantly less IL-1β and TNF-α when stimulated with lipopolysaccharide [28, 29]. Women have also been shown to possess better immune functions in sepsis and even have a better prognosis than men [30]. Studies have also demonstrated that women have longer disease free period and overall survival after operations or treatments for colorectal cancer [31].

The reasons behind the gender difference of cytokine production are still unclear. However, the phenotype can be seen as an outcome from interactions between genotype and environmental factors. From this perspective, some phenomena could be attributed to polymorphism in cytokine genes. Childhood responses to vaccine are highly heritable and polymorphisms in genes of cytokine are associated with these responses [32, 33]. In some cases, external environmental factors play a key role in gender differences of cytokine productions. Many factors could be treated as the incentives of cytokine expression differences, such as burn injury [26], acute psychological stress [34] and cytomegalovirus infection [35].

In summary, the present study show that there is a gender difference in the production of IL-1 and TNF-α in healthy individuals of Jiangsu Province, China. It may reflect specific biological characteristics of both males and females. Further clinical relevance and what can be used from this finding in future clinical practice still need to be paid attention to and discussed. Gender difference of plasma cytokines levels may be significant to clinical medicine development, providing chances and challenges to clinical trials and drug applications, although the precise mechanisms responsible for these gender-dependent differences need further research.

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Conflict of interest: Authors state no conflict of interest

References

[1] Stanley AC, Lacy P. Pathways for cytokine secretion. Physiology 2010; 25:218-229
[2] Opal SM, DePalo VA. Anti-inflammatory cytokines. Chest 2000; 117:1162-1172
[3] Dinarello CA. Biologic basis for interleukin-1 in disease. Blood 1996; 87:2095-2147
[4] Tocci MJ. Structure and function of interleukin-1 beta converting enzyme. Vitamins and hormones 1997; 53:27-63
[5] Rachal Pugh C, Fleshner M, Watkins LR, Maier SF, Rudy JW. The immune system and memory consolidation: a role for the cytokine IL-1beta. Neuroscience and biobehavioral reviews 2001; 25:29-41

[6] Shieh JH, Peterson RH, Moore MA. IL-1 modulation of cytokine receptors on bone marrow cells. In vitro and in vivo studies. Journal of immunology 1991; 147:1273-1278
[7] Dinarello CA, Wolff SM. The role of interleukin-1 in disease. The New England journal of medicine 1993; 328:106-113
[8] Dinarello CA. The interleukin-1 family: 10 years of discovery. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 1994; 8:1314-1325
[9] Hannum CH, Wilcox CJ, Arend WP, Joslin FG, Dripps DJ, Heimdal PL, et al. Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. Nature 1990; 343:336-340
[10] Arend WP. Interleukin 1 receptor antagonist. A new member of the interleukin 1 family. The Journal of clinical investigation 1991; 88:1445-1451
[11] Dinarello CA, Thompson RC. Blocking IL-1: interleukin 1 receptor antagonist in vivo and in vitro. Immunology today 1991; 12:404-410
[12] Eisenberg SP, Brewer MT, Verderber E, Heimdal P, Brandhuber BJ, Thompson RC. Interleukin 1 receptor antagonist is a member of the interleukin 1 gene family: evolution of a cytokine control mechanism. Proceedings of the National Academy of Sciences of the United States of America 1991; 88:5232-5236
[13] Beutler B, Cerami A. Cachectin: more than a tumor necrosis factor. The New England journal of medicine 1987; 316:379-385
[14] Dayer JM, Beutler B, Cerami A. Cachectin/tumor necrosis factor stimulates collagenase and prostaglandin E2 production by human synovial cells and dermal fibroblasts. The Journal of experimental medicine 1985; 162:2163-2168
[15] Vlassara H, Brownlee M, Manogue KR, Dinarello CA, Pasagian A. Cachectin/TNF and IL-1 induced by glucose-modified proteins: role in normal tissue remodeling. Science 1988; 240:1546-1548
[16] Ettehadi P, Greaves MW, Wallach D, Aderka D, Camp RD. Elevated tumour necrosis factor-alpha (TNF-alpha) biological activity in psoriatic skin lesions. Clinical and experimental immunology 1994; 96:146-151
[17] Krueger G, Callis K. Potential of tumor necrosis factor inhibitors in psoriasis and psoriatic arthritis. Archives of dermatology 2004; 140:218-225
[18] Cohen J. The immunopathogenesis of sepsis. Nature 2002; 420:885-891
[19] Goodman RB, Pugin J, Lee JS, Matthay MA. Cytokine-mediated inflammation in acute lung injury. Cytokine & growth factor reviews 2003; 14:523-535
[20] Park WY, Goodman RB, Steinberg KP, Ruzinski JT, Radella F, 2nd, Park DR, et al. Cytokine balance in the lungs of patients with acute respiratory distress syndrome. American journal of respiratory and critical care medicine 2001; 164:1896-1903
[21] Tyburski JG, Dente C, Wilson RF, Steffes C, Devlin J, Carlin AM, et al. Differences in arterial and mixed venous IL-6 levels: the lungs as a source of cytokine storm in sepsis. Surgery 2001; 130:748-51; discussion 751-752
[22] Hollenberg SM, Cunnion RE, Parrillo JE. The effect of tumor necrosis factor on vascular smooth muscle. In vitro studies using rat aortic rings. Chest 1991; 100:1133-1137
[23] Desai TR, Leeper NJ, Hynes KL, Gewertz BL. Interleukin-6 causes endothelial barrier dysfunction via the protein kinase C pathway. The Journal of surgical research 2002; 104:118-123
[24] Sedgwick JB, Menon I, Gern JE, Busse WW. Effects of inflammatory cytokines on the permeability of human lung microvascular endothelial cell monolayers and differential eosinophil transmigration. The Journal of allergy and clinical immunology 2002; 110:752-756
[25] Maruo N, Morita I, Shirao M, Murota S. IL-6 increases endothelial permeability in vitro. Endocrinology 1992; 131:710-714
[26] Plackett TP, Gamelli RL, Kovacs EJ. Gender-based differences in cytokine production after burn injury: a role of interleukin-6. Journal of the American College of Surgeons 2010; 210:73-78
[27] Sinha I, Cho BS, Roelofs KJ, Stanley JC, Henke PK, Upchurch GR, Jr. Female gender attenuates cytokine and chemokine expression and leukocyte recruitment in experimental rodent abdominal aortic aneurysms. Annals of the New York Academy of Sciences 2006; 1085:367-379
[28] Imahara SD, Jelacic S, Junker CE, O'Keefe GE. The influence of gender on human innate immunity. Surgery 2005; 138:275-282
[29] Aulock SV, Deininger S, Draing C, Gueinzius K, Dehus O, Hermann C. Gender difference in cytokine secretion on immune stimulation with LPS and LTA. Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research 2006; 26:887-892
[30] Zellweger R, Wichmann MW, Ayala A, Stein S, DeMaso CM, Chaudry IH. Females in proestrus state maintain splenic immune functions and tolerate sepsis better than males. Critical care medicine 1997; 25:106-110
[31] Wichmann MW, Muller C, Hornung HM, Lau-Werner U, Schildberg FW, Colorectal Cancer Study G. Gender differences in long-term survival of patients with colorectal cancer. The British journal of surgery 2001; 88:1092-1098
[32] Baynam G, Khoo SK, Rowe J, Zhang G, Laing I, Hayden C, et al. Parental smoking impairs vaccine responses in children with atopic genotypes. The Journal of allergy and clinical immunology 2007; 119:366-374
[33] Wiertsema SP, Khoo SK, Baynam G, Veenhoven RH, Laing IA, Zielhuis GA, et al. Association of CD14 promoter polymorphism with otitis media and pneumococcal vaccine responses. Clinical and vaccine immunology : CVI 2006; 13:892-897
[34] Prather AA, Carroll JE, Fury JM, McDade KK, Ross D, Marsland AL. Gender differences in stimulated cytokine production following acute psychological stress. Brain, behavior, and immunity 2009; 23:622-628
[35] Villacres MC, Longmate J, Auge C, Diamond DJ. Predominant type 1 CMV-specific memory T-helper response in humans: evidence for gender differences in cytokine secretion. Human immunology 2004; 65:476-485