STUDY OF THE EFFECT OF ACTIVE INFECTIONS ON DIHYDRORHODAMINE (DHR) TEST IN CHRONIC GRANULOMATOUS DISEASE PATIENTS (CGD)

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Introduction

Chronic granulomatous disease (CGD) is the most common type of primary innate immune deficiency caused by defects in the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme in the neutrophils. [1] It's considered as a fatal granulomatous disease of childhood as most patients with CGD die by 10 years of age. [2] Any mutation at one of the genes encoding for glycoproteins that are forming the five subunits of the NADPH oxidase enzyme leads to CGD. If the mutation leaves some residual NADPH oxidase activity intact the clinical expression of the disease is attenuated and prognosis is better. [3] CGD can be treated by conventional treatment, stem cell transplantation or genetic therapy. [4] The (DHR) test was approved to be the gold standard for diagnosis of CGD. [5]

Aim of the work

The aim of the present study is to detect the effect of active infections on the interpretation of DHR test results in chronic granulomatous disease patients and patients with active infections with no evidence of CGD.

Subjects

The present study was conducted on 24 patients previously diagnosed as CGD and 24 patients with active infection with no evidence of CGD (control group). All patients were recruited from outpatient clinic of El-Shatbi pediatric hospital Alexandria University during the period from 10/2018 till 10/2019. Patients with neutropenia were excluded from the study.

Methods

The dihydro-rhodamine test (DHR) was done for every patient while in active infection period and was also repeated again when the patient recovered and became completely free. In DHR test, neutrophils were incubated with DHR123 (2500 µg/mL in DMSO, SIGMA) then stimulated by PMA (100 µg/mL in DMSO, SIGMA) which stimulates the production of oxidative burst in the form of hydrogen peroxide by the action of NADPH oxidase enzyme. Products of oxidative burst oxidize DHR to rhodamine 123 which emits fluorescence. Fluorescence emitted is measured and quantified by flowcytometry device (BD FACSCalibur), CELL QUEST software and expressed as mean fluorescence channel. Stimulation index is calculated as a ratio of mean fluorescence channel after stimulation to mean fluorescence channel before stimulation. (Figures 1, 2, 3 and 4)

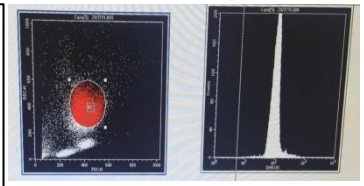

Figure (1): DHR test result for patient with active infection with no evidence of CGD before stimulation by PMA.

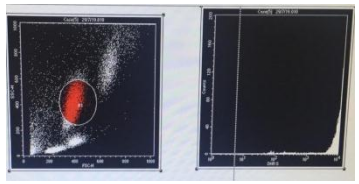

Figure (2): DHR test result for patient with active infection with no evidence of CGD after stimulation by PMA .

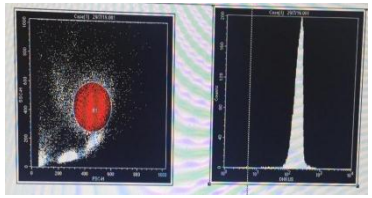

Figure (3): DHR test result for patient with CGD before stimulation by PMA.

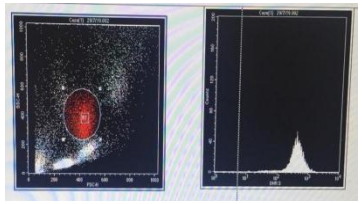

Figure (4): DHR test result for patient with CGD after stimulation by PMA .

Table (1): Comparison between the two studied groups according to Stimulation index

Stimulation index	Cases	Control	U	P
Infection	**(n = 21)**	**(n = 24)**		
Min. – Max.	0.24 – 2.64	35.0 – 806.0		
Mean ± SD.	0.95 ± 0.64	128.3 ± 159.7	0.000*	<0.001*
Median (IQR)	0.81 (0.61 – 0.99)	67.50 (47.50–172.0)		
Free	**(n = 19)**	**(n = 24)**		
Min. – Max.	0.71 – 3.92	34.0 – 795.0		
Mean ± SD.	1.40 ± 0.80	125.1 ± 157.6	0.000*	<0.001*
Median (IQR)	1.11 (0.88 – 1.76)	64.50 (46.50 –167.5)		
Z (p_0)	1.448 (0.148)	3.453*(0.001*)		

U: Mann Whitney test
Z: Wilcoxon signed ranks test
p: p value for comparing between the studied groups
p0: p value for comparing between Infection and Free in each group
*: Statistically significant at $p \leq 0.05$

Results

No significant difference was found in the group of CGD patients regard stimulation index between infection period and infection free period. On the other hand stimulation index of control group was significantly higher during infection period than infection free period . (Table 1)

Conclusion

This study demonstrated that DHR test is a rapid, cheap and high sensitive test for diagnosis of CGD and mild to moderate Infection does not affect the DHR result. However severe infections can affect DHR result causing false negative DHR result resulting in false positive diagnosis of CGD.

References

1. Pac M, Bernatowska E. Comprehensive activities to increase recognition of primary immunodeficiency and access to immunoglobulin replacement therapy in Poland. Eur. J. Pediatr. 2016 Aug;175(8):1099-105.
2. Marciano BE, Spalding C, Fitzgerald A et al. Common severe infections in chronic granulomatous disease. Clin Infect Dis 2015; 60:1176–83.
3. Kulkarni M, Hule G, de Boer M, van Leeuwen K, Kambli P, Aluri J, Gupta M, Dalvi A, Mhatre S, Taur P, Desai M. Approach to molecular diagnosis of chronic granulomatous disease (CGD): an experience from a large cohort of 90 Indian patients. Journal of clinical immunology. 2018 Nov 1;38(8):898-916.
4. Dirk Roos, Chronic granulomatous disease, British Medical Bulletin, Volume 118, Issue 1, June 2016, Pages 50–63.
5. Fleisher TA, Madkaikar M, Rosenzweig SD. Application of flow cytometry in the evaluation of primary immunodeficiencies. Indian J Pediatr 2016; 83:444–9.