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Measurement of reference value range of serum inflammatory cytokines in normal population in Baoding area

Xiaomeng Zheng[1], Xiaodong Li[2], Yanan Meng[1,*]

[1]Department of Clinical Laboratory, Affliated Hospital of Hebei University, Baoding, 071000, China
[2]Department of Nuclear Medicine, Affliated Hospital of Hebei University, Baoding, 071000, China
[*]Corresponding author

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Abstract: Cytokines are a general term for polypeptide cell regulatory substances, including interleukin, interferons, growth factors, cell stimulating factors, tumor necrosis factor and so on. It is mainly composed of immune cells (T cells, B cells, macrophages, etc.) and some non-immune cells (endothelial cells, fibroblasts), a kind of small molecule proteins with a wide range of biological activity, with various functions such as regulating immunity, promoting blood cell generation, cell growth and repair of damaged tissues. Cytokines also play an important role in the pathogenesis, diagnosis, drug efficacy and prognosis evaluation of hematological and immune system diseases. However, in the clinical reports, the normal control has a small sample size, narrow coverage, regional differences and different detection techniques, and the reported results are lack of comparable, so it is very important to establish the reference value range of cytokines in the normal population in this region. At present, there is no epidemiological report of inflammatory cytokine levels in normal population in Baoding. Therefore, the flow cytometry microsphere array method (cytometric bead arry, CBA) can detect several soluble proteins at one time, The serum levels of IL-1 β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-8, IL-10, IL-12P70, IL-17A, IFN- α, TIFN- α and IFN- γ in multiple levels of cytokines, To provide a basis for determining the normal reference values of these factors in the Baoding area, It also lays the foundation for the subsequent research of related diseases.

1. Materials and methods

1.1 Experimental subject

Select the individuals recognized as healthy by the Physical Examination Center of the Affiliated Hospital of Hebei University. A total of 160 subjects, aged 16-78 years, were included in the study. These subjects had no acute or chronic diseases, no infectious diseases, and no history of exposure to special elements. The subjects comprised 88 males and 72 females. Based on age (by year), they were divided into two groups: 18-45 years in the young group and 46-69 years in the middle-age group.

1.2 Test equipment and reagents

BD Company FACS CantoPlus flow cytometer IL-1 β, IL-2, IL-4, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12P70, IL-17A, IFN- α, TNF- α, IFN- γ)

1.3 Test Method

① Sample pretreatment: 2 mL of early morning fasting venous blood was collected from 160 subjects using an EDTA-K2 anticoagulant tube. To avoid cytokine breakdown consumption in serum, blood samples were required to be processed within 4h. Centrige at 3000 r/min and 4℃ for 20min, pack 100 μ L of supernatant into 1.5 mL EP tube in-20℃ refrigerator and keep the remaining serumin-8 0℃ refrigerator for later testing.② Standard preparation: use 2 mL sample dilution solution, gently mix and stand for 15min, and dilute the standard to produce 1:2,1:4,1:8... 1:2048 concentration gradient.25 μ L capture pellets and 100 μ L PBS were mixed as negative controls.③ Treatment and incubation of serum and standards: At the dose of 25 μ L of the capture pellet of each test tube, after centrifugation (200g, 5min), the pellet was resuspended with the same volume of the pellet buffer as the suction supernatant, and incubated for 15min. After 25 μ L of capture microsphere mixture, 25 μ L of the detection antibody at room temperature, 1 mL of PBS solution was washed, the supernatant was centrifuged, the pellet was resuspended with 100 μ L of PBS solution, and the level of each cytokine was detected by computer.④ Flow cytometer to obtain the detection data: adjust the flow cytometer according to the relevant model steps, test the standards and samples respectively, export the FCS data files, analyze them with FCAP software, draw the standard curve, and accurately calculate the cytokine level in the samples.

1.4 Data Analysis

Apply SPSS 23.0 to analyze the test results. Q test method removed outlier values; since the serum distribution of all cytokines, the range of 95% medical reference value was calculated; Man Whitney U-test was used to compare the differences of factor levels between different genders and age groups; the significance level was 0.05.

2. Results

2.1 Comparison of test results of serum inflammatory cytokines in different gender and age distribution populations

The differences in IL-6 levels were significant by sex, while the remaining 11 cytokines and the 12 cytokines were not significant by sex. See Table 1, 2.

Table 1: Comparison of each inflammatory factor in serum between men and women in the normal population

Divide into groups	n	IL -2	IL -4	IL -5	IL -6	IL -8	IL -1β	IL-17A	IL -10	IFN -α	TFN -α	IL -12p70	IFN -γ
The male sex	88	.3271 (0,1).506.298	.99610 (0,1). 300.632	.4700 (0,0). 203.636	.4863 (1,4). 77.68	.3815 (2.873,6). 334	.8091 (0,2). 68.03	10.068 (4,14.314) .600	.2772 (1,2). 407.368	.9781 (0,1). 445.649	.8291 (0,1). 467.944	.2362 (1,2). 138.453	.4351 (0,1). 74.748
Femininity	72	.8550 (0,1). 460.165	.8920 (0,1). 380.250	.4350 (0,0). 160.573	.162(1,2). 490.580	.8083 (2,4). 593.658	.1461 (0,1). 448.578	.929.1486 (2,10.173)	.5921 (1,2). 138.208	.5511 (0,1). 528.378	.2821 (0,1). 543.961	.6621 (0,2). 863.355	.0771 (0,1). 683.390
P		0.787	0.809	0.900	0.011	0.170	0.216	0.050	0.054	0.858	0.934	0.474	0.152

Data in the table is M (P_{25}, P_{75}), Significance level of 0.05

Table 2: Comparison of each inflammatory factor in serum in normal population of different age groups

Divide into groups	n	IL -2	IL -4	IL -5	IL -6	IL -8	IL -1β	IL -17A	IL -10	IFN -α	TFN -α	IL -12p70	IFN -γ
18-45 Years old	112	.1821 (0,1). 494.260	.9000(0,1). 300.250	.4530(0,0). 200.591	.5422(1.420,2).885	.8573(2,4). 350.759	.6291(0,2 .640.030	.052.7108(3,10.628)	.9961(1,2). 360.270	.7951(0,1). 445.573	.6581(0,2).543.048	.8801(0,2).885.430	.2251(0,1).725.635
46-69 Years old	48	9560 (0,1). 485.128	.0601(0,1). 473.537	.4570(0,0). 175.693	.0753(2,4).085.315	4.277(3,5). 037.086	.2331(0,1).560.561	10.063(3,16.862).839	.9071(1,2). 118.515	.7641(0,1). 556.505	.4081(0,1).432.587	.2072(1,2).146.415	.3881(0.698,1).726
P		0.971	0.272	0.69	0.108	0.221	0.450	0.536	0.741				

Data in the table is M (P_{25}, P_{75}), Significance level of 0.05

2.2 Reference range of each cytokine in normal human serum, as shown in Table 3

Table 3: Reference ranges for each cytokine in normal human serum

Cell factor	IL -2	IL -4	IL -5	IL -6	IL -8	IL -1β	IL -17A	IL -10	IFN -α	TFN -α	IL -12p70	IFN -γ
Reference Range (ng / L)	0-4.18	0-2.13	0-1.04	0 – 7.29 for males 0 – 3.78 for women	0-12.79	0-4.48	0-20.27	0-4.40	0-7.09	0-4.75	0-4.80	0-2.68

3. Discussion

In recent years, many literatures [1-6] have reported the relationship between cytokines and various diseases. Various cytokines in the serum are involved in the physiological function of the body, and the normal and stable cytokine level is an important factor to maintain the stability of the environment in the human body. This study showed that serum cytokine levels in normal population are not affected by sex except IL-6 is affected by sex, in addition, serum cytokines are not affected by age in normal population. According to the data of this study, the reference range of normal human serum IL-1 β, IL-2, IL-2, IL-4, IL-5, IL-6, IL-8, IL-8, IL-10, IL-12P70, IL-17A, IFN- α, TNF- α, IFN- γ, They were 0-4.18,0-2.13,0-1.04,0-7.29 (male) & 0-3.78 (female) and 0-12.79,0-4.48,0-20.27,0-4.40,0-7.09,0-4.75,0-4.80,0-2.68ng/L, respectively.

Serum factor 12 in this study and a study in Zhengzhou area[7]. It may be related to geographical factors such as living environment, eating habits and so on. In the related studies, there were two articles, respectively[8-9]Reported the level of inflammatory cytokines in the child population. The data of this study were quite different from their research results, and the level of inflammatory cytokines in children was significantly lower than that of adults. Therefore, the normal reference value of children cytokines still needs to be collected in large samples. In addition, there are also differences in the data between the two documents. In addition to regional differences, small sample size and specimen error, the content of cytokines is low in the body, so there may be many factors in the detection process, leading to the large difference in normal control results in each report.

Furthermore, ZhaohaoHuang et al showed that males and females differ in the composition and proportion of peripheral blood immune cells, sex differences are widespread in immune cells, and females tend to have more T cell numbers as well as upregulated B cell activation, while males tend to exhibit a higher inflammatory state [10]. This may be one of the reasons why IL-6 levels were

statistically different between sexenders and the IL-6 levels were lower in normal women than in normal men.

At present, there are many conventional methods to detect soluble factors, including ELISA quantitative detection and Western blot qualitative detection. However, ELISA can only detect one cytokine at a time, which is not only time-consuming and laborious, but also requires a large demand for samples, so the scope of application is limited. CBA using a series of different fluorescence intensity spheres, on the bread is specifically capture antibody, incubated with the sample formed "sandwich" complex, according to the standard curve calculated by the software for the target protein accurate quantification, easy to operate, small operation error, and less sample dosage, a variety of indicators. In this study, CBA determined the levels of 12 inflammatory cytokines (IL-1 β, IL-2, IL-4, IL-5, IL-6, IL-6, IL-8, IL-10, IL-12P70, IL-17A, IFN- α, IFN- α, IFN- γ), and successfully established a stable CBA system for detecting inflammatory cytokines, providing a solid experimental and data basis for the further study of dynamic changes of blood cytokine network, the pathogenesis of various diseases and the significance of diagnosis, monitoring and therapeutic prognosis.

According to multiple reports normal control cytokine levels are inconsistent, normal population cytokine levels affected by many factors, so simply by provincial normal value to measure Baoding area is obviously unscientific, the conclusion is not reliable, so establish the region of the normal population serum inflammatory cytokines reference value range for normal body and a variety of related diseases and cytokines is of great significance.

This study also has certain limitations, due to the limited number of this experiment, especially the statistics of normal healthy middle-aged data, the results may not fully reflect the age and gender factors on normal population serum inflammatory cytokines, in addition, not statistical children (0-6 years), children (7-12 years), teenagers (13-17 years) and old (69 years old) range, so more extensive epidemiological investigation, expand the age range for statistical analysis.

The level of cytokines in the body will play an important role in the immune response, blood cell biogenesis and tissue damage repair, while the level of inflammatory cytokines in the normal population is affected by many factors. Therefore, it is suggested that in ordinary life, attention should be paid to cultivating reasonable living habits, scientific diet collocation, pay attention to strengthening physical exercise to enhance physical fitness, improve immune function, so that the body serum cytokines are in a reasonable balance state.

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