



Research Article

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Advanced Laboratory Investigations Conducted at Medical Research Unit Laboratory, MHWT, Uttara Model Town, Dhaka, Bangladesh

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Abstract

Among the medical sciences, Diagnostic Immunology has become a versatile branch due to availability of monoclonal antibody (MCA)-based advanced techniques such as radioimmunoassay (RIA), enzyme immunoassay (EIA), fluorescence activated cell sorter (FACS), Nephelometry, Immunochromatographic technique (ICT), Immunofluorescence technique (IFT), etc. Laboratory diagnosis and follow-up of immunologically-mediated as well as other diseases are made now-a-days with high precision and accuracy with the application of these advanced techniques. Diagnostic immunology was reorganized and more versatile investigations were introduced at the Medical College for Women & Hospital (MCW&H) laboratory, Uttara, Dhaka in August 2002. The idea was to provide high quality laboratory services for specialized diagnostic immunology tests in a cost effective way. In 2005 Medical Research Unit (MRU) had been set up in at MCW&H building to conduct high quality clinical and biomedical research and provide further specialized biochemical and immunological laboratory tests at affordable costs. Many diagnostic tests were introduced and carried out regularly at MRU (Diagnostic) Laboratory to confirm and pin-point the clinical (provisional) diagnosis of human diseases. The analysis of the results of some advanced laboratory investigations and also the immunodiagnostic tests in various antibody classes for TORCH/TORCH infections conducted at MRU Laboratory have been presented in the present article and discussed accordingly.

Keywords: Diagnostic immunology, Hepatitis marker, Autoantibody, Tumour marker, Immunoglobulin E, Complement, Hormone, ToRCH, TORCH

Background

Since the establishment of radioimmunoassay (RIA), enzyme immunoassay (EIA), discovery of monoclonal antibody (MCA) and fluorescence activated cell sorter (FACS), tremendous progress have been made in the field of diagnostic immunology. MCA based advanced techniques such as RIA, EIA, FACS, nephelometry, immunofluorescence technique, immunochromatographic technique (ICT), etc are presently used in modern diagnostic immunology. With the application of these powerful techniques, laboratory diagnosis and follow-up of immunologically-mediated as well as other diseases are made now-a-days with high precision and accuracy [1-6].

Immunology laboratory were reorganized and more versatile tests were introduced at the Medical College for Women & Hospital (MCW&H) in August 2002. The idea was to provide high quality laboratory service for specialized diagnostic immunology tests in a cost effective way. In 2005 Medical Research Unit (MRU) had been set up in at MCW&H building to conduct high quality clinical and biomedical research and provide further specialized biochemical and immunological laboratory tests at affordable costs. Many diagnostic tests were introduced and carried out regularly at MRU (Diagnostic) Laboratory to confirm and pin-point the clinical (provisional) diagnosis of human diseases. The analysis of the results of some advanced laboratory investigations



and also the immunodiagnostic tests in various antibody classes for TORCH/ToRCH infections carried out at MRU (Diagnostic) Laboratory have been presented in the present article. Some of the other immunological test results produced at MRU (Diagnostic) Laboratory such as antinuclear antibody (ANA), anti-dsDNA antibody (Anti-dsDNA), complements (C3, C4), immunoglobulin E (IgE), etc had been published in 2005 previously [6].

Materials and Methods

Immunodiagnostic Tests

The diagnostic tests introduced and routinely carried out at MCW&H since August 2002 were the following: Antinuclear antibody (ANA), Anti- dsDNA antibody (Anti-dsDNA), Complement 3 (C3), Complement 4 (C4), Immunoglobulin MCW&H since August 2002 were the following: ANA, anti-dsDNA, C3, C4, IgE, PSA, CEA, AFP, T3, T4, TSH, PRL, TEST, HBsAg, HBc IgM Ab, HBeAg, HBsAb, HCV-Ab, HAV-IgM Ab and HEV-IgM Ab [6]. The laboratory procedures adopted were the following.

Autoantibody Profile

The diagnostic Kits for autoantibodies were based on indirect solid phase enzyme immunometric assay (EIA) [7-9]. C3, C4 and IgE: Serum complement components (C3, C4) were quantitatively determined using diagnostic kits based on liquid phase immunoprecipitation assay with nephelometric end point detection [1-4]. The quantitative estimation of total IgE in human serum was made using assay kit based on EIA [6,10].

Tumour/Cancer Markers

The tumour markers (PSA, CEA, AFP) were quantitatively analysed using diagnostic kits based on third generation EIA in

human serum [11,12,13]; Hormone Profile: The analysis of hormones (T3, T4, TSH), were carried out using indirect and competitive EIA based assay kits [6,14-17]. Hepatitis Virus Profile: All the hepatitis virus infection tests were done using diagnostic kits based on third generation EIA obtained from different internationally reputed companies [18,19].

TORCH Panel

The immunodiagnostic tests in IgG and IgM class antibodies were done at MRU from January 2017 to September 2018 against Toxoplasma (TO), Rubella virus (R), Cytomegalo virus (C) and Herpes simplex viruses 1 & 2 (H) using kits based on solid phase indirect and competitive binding EIA supplied by reputed international companies from USA, UK, Germany, Belgium, China, etc [20-26].

Results

The results of the serum specimens analysed from June 2003-July 2004 for autoantibody profile, C3, C4 and IgE, tumour/cancer markers, hormone profile and hepatitis virus profile are presented in Table 1. The results of the complete TORCH panel are stated in Table 2. The results of tests in serum for one or the other component (organism) of TORCH infections are stated in Table 3. Table 2 shows that all 30 specimens analysed for TORCH infections were females (N: 30, Age range: 18-38 years; Mean age + SD: 28+20 years). It was evident from Table 2 that only 3 specimens (i.e. specimen no 06, 07, 25) and 1 specimen (i.e. specimen no 39) were positive for current/recent infection with Toxoplasma and CMV respectively. All other specimens were positive for past exposure to either all components or negative for one or more components of TORCH infections.

Table 1: Results of the serum specimens analysed from June 2003 to July 2004.

SI. No.	Name of the test	Number of specimens analysed					Total
		Normal	High	Low	-ve	+ve	
1.	Antinuclear antibody (ANA)				26	3	29
2.	Anti-ds DNA antibody (Anti-dsDNA)				6	1	7
3.	Complement 3 (C3)	3	1	16			20
4.	Complement 4 (C4)	10	1	1			12
5.	Immunoglobulin E (IgE)				11	2	13
6.	Prostate specific antigen (PSA)				32	3	35
7.	Carcinoembryonic antigen (CEA)				21	0	21
8.	Alpha-fetoprotein (AFP)				11	1	12
9.	Triiodothyronine (T3)	62	15	7			84
10.	Thyroxin (T4)	109	14	12			135
11.	Thyroid stimulating hormone (TSH)	111	24	17			152
12.	Prolactin (PRL)	6	6	0			12
13.	Testosterone (TEST)	23	0	9			32
14.	Hepatitis BsAg (HBsAg)				785	43	828

15.	Anti-HBcAg IgM class (HBc-IgM ab)				11	3	14
16.	Hepatitis BeAg (HBeAg)				16	3	19
17.	Anti-HBs antibody (HBs Ab)				9	1	10
18.	Anti-HCV antibody (HCV-Ab)				42	5	47
19.	Anti-HAV IgM class (HAV-IgM ab)				46	4	50
20.	Anti-HEV IgM class (HEV-IgM ab)				30	44	74
Total :		324	61	62	1046	113	1606

Table 2: Laboratory investigations for antibody profile (IgG & IgM Classes) for TORCH infections.

Patient/ SI No	Age/Sex	Antibody Profile Against TORCH Infections*									
		A	B	C	D	E	F	G	H	I	J
01	30 Yr/F	-	-	+	-	+	-	+	-	-	-
02	30 Yr/F	+	-	+	-	+	-	+	-	+	-
03	27 Yr/F	-	-	-	-	+	-	+	-	+	-
04	30 Yr/F	+	-	+	-	+	-	+	-	+	-
05	33 Yr/F	-	-	+	-	+	-	+	-	+	-
06	26 Yr/F	+	+	+	-	+	-	+	-	+	-
07	25 Yr/F	+	+	+	-	+	-	+	-	-	-
08	26 Yr/F	-	-	+	-	+	-	+	-	+	-
09	28 Yr/F	-	-	+	-	+	-	+	-	-	-
11	30 Yr/F	+	-	+	-	+	-	+	-	-	-
13	24 Yr/F	-	-	+	-	+	-	+	-	-	-
14	23 Yr/F	-	-	-	-	-	-	+	-	-	-
19	27 Yr/F	-	-	+	-	+	-	+	-	-	-
21	25 Yr/F	-	-	+	-	+	-	+	-	-	-
25	38 Yr/F	-	+	+	-	+	-	+	-	-	-
26	18 Yr/F	-	-	+	-	+	-	+	-	-	-
29	20 Yr/F	-	-	+	-	+	-	+	-	+	-
33	34 Yr/F	-	-	+	-	+	-	+	-	-	-
35	62 Yr/F	+	-	-	-	+	-	+	-	-	-
37	21 Yr/F	-	-	-	-	+	-	-	-	-	-
38	21 Yr/F	-	-	+	-	+	-	+	-	-	-
39	23 Yr/F	+	-	+	-	+	+	+	-	-	-
41	24 Yr/F	+	-	+	-	+	-	+	-	-	-
49	37 Yr/F	+	-	+	-	+	-	-	-	-	-
50	25 Yr/F	+	-	+	-	+	-	+	-	-	-
51	22 Yr/F	+	-	+	-	+	-	+	-	-	-
53	31 Yr/F	+	-	+	-	+	-	+	-	-	-
54	30 Yr/F	-	-	-	-	+	-	+	-	-	-
58	21 Yr/F	-	-	-	-	+	-	+	-	-	-
62	31 Yr/F	+	-	+	-	+	-	+	-	-	-
N=30											

*A: Toxo-IgG, B: Toxo-IgM; C: Rubella-IgG, D: Rubella-IgM; E: CMV-IgG, F: CMV-IgM; G: HSV1-IgG, H: HSV1-IgM; I: HSV2-IgG, J: HSV2-IgM

Table 3: Laboratory investigations for components of TORCH i.e. Toxo, Rubella, HSV1 and HSV2 infections singly or together

Patient SI No	Age/Sex	Toxo, Rubella, HSV1 and HSV2 antibody *									
		A	B	C	D	E	F	G	H	I	J
10	26 Yr/F	+	+								
15	27 Yr/F	+	+								
16	25 Yr/F	+	+								
17	26 Yr/F	+	+								
18	34 Yr/F	-	-								
20	26 Yr /M							-	-	-	-
22	30 Yr /M							+	-	+	-
23	35 Yr /M							+	-	-	-
24	21 Yr /M							-	-	-	-
27	25 Yr /M			+	-						
28	42 Yr /M							+	-	+	-
30	52 Yr /M							+	-		
31	52 Yr /M									-	-
32	25 Yr /M							+	-		
34	20 Yr /M							-	-		
36	29 Yr /M							+	-	-	-
40	22 Yr /M							+	-	-	-
42	26 Yr/F	-	-								
43	32 Yr /M							+	-	-	-
44	25 Yr/F	+	-								
45	14 Yr /M	+	-								
46	27 Yr /M							+	-		
47	28 Yr/F							+	-	-	-
48	43 Yr /M							+	-	+	-
52	33 Yr /M							-	-	-	-
56	48 Yr /M							+	-	+	-
57	43 Yr /M							-	-	-	-
59	32 Yr /M							+	-	+	-
60	13 Yr/F							+	-	-	-
63	35 Yr /M							+	-	-	-
64	30 Yr/F			+	-						
65	30 Yr /M							+	-	-	-
N=32											

*A: Toxo-IgG, B: Toxo-IgM; C: Rubella-IgG, D: Rubella-IgM; E: CMV-IgG; F: CMV-M; G: HSV1-IgG, H: HSV1-IgM; I: HSV2-IgG, J: HSV2-IgM

As per request from the ward or OPD, 32 specimens were analysed for one or more components of TORCH infections separately rather than the complete profile Table 3. Of the 8 specimens analysed for Toxoplasma infection only, 04 of them (i.e. No. 10, 15, 16, 17) were positive for both IgG and IgM classes of antibodies indicating acute/recent infection, 02 specimens were positive for IgG class antibody suggestive of past infection and 02 specimens were negative for both classes (IgG and IgM) of antibodies indicating no infection with Toxoplasma. Only 2 specimens (i.e. specimen no. 27, 64) were analysed for Rubella antibodies alone which were positive for IgG class antibody only suggesting past infection or vaccination with rubella virus and no specimen for only CMV antibodies. Out

of the remaining 22 specimens, 17 were analysed for both HSV1 and HSV2, 4 were analysed for HSV1 alone and 1 was analysed for HSV2 alone as per request. Of the 17 specimens, 5 were positive for IgG class and negative for IgM class antibodies indicating past/old infections for both HSV1 and HSV2, 8 were positive for past infections with HSV1 but negative for HSV2 and 4 specimens were negative for both HSV1 and HSV2 infections. Out of the 3 specimens analysed for HSV1 only, 2 were indicative of past infection and 1 was negative for HSV1 infection. The 01 specimen analysed for HSV2 alone was found negative for both classes of antibodies indicating no exposure to HSV2.

Discussion

Since diagnostic immunology laboratory was reorganized and some more versatile tests were introduced at MCW&H in August 2002 with competitive prices, a large number of requests were received for various biochemical and immunodiagnostic tests. The highest number of specimens received were for HBsAg followed by TFTs and other tests as stated in Table 1.

Regarding the autoantibody profile, 3/29 (10.3%) and 1/7 (14.3%) were positive for ANA and anti-dsDNA respectively. The anti-dsDNA antibody test is highly specific for differential diagnosis of systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). Although ANA test is nonspecific, it is very useful and diagnostic of many rheumatoid and inflammatory autoimmune disorders. Among the complements, C3 was low in 16/20 (80.0%) specimens. Low C3 level is considered as an useful indicator for diagnosis of a wide range of diseases such as SLE, RA with vasculitis, post-streptococcal glomerulonephritis (PGN), membranoproliferative glomerulonephritis (MPGN), acute and chronic infections, inflammatory disorders, complement deficiencies and pregnancy [1-3]. Serum total IgE level was positive (>100 iu / ml) in 22/33 (66.1%) specimens indicating probable allergic and parasitic infections Table 1 [7-11].

Among the tumour markers, PSA received highest interest and 3/35 (20.0%) were positive indicating prostatic hyperplasia or prostatic carcinoma. Among the hormones TFTs were most popular, efficient and cost effective in diagnosis and follow-up of patients with hyperthyroidism (thyrotoxicosis), hypothyroidism and enthyroidism [1,2]. The T3 levels were normal, higher and lower in 62/84 (73.5%), 15/84 (18.0%) and 7/84 (8.5%) specimens respectively. The T4 levels were normal, higher and lower in 109/135 (82.2%), 14/135 (10.4%) and 12/135 (8.7%) specimens respectively. The TSH level were normal, higher, and lower in 111/152 (73.1%), 24/152 (15.7%) and 17/152 (11.2%) respectively. These results of TFTs clearly indicated their importance in the diagnosis and follow up of patients with thyroid diseases. The other hormones analysed were PRL and TEST. PRL level was higher in 6/12 (50.0%) specimens and TEST level was lower in 9/32 (28.1%) specimens suggestive of probable infertility cases. Hyperprolactinemia is also associated with amenorrhea-galactorrhea syndrome, decreased libido and, in extreme cases pituitary prolactinoma (serum PRL level ≥ 100 ng/ml). However, serum PRL level in all the specimens were <100 ng/ml, excluding the possibility of pituitary hyperprolactinoma in any of our patients. However, possibility exists that mild hyperprolactinoma may have an important role in the actiopathogenesis of psoriasis vulgaris as suggested by many investigators recently Table 1 [17-19].

Among the hepatitis profile, HBsAg, HBc-IgM ab and HBeAg were positive in 43/828 (5.2%), 3/14 (21.5%) and 3/19 (15.5%) respectively indicating the importance of these parameters in the

diagnosis and follow-up of patients with HBV infection. HBs ab was positive in 1/10 (10%) specimens indicating probable natural recovery of these patients from HBV infection eventually. HCV-ab and HAV-IgM ab and HEV-IgM ab were positive in 5/47 (12.1%), 4/50 (8.0%) and 44/74 (59.4%) respectively suggesting high rate of exposure to other hepatitis viruses particularly HEV. HEV-IgM ab was positive in 59.4% of our specimens and this high incidence was probably due to the fact that HEV is an enterically transmitted virus Table 1 [3,4,20-23].

TORCH infections are some of the most common infections associated with congenital anomalies. Most of the TORCH infections cause mild maternal morbidity, but have serious foetal consequences. Treatment of maternal infection frequently has no impact on foetal outcome [20,21]. Maternal TORCH infections during pregnancy are a threat to pregnancy because they can be transmitted to the foetus while in the womb [20,22]. Also, congenital malformations caused by TORCH infections are important causes of morbidity, mortality and disability found in large percentage of neonates [20,23]. The consequence of these infections on foetus depend upon the type and virulence of the infecting agent and the stage of pregnancy. Therefore, recognition of maternal disease and then foetal monitoring are important for all relevant clinicians [23-25]. The prevalence of these infections varies from one geographical location to another [20,27,28]. Any patient infected with the TORCH agents, mainly two types of antibodies i.e. IgM and IgG classes are produced against the infective organisms. By measuring the antibody classes in mother's blood, one can identify the type of infection [20,23]. When IgM class antibody is present with or without IgG class antibody, it invariably suggests acute/recent infection and the presence of only IgG class antibody suggests mainly past or present active infection [20,29]. In the present study, an attempt was made to determine the prevalence of seropositivity in of TORCH in requested specimens sent to MRU Diagnostic Laboratory for investigations. However, laboratory evidence for TORCH infections, either multiple or single, was very low in the present study (Table 2, 3) contrary to some other reports from Bangladesh [20,22,25]. The probable reason may be that samples received at our MRU laboratory were from heterogeneous sources rather than concentrated from either Obstetrics and Gynecology or Neonatal units of hospitals and clinics.

American College of obstetrics and Gynecology (ACOG) currently recommended screening pregnant women for TORCH at the first prenatal visit. Clinicians must be well acquainted with the presentations of these infections, so that proper diagnosis can be made and treatment initiated, thus decreasing the likely wood of maternal and neonatal morbidity and mortality [20,28-30].

In conclusion, we were encouraged by the interest and confidence shown by the patients as well as the clinicians in the advanced tests introduced at MRU, MHWT, MCW&H Building, Uttara, Dhaka. The costs of these tests in our laboratory were one of

the lowest and in the near future, we are inclined to introduce more immunodiagnostic tests with competitive prices. It is of paramount importance, however, to take clinical data into consideration whenever making interpretations of the laboratory (diagnostic) test results. Therefore, it would have been clinically and scientifically more meaningful if the specimens were sent to the laboratory with more clinical details as per as practicable, particularly the provisional diagnosis and other most relevant clinical features of the patients.

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