EVALUATION OF SERUM IgG, IgA, IgM AND C3 VALUES BY NEPHELOMETRIC METHODS*

Nefelometrik ve İmmunoturbidimetrik Metodlar ile Serum IgG, IgA, IgM ve C3'ün Değerlendirilmesi

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Summary:

Purpose: *Serum measurements of IgG, IgA, IgM and complement 3 (C3) are useful for assessment of immunological disorders, abnormal protein metabolism and the body's lack of ability to resist infectious agents. In this study, our aim was to compare serum IgG, IgA, IgM and C3 values measured by the nephelometric method (Beckman Array System) and by the immunoturbidimetric method (Incstar Corp.).*

Material and Methods: *The assay samples were obtained from 35 patients from the Internal Medicine Department and from Bio-Rad Immunology Control Levels 1 and 2 (LiquicheckTM, Bio-Rad). All units are given as mg/dL.*

Results: *The results of the correlation analysis between the two methods, Beckman Array System (x) and Incstar (y) were as follows: y=0.727x+241 (r=0.906) for IgG, y=1.01x+11.8 (r=0.978) for IgA, y = 0.962x+(-0.46) (r=0.956) for IgM and y=0.851x+14.7 (r=0.900) for C3. Intra and interassay precisions were performed with control sera at two levels analyzed ten times. Briefly, intraassay CV percentages for these parameters were between 1.2-7.0% and 2-12.5%, for Beckman Array System and Incstar, respectively. Additionally, interassay CV percentages were between 2.3-8.2% for Beckman Array System and 5.1-15.2% for Incstar.*

Conclusion: *These results indicate that the nephelometric method (Beckman Array System) offers a more accurate, precise, and convenient method for measuring IgG, IgA, IgM and C3 in human serum when compared to the immunoturbidimetric method (Incstar Corp.).*

Key Words: *Methods, Nephelometry,Turbidimetry*

Özet:

Amaç: *Serum IgG, IgA, IgM ve kompleman 3 (C3) ölçümleri, immunolojik bozuklukların, anormal protein metabolizmasının ve vücudun infeksiyöz ajanlara direnç gösterme yeteneğinin yokluğunun değerlendirilmesinde faydalıdır. Bu çalışmada, serum IgG, IgM, IgA ve C3 değerlerini nefelometrik method (Beckman Array System) ve immunoturbidimetrik (Incstar Corp.) metodlarla karşılaştırmayı amaçladık.*

Gereç ve yöntem: *Örnekler Dahiliye Kliniğindeki 35 hastadan ve Bio-Rad İmmunolojik kontrol 1 ve 2 düzeylerinden (LiquicheckTM Bio-Rad) elde edildi. Bütün üniteler mg/dL olarak verildi.*

Bulgular: *Hasta örnekleri her iki metotla ölçüldü ve Beckman Array System (x) ile Incstar (y) metotları arasındaki karşılaştırma sonuçlarına göre şu korelasyonlar elde edildi: IgG için y=0.727x+241 (r=0.906), IgA için y=1.01x+11.8 (r=0.978), IgM için y = 0.962x+(-0.46) (r=0.956) ve C3 için y=0.851x+14.7 (r=0.900). Intra ve interassay % CV ler iki seviyedeki kontrol serumlarıyla on kez ölçüm yapılarak elde edildi ve özet olarak bu parametreler için intraassay %CV'ler sırasıyla Beckman Array System için %1.2-7.0 arasında ve Incstar için %2.0-12.5 arasında idi. Ayrıca interassay %CV'ler Beckman Array System için %2.3-8.2 ve Incstar için %5.1-15.2 idi.*

Sonuç: *Bu sonuçlar, immunoturbidimetrik metotla (Incstar Corp.) karşılaştırıldığı zaman nefelometrik metodun (Beckman Array System) insan serumunda IgG, IgA, IgM ve C3 ölçümü için daha güvenli, doğru ve uygun bir metod olduğunu göstermektedir.*

Anahtar Kelimeler: *Metod , Nefelometri, Turbidimetri*

Immunoglobulins are heterogenous glycoproteins which consist of two identical heavy and two identical light polypeptide chains, joined by interchain disulfide bonds and noncovalent forces. There are five major groups of immunoglobulins in the serum: IgG, IgA, IgM, IgD and IgE. Although most serum proteins are synthesized in the liver, immunoglobulins are synthesized and secreted by plasma cells (1,2).

Complements are a group of serum proteins which

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destroy infectious agents. Measurement of these proteins is of value in the diagnosis of immunologic disorders, particularly those associated with deficiencies of complement components. Among these components, C3 is the most important (1-3).

Measurements of IgG, IgA, IgM and C3 place a great demand on the clinical laboratory: The method of choice should measure these parameters very precisely; should be economical, automatable, and simple to perform, and should yield results that are comparable between different laboratories.

Historically, immunoglobulins have been measured only as part of total globulins present in human serum. The introduction of electrophoretic separation techniques allowed the fractionation of globulins into their components. The fraction consists almost completely of immunoglobulins. Different immunochemical procedures have been developed for the quantitation of these immunoglobulin classes. The most commonly used immunochemical methods for the quantitation of immunoglobulins (particularly IgG, IgA, and IgM) are single radial immunodiffusion, nephelometry (rate or end point), immunodiffusion, electroimmunodiffusion and turbidimetry. The quantitation of immunoglobulins by nephelometry is a more recent development (2). Among these methods, turbidimetry and nephelometry are used to measure the light scattering due to immune complexes that form when an antigen is mixed with its antibody in appropriate proportions (4).

Turbidity causes the attenuation of the intensity of the incident beam of light as it passes through a solution of particles. The measurement of this decrease in intensity of the incident light beam that is caused by scattering, reflectance and absorption of light is called immunoturbidimetry. Immunoturbidimetric measurements of serum IgG, IgA, IgM and C3 were performed with an autoanalyzer (Mitsubishi Super Z 818, Japan).

Immunonephelometry is defined as the detection of light energy scattered or reflected toward a detector that is not in the direct path of the transmitted light. Common nephelometers measure scattered light at right angles to the incident light. Some are designed to measure scattered light at an angle other than 90 degrees to take advantage of increased toward-scatter intensity caused by light scattering from larger particules such as immune complexes (5).

Comparisons of two methods of measurement, particularly two assays, are very common in clinical biochemistry (6). In the present study, we aimed to compare two commercially available kits (nephelometric versus immunmoturbidimetric method) for the determination of serum IgG, IgA, IgM and C3.

MATERIALS and METHODS

Specimens were collected from 35 patients (23 males, 12 females; ranging in ages from 18 to 57 years) from Internal Medicine Department of Medical Faculty, Atatürk University.

Venous blood samples were obtained, after an overnight fast, between 0800 -0900 h. in vacutainer tubes. Blood samples were centrifuged at 2000 g for 10 minutes and serum samples obtained were analyzed immediately. Additionally, control serum samples from Bio-Rad Immunology Control Levels 1 and 2 (LiquicheckTM, BioRad) were analyzed for determination IgG, IgA, IgM and C3 levels. Both patients' sera and control samples were studied with nephelometric and immunoturbidimetric methods.

For the comparison of two methods (rate nephelometry vs immunoturbidimetry), the parameters were measured in human sera and control sera, using commercially available kits from Beckman and Incstar Companies.

The precision of the assays was assessed by measuring normal and pathological concentrations of these parameters in commercially prepared control sera, 10 times during the same assay (intraassay precision) and on 10 consequtive days (interassay precision). The precision was expressed

as the coefficient of variation.

The results were expressed as mean ± standard deviation. The "between-methods" correlations of IgG, IgA, IgM and C3 values were evaluated by linear regression analysis. Differences of two methods in terms of these parameters were compared by "Paired t test". A p value less than 0.05 was considered as statistically significant. All statistical procedures were performed using statgraphics packet programme on an IBM computer.

Table I. Mean serum IgG, IgA, IgM and C3 levels in patient population. (n=35)

Methods	IgG(mg/dl)	IgA(mg/dl)	IgM(mg/dl)	C3(mg/dl)
Beckman(MeanSD) (Range)	1273± 399.6 (669-2120)	216.9± 115.1 (47.4-521)	157.0± 68.3 (87-327)	128.4± 36.9 (43-188)
Incstar (MeanSD) (Range)	1419.5± 497.6 (675-2224)	227.3± 111.8 (44-488)	163.7± 67.9 (63-345)	133.7± 39.0 (39-194)
t	3.97 p≤0.0004	-1.98 p≥0.05	1.15 p≥0.05	1.99 p≥0.05

Table II. Intra-and interassay CVs of both methods (nephelometry and immunoturbidimetry)

			(Intraassay±n=10)				(Interassay±n=10)			
		Control serum range of Bio-Rad	Mean (±SD) Beckman	Incstar	CV % Beckman	Incstar	Mean (±SD) Beckman	Incstar	CV % Beckman	Incstar
level 1	IgA(mg/dl)	112 (89-134)	117(5.8)	104.2(22.5)	4.9	12.5	121.5(6.8)	107(10.6)	5.6	10
level 1	IgG(mg/dl)	561 (449-673)	561.2(6.0)	525.8(35.3)	2	6.7	559.3(14.5)	534.3(34.7)	2.6	6.5
level 1	IgM(mg/dl)	60 (47-72)	57.2(2.9)	47.2(12.3)	5	12.5	55.3(4.5)	59.5(9)	8.2	15.2
level 1	C_3(mg/dl)	72.5 (58.0-87.0)	72.4(5.1)	77.4(5.9)	7.0	7.6	75.6(5.9)	68.1(4.3)	7.8	6.4
level 2	IgA(mg/dl)	347 (277-416)	336.4(8.9)	320.6(21.8)	2.6	6.7	341.5(9.9)	330.4(28.4)	2.9	8.6
level 2	IgG(mg/dl)	1574 (1259-1888)	1571.8(12.3)	1536.2(31.3)	1.2	2	1566(36)	1543.6(78.7)	2.3	5.1
level 2	IgM(mg/dl)	202 (162-243)	213.0(5.6)	221.0(21.4)	2.6	9.6	209.5(7.1)	209.6(29.3)	3.4	14
level 2	C_3(mg/dl)	225 (180-270)	219.2(7.6)	196.2(11.0)	3.4	5.6	223.8(8.7)	205.5(15)	3.9	7.3

Table III. Correlation analysis of two methods.(n=35)

Parameter	Slope	Intercept	r
IgG	0.73	241	0.906
IgA	1.01	-11.8	0.978
IgM	0.96	-0.46	0.956
C3	0.85	14.7	0.900

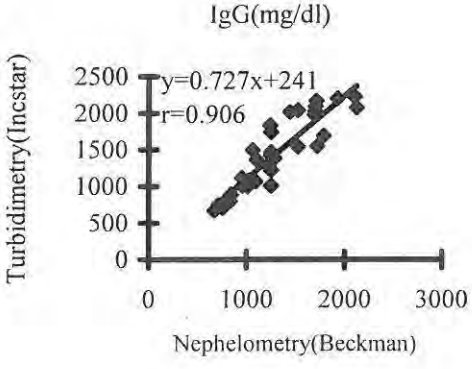

Fig 1. The correlation between nephelometric (Beckman Arry System) and immunoturbidimetric (Incstar) IgG levels in patients' sera

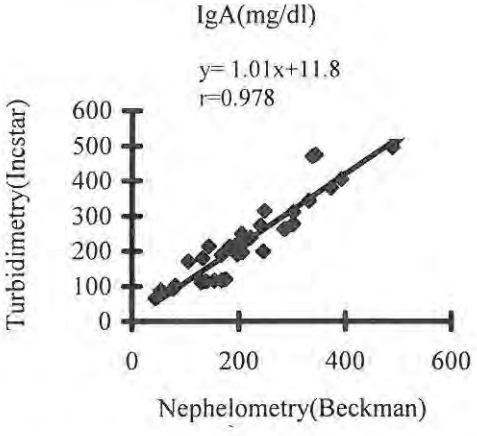

Fig 2-The correlation between nephelometric (Beckman Array System) and immunoturbidimetric (Incstar) IgA levels in patients' sera

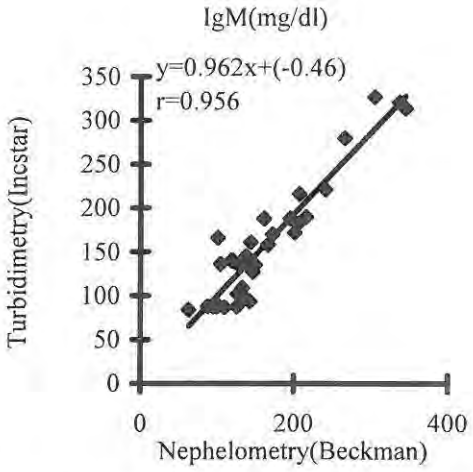

Fig 3. The correlation between nephelometric (Beckman Array System) and immunoturbidimetric (Incstar) IgM levels in patients' sera

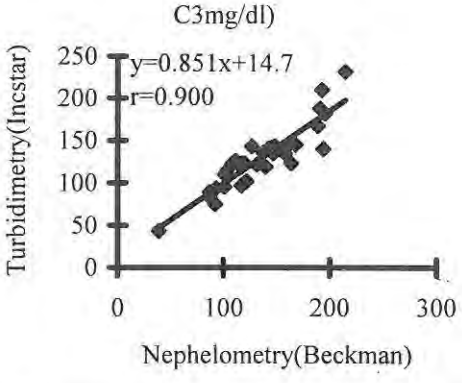

Fig 4. The correlation between nephelometric (Beckman Array System) and immunoturbidimetric (Incstar) C3 levels in patients' sera

RESULTS

Mean serum IgG, IgA, IgM and C3 levels measured by both nephelometric and immunoturbidimetric methods in patient population are given on Table I. There was no significant difference between two methods with respect to these parameters,except IgG. As seen from Table I, IgG, IgA, IgM and

C3values obtained by nephelometry were comparable to those obtained by immunoturbidimetry for patients' sera. Additionally, the measurements with Incstar were 5-10% higher than the measurement with Beckman Array System.

Correlation results of both methods studied are summarized on Table II, and shown in Figures 1-4.

DISCUSSION

There are five major classes of immunoglobulins, namely IgG, IgA, IgM, IgD and IgE. The first three are primarily involved with combatting infections. IgG is the immunoglobulin present at the highest concentration followed by IgA and IgM. C3 complement is part of a complex series of serum proteins which interact to promote some of the functions of the immune system (7).

Nephelometry is the measurement of light scattered by a particulate solution. Immunoturbidimetry measures light scattering as a decrease in the light transmitted through the solution (1,2). The choice between turbidimetry and nephelometry depends on the application and the available instrumentation. Until recently, the statement was often made that for relatively clear solutions in which the transmission of light in the forward direction is greater than 95%, small changes in absorption due to turbidity were difficult to measure with precision, and nephelometry was the method of choice. However, with the advent of stable high-resolution photometric systems, turbidimetric measurements have become competitive in sensititivity with nephelometric methods for immunological quantitation of serum proteins. Nephelometry, however, still offers some advantage in sensitivity when measuring low-level antigen-antibody reactions (8). There have been few reports on the analytical and clinical evaluation of these assays.

Fairly good correlations were observed between both assays for patients' serum samples.

Table III shows mean ± standard deviation values and the mean value of Bio-Rad control serum samples at two levels and intra- and inter - assay CVs for each method. Intra- and interassay CVs of both methods were within acceptable limits and those observed for nephelometry were lower than those observed for immunoturbidimetry for each parameter both intra and interassay CV values (except for C3 interassay CV which is lower in immunoturbidimetry than in nephelometry). In Beckman, intraassay CVs for control serum level 1 were between 2 - 7% and for control serum level 2 were between 1.2 - 3.4 %. These values for Incstar were between 6.7 -12.5 % and 2 - 9.6 %, respectively. In evaluation of interassay CVs; Beckman had 2.6 - 8.2 % and 2.3 - 3.9 % and Incstar had 6.4 - 15.2 % and 5.1 - 14 % for control serum levels 1 and 2, respectively. Additionally, the measurements of Beckman were closer (more appropriate) to the values reported by the manifacturer than those of Incstar. Therefore, it may be claimed that nephelometry is more precise than immunoturbidimetry in measurements of IgG, IgA, IgM and C3 in this study.

The main problem in the accurate quantification of immunoglobulins is the extreme heterogeneity of the group (9). Only immunochemical methods are sensitive enough to detect or quantitate immunoglobulins at normal levels. Although radial immunodiffusion or electroimmunoassay gel techniques may be used, nephelometry or immunoturbidimetry is preferred now that very specific antisera with high titer and affinity are available. The latter methods require few manipulations and are more rapid and precise (10).

As a result, the findings of the present study showed that immunonephelometric method (Beckman Protein Array System) offers more accurate, precise and convenient method for measuring IgG, IgA, IgM and C3 in human serum when compared to immunoturbidimetric method (Incstar Corp.).

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