Determination of reference values of humoral and cellular immunological indices in healthy people in Basrah province

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Abstract

The Present study was aimed to estimate normal reference values of some humoral immune indices such as Immunoglubulins (IgG, IgA and IgM) and complement proteins (C3, C4) and cellular immune indices like the percentage and absolute number of T-cells of healthy people with the age (20-49) in the Basrah province center.

A single radial Immunodiffussion method was applied to determine the concentration of IgG,IgA,IgM,C3 and C4,while resetting quantification was used to calculate the percentage and absolute number of T-lymphocytes.

According to the results of single radial Immunodiffusion test, IgG, IgM and C3 means were significantly associated with sex of studied persons($P < 0.05$) and not significantly with age . The higher values for IgG and IgM means were observed in females compared to males. In contrast IgA and C4 means values were statistically different ($p<0.05$) according to age of persons but there was no effect for the sex on the IgA means.

The results of E-rosette test, revealed that there was significant effect ($P < 0.05$) for the sex and age on the percentage of T-lymphocytes .the statistical analysis to absolute number of T-lymphocytes revealed that there was significant($P < 0.05$) affected by the sex in case of females in contrast to males in all age groups.

Keyword: reference value ,immunoglobulines ,complement components ,T-cell, healthy people

Introduction

In clinical immunology, the knowledge of immune system components and their normal values is vital. In this field, many studies have been performed (Kratz *et al.*,1998 and Richie *et al.*,1998). Reference values of immunoglobulin's (Ig) and complement components (CCs) might be different in each population. These data are essential for researches and making clinical diagnosis in every population (Aksu *et al.*,2004).

Usually, human subjects all over the world show variations, albeit little, in serum immune factor levels, this could be probably due to health, nutritional, environmental and racial variation (Oyeyinka *et al.*,1995). The studies from different countries not only indicate the presence of variation in quantities of lymphocytes and their subsets but also show that reference ranges obtained from studies in one population may not be used for another population although similar methods were used (Webster *et al.*,2003).

This reference ranges are crucial for the establishment of the precise diagnosis and prognosis. It is suggested that different factors such as environmental factors (infections, smoking and nutrition, sex, age and race) may be account for the variation between populations in lymph-ocyte subsets (Adetifa *et al.*,2009). The objective of the present study was to establish reference values for lymphocyte and immunoglobulin levels (IgG, IgA ,IgM) complement proteins (C3,C4) in healthy adults people in Basrah province.

Materials and Methods

A total of (300) healthy participants comprising (150) male and (150) female aged from(20-49) years were enrolled in this study. The participants were then grouped into three age categories group 1: (20-29) years; group 2: (30-39) years; group3: (40-49) years. The participants had to fill a questionnaire regarding infection in the past 4 weeks including viral, bacterial, fungal, and other pathogens, use of antibiotics in the past 4 weeks and history of medication, including analgesics, non-steroidal anti-inflammatory agents, anti-ulcer drugs, anti-hypertensive drugs, and other cardiovascular drugs. Subjects who reported a positive history for any of these items were excluded from this study.

Five milliliters of peripheral venous blood was withdrawn from each person and each blood sample

was divided into the following:-

1. Two ml of heparinized blood was transferred into a sterile test tube for lymphocyte separation.
2. Three ml of venous blood was used to provide sufficient serum for immunoglobulins (IgG, IgM, IgA) and complement (C3, C4).

Lymphocytes separation: -

Depending on a density gradient of lymphocyte cells, lymphoprep was used to separate lymphocyte from whole blood for in vitro testing (Lefkovit,1997). Human thymus derived lymphocytes (T-cells) were detected via attachment of sheep erythrocytes to specific receptors on the cell membrane, a technique known E-rosetting quantitation of T- cells in a mononuclear suspension done by (Jondal *et al.*,1972) modification of methods employed by some other .

Measurement of (IgG, IgA, IgM) and Complement component(C3, C4)

(SRID) was done by using plates produced by (Lat.S.R.L-Via Milano,15\F Kit), the wells were filled with (5) microliters of testing sera the sample diffuse radially through gel and the antigen form precipitin ring with the monospecific antiserum. The result can be calculated easily from the table of diameters provided with plates.

Statistical analysis

The present results were analyzed by Superior Performance Statistical Software (SPSS, version17) program.

Results

In relation to the results of humoral immune indices , data showed significant differences ($P < 0.05$) in the mean concentration of IgG and IgM between males and females, where females were higher than males While the total range for IgG was (659-2570) and (800-1800) of the using kit, and the median values of IgG (1614.5) higher than those of kit(1300) and the total range of IgM was (58- 397) in comparison with values of the using kit (60- 280). But the median values (227.5) were higher than the median of kit (170).

The mean concentration of IgA did not show any significant differences with the age and sex, but there was a significant correlation between the age and their concentration , as there was an increasing in concentration of IgA with age in both sexes. While the total range was (87- 530) in comparison with those of kit (90-450), and the median of the recent values (308.5) higher than those of kit(270).

Whereas the concentration of complement protein fragment C3 showed a significant differences between both sexes , in the case of the age effect there have been no significant differences among age groups, While the total range was (61-208) in comparing with those of the kit (91- 156) while the median values of C3 (134.5)were higher than median of kit (123.5).

While C4 did not show any significant differences between both sexes With an observation of a high significant correlation ($P < 0.01$) between their concentration and age. and the total range was (14-62) when compared with those of kit (20-50) the median of the values of C4 (38) were higher than the median values of the kit (35) table(1).

Results related with measuring normal values of cellular indices representing absolute number and percentage of T-lymphocytes, the results recorded that there were a significant differences in the percentage of T- cells between both sexes ,also with an observation of presence of significant correlation (decreasing) between age and their percentage, in addition the total range of recorded data reached to (50-84) , while the ranges of normal values reached to (68-80).Moreover, the median values (67) were lower than those of normal values (74). Statistical analysis also indicate that there was a significant differences in the mean of T-cells absolute number in all females age groups . In males, the results didn't recorded any significant difference in all age groups but a decline (not significant) was observed in their mean absolute number. the total range of recorded data reached to (50-84) , while the total range of the recorded data reached to (196.5-3021) and ranges of normal values were (800-2200).Moreover, the median values (1609.125) were higher than those of normal values (1500) table(2).

Table(1): immunoglobulins levels and complement component in serum of healthy people and comparison with normal values of using kit

Parameters	Sex	N	Mean ± SD	Total ranges and median	Normal values of using kit and their median
IgG	F	150	1482.44±508.543*	(659-2570) 1614.5	(800-1800) 1300
	M	150	1290.62±386.88*		
	Total	300	1386.53±461.19		
IgA	F	150	273.78±105.85	(87-530) 308.5	(90-450) 270
	M	150	257.82±114.809		
	Total	300	265.80±110.526		
IgM	F	150	257.86±89.535*	(58-397) 227.5	(60-280) 170
	M	150	235.76±91.989*		
	Total	300	246.81±91.292		
C3	F	150	135.23±43.770*	(61-208) 134.5	(91-156) 123.5
	M	150	125.43±38.660*		
	Total	300	130.21±41.74		
C4	F	150	27.52±11.397	(14-62) 38	(20-50) 25
	M	150	26.32±11.242		
	Total	300	26.92±11.317		

Table(2): Percentage and absolute T-cells of healthy people and comparison with normal values

Parameters	Sex	N	Mean ± SD	Total range and median	Normal values of T-cells and their median
T-cell%	F	150	68.31±5.83*	(50-84) 67	(68-80) 74
	M	150	66.84±5.61*		
	Total	300	67.58 ± 5.760		
T-cell absolute	F	150	1573.92±579.142	(196.5-3021.75) 1609.125	(800-2200) 1500
	M	150	1355.51±442.87		
	Total	300	1405. ±541.765		

Discussion

Humoral immune parameters including immunoglobulins classes such as (IgM, IgG and IgA) as well as complement components represented by (C3 and C4) were considered as useful parameters in assessing the immunological responses in human. A single radial immune diffusion method was applied to determine the concentration of each fraction. Concentration of (IgG, IgA, IgM) and (C3,C4) have long been recognized to vary from person to person. IgG and IgM were showed a significant difference between both sexes. thus, the means of IgG and IgM in females were higher than for males ($p < 0.05$) but not showed differences with age. The higher levels of IgM in three age groups agree with previous studies, as some studies found a relationship between the number of X chromosomes and IgM concentrations (Grandbacher,1972 and Hatagima *et al* .,1999).

while IgA level didn't show any statistical significant differences with sex but correlation coefficient tests showed a significant positive correlation between age and serum IgA concentrations ($P<0.05$) .

Cellular immune response including determination of the percentage and absolute of T-lymphocytes by using E- rosette test to calculate the percentage of positive E- rosette forming cell (ERFC).Recent data documented that there was no significant difference between absolute counts of T-lymphocytes in males and females but only with age ($P > 0.05$). while the percent of T-lymphocytes were showed significant differences between both sex ($p> 0.05$). Results also noted that there was a decreasing in percentage of T-cells due to acceleration of thymocyte apoptosis by androgens and profile the peripheral T-cells collection (Olsen *et al.*,1998). Variation in immune cell counts may be due to gender and sex hormones. Sex hormones can affect the immune cell populations in the indirect pathways like the thymic pathways (Ready *et al*.,2002)

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تحديد القيم المرجعية لمؤشرات المناعة الخلوية والخلطية في الأشخاص الأصحاء في محافظة البصرة

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الخلاصـــــــــــــــــــــــــــــــة

تهدف الدراسة الحالية إلى تقدير القيم المرجعية الطبيعية لبعض مؤشرات الاستجابة المناعية منها الخلطية والمتمثلة بالكلوبيولينات المناعية (IgG ،IgA وIgM) بروتينات المتمم complement proteins (C3 وC4)،والخلوية والمتمثلة بالفعالية البلعمية والنسبة المئوية والعدد المطلق للخلايا اللمفاوية في الأشخاص الأصحاء وبعمر (20-49)في مركز محافظة البصرة .

استخدمت طريقة الانتشار المناعي القطري المنفرد Single Radial Immunodiffusion لقياس تراكيز الكلوبيولينات (IgM, IgG وIgA) وبروتينات المتمم (C3 وC4) بينما استخدمت طريقة (E-rosette) لحساب العدد المطلق والنسبة المئوية للخلايا اللمفاوية التائية T-lymphocytes .

وفي ما يتعلق بنتائج اختبار الانتشار المناعي القطري المنفرد أظهر معدل تركيزIgG،C3وIgM اختلافاً معنوياً($P<0.05$) بين الذكور والإناث وذلك لارتفاعهما في الإناث أكثر من الذكور، ولم تظهر معدلاتهم اختلافات معنوية مع العمر، أما معدل تركيز IgA فلم يظهر أية فروقاً معنوية مع العمر والجنس ، لكن لوحظ وجود ارتباط معنوي ($P<0.05$) بين العمر وتركيز IgA اذ لوحظ ان معدل تركيزه يزداد مع العمر في كلا الجنسين. بينما لم يظهر C4 أي فروقا معنوية بين كلا الجنسين مع ملاحظة وجود ارتباط معنوي عالي ($P<0.01$) بين تركيزC4 و العمر.

وفي ما يتعلق بنتائج اختبار E-rosette،فقد أكدت النتائج وجود فروقا معنوية($p<0.05$) في النسبة المئوية للخلايا اللمفاوية التائية بين كلا الجنسين مع ملاحظة وجود ارتباط (انخفاض) معنوي بينها وبين العمر .

الكلمات المفتاحية: القيم المرجعية، الكلوبيولينات المناعية، المتمم ،الخلايا التائية ،الاشخاص الاصحاء