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STUDY ON HUMORAL AND CELLULAR MEDIATED IMMUNITY AGAINST *SHIGELLA DYSENTERIAE* TYPE 1 FOLLOWING THEIR EXPERIMENTAL INFECTION IN WHITE MICE AND GUINEA PIGS

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ABSTRACT
Shigellosis is an infectious disease problem affecting man and many species of animal. For the importance of this disease, this study aimed to identify both the humoral and cell mediated immunity against shigellosis. For this reason, a local strain of *Shigella dysenteriae* type 1 obtained from the Central Public Health Laboratory, Ministry of Health, Iraq. The strain was reidentified to be sure *shigella dysenteriae* type 1 using cultural, biochemical, API-20 test and sereny test. The LD50 was 8 $x10^3$ CFU in (0.25 ml of bacterial suspension were 1/P inoculated into white mice and 0.5 ml bacterial suspension ($16x10^3$ CFU /ml) S/C inoculated in Guinea pigs at the 1st, 3rd, 5th weeks, the results revealed that high levels of antibodies detected by Elisa test and the maximum level of antibodies detected at 28th day post inoculation in white mice also, this study revealed high level of cellular mediated immunity detected by DTH-skin test at 24 hrs post inoculation of the Ag intradermally giving thickened erythematous areas at the site of inoculation. Macrophage migration inhibition test gave high level of migration inhibition of peritoneal macrophages in immunized group of Guinea pigs comparable to the control group (non-immunized) gave complete migration of peritoneal macrophages in tissue culture plates . Lymphocytes transformation test was also gave high level of mitotic index in the lymphocytes in immunized guinea pigs comparable to non-immunized group.

KEYWORDS: *Shigella dysenteriae* type 1, erythematous, inoculation, Lymphocytes transformation.

INTRODUCTION

Shigellosis is an infectious disease affecting children at different ages, associated with a dysenteric diarrhea admixed with mucous and blood and abdominal pain and fever. It distributed all over the world accompanied by high mortalities in children[1] especially in poor and crowded countries[2]. The infection with *Shigella dysenteriae* type 1 considered to be highly fatal associated with the different inflammatory lesions and micro abscesses occurred in the intestinal mucosa induced by this microbe a highly pathogenic Shigella species in addition to hemorrhage and pseudodiphtheric membrane on intestinal mucosal surface[3]. Monkeys considered to be the natural host for shigella in addition to human being, also in Guinea pigs and other laboratory animals might be affected by this microbial agent[4]. Regarding the immunity against shigellosis still limited especially humoraland cellular immunity[5] as for the *Shigella dysenteriae* type 1 as an intra and extracellular pathogen, for this reason cellular mediated and humoral immunity is important , so this study aimed to identify both humoral and cell mediated immunity in white mice and guinea pigs against these bacteria.

MATERIALS & METHODS

A local strain of shigella dysenteriae type 1 obtained from public health laboratory. The strain were re-identified to be sure shigella dysenteriae type 1[6] using cultural, biochemical test, API -20 tests slide agglutination test and Sereny test in Guinea pigs causing Keratoconjunctivitis[7]. The LD 50 –for this organism were 8×10^3 CFU[8]. Then 45 mice were taken and injected 1/p with 8×10^3 CFU (0.25ml of bacterial suspension) three mice were sacrificed at 2 days intervals and blood samples taken for measuring the humoral immunity by Elisa test[9]. Regarding guinea pigs immunization were done according to[10] Guinea pigs were injected S/C with 0.5 ml of bacterial suspension ($16\ x10^3$ CFU/ml), booster doses at 3rd, 5th week with 1ml of bacterial suspension ($16x10^3$ CFU) were given S/C also. The control group S/C injected with phosphate buffer saline. Five animals were taken from each group to measure cell mediated immunity (Delayedtype hypersensitivity skin test and macrophage migration inhibition test)[10] and for lymphocyte transformation test in blood leukocytes[10].

RESULTS

Humoral Immunity

Enzyme linked immunosorbant Assay (Elisa)

The results indicated that the proper serum dilution 1/100 characterized the negative and positive cases and proper Ag dilution 1:1600 (2 μg/ml), characterized between negative and positive sera specimens with a conjugate dilution 1: 200. The cut off mean values were 0.673 ±0.138 for negative sera and 0.949 for control sera specimen and 1.694 ± 0.365 for positive sera .After doing Elisa test on positive, negative

control and on sera specimens and the test was positive if optical density above cut off value and negative bellow cutoff value. (Table 1, 2).

TABLE 1: Upper lower and mean values of optical density of Elisa test during periods after 1/P inoculation of *Shigella dysenteriae* type -1 in mice

Groups	Periods	Upper limits	Lower limits	Mean
1	3	1.171	0.943	1.057
2	7	1.223	0.987	1.105
3	10	1.475	1.090	1.280
4	13	1.522	1.1300	1.326
5	16	1.641	1.201	1.421
6	19	1.724	1.322	1.523
7	22	1.840	1.390	1.615
8	25	1.904	1.486	1.695
9	28	2.302	1.600	1.951
10	40	2.103	1.625	1.864

TABLE 2: Upper, lower and mean values of optical density of Elisa test during periods after oral giving of Shigella *dysenteriae* type 1 in mice

Groups	Periods	Upper limit	Lower limit	Mean
1	3	0.85	0.45	0.65
2	7	0.90	0.50	0.70
3	10	0.95	0.60	0.77
4	13	1.00	0.65	0.825
5	16	1.10	0.70	0.9
6	19	1.20	0.80	1.00
7	22	1.30	0.90	1.10
8	25	1.60	1.20	1.40
9	28	1.80	1.40	1.60
10	40	1.80	1.40	1.60

Cellular mediated immunity

Delayed type hypersensitivity (DTH) skin test

The mean values of erythema diameters were 12.6mm, 9 mm and 6.4mm after 24 hrs, 48 hrs and 72hrs respectively in immunized animals at 400 μg/ml concentration of Ag Whereas, the mean diameters values were reduced at 40 μg/ml and 4 μg/ml to (8.6, 7.8, 4.6 and 6.2, 5.4, 2.4 respectively) after 24 hrs, 48 hrs and 72 hrs (Table 3).

TABLE 3: DTH- Skin test (erythema diameters) in immunized and control group of G. pigs against 1/D *Shigella dysenteriae* type-1 Ag inoculation

Time / hours												Groups
72hrs				48 hrs				24hrs			Diameter	
Ag Coneant PBS				Ag coneant PBS				Ag coneant PBS			mm	
	4	40	400		4	40	400		4	40	400		
0	2-3	4-5	5-7	0	5-6	6-9	7-10	0	5-7	7-10	9-15	Range	Immunized
	2.4	4.6	6.4	0	5.4	7.8	9		6.2	8.6	12.6	Mean	group
	±	±	±		±	±	±		±	±	±	±	
0	0.591	0.547	0.849		0.547	1.303	1.224	0	0.836	1.105	2.313	S.D	

Also the skin thickness differences mean values were 2.7mm after 24 hrs at injected area with Ag concentration (400 μg/ml) and the skin thickness differences mean values reduced to 2.3 mm and 1.4 mm after 48 hr and 72 hrs respectively in immunized animals comparable to control group (Table 4).

TABLE 4: DTH –skin test (skin thickness) in immunized and control group of G. pigs against *Shigella dysenteriae* type 1 Ag

Skin thickness differences 72hrs	Skin thickness differences 48hrs	Skin thickness differences 24hrs	Skin thickness (normal)	Animal number
1.3	2.8	3.2	2.1	1
1.9	2.6	2.4	1.6	2
0.7	1.4	2.3	1.8	3
1.4	2.3	3.1	1.9	4
1.8	2.7	2.5	1.7	5
1.4	2.3	2.7	1.8	mean

Macrophage migration inhibition test (MMIT)

The immunized group of Guinea pigs showed inhibition of peritoneal macrophages migration in tissue plates after 24 hrs following addition of 400 μg /ml , 40 μg /ml and 4 μg / ml Ag concentrations (table –5) in the immunized group the mean value of migration inhibition decreased gradually with the decrease of Ag concentration . The mean values were 0.477 ±0.268 at 400 μg /ml Ag concentration and decreased to 0.610 ± 0.181 and 0.641 ± 0.145 at 40 μg /ml and 4 mg /ml respectively comparable to 1.258± 0.387, 1.717 ± 0.641 and 2.746± 1.119 at 400 μg / ml, 40 μg / ml and 4 μg /ml respectively in control group (non-immunized).

TABLE 5: migration inhibition areas diameters in immunized and control groups against *Shigella dysenderiae* type-1 Ag

PHA	Ag content			No of animal	Groups
(10mg/ml)	4	40	400	No .animal	
0	0.725	0.725	0.544	1	Immunized group
0	0.632	0.591	0.429	2	
0	0.786	0.786	0.671	3	
0	0.759	0.740	0.740	4	
0	0.681	0.681	0.640	5	
0	0.713	0.702	0.409	6	
0	0.441	0.287	0.160	7	
0	0.392	0.374	0.230	8	
0	0.641±0.145	0.610±0.181	0.477± 0.268		Mean ± S.D
0	2.050	1.000	0.833	1	Control groups
0	1.790	1.120	1.000	2	
0	4.220	2.140	1.140	3	
0	2.990	1.850	1.560	4	
0	2.680	2.478	1.759	5	
	2.746 ±1.119	1.717 ±0.641	1.258 ±0.387	Mean ± S.D	

Lymphocytes transformation test

The results indicates that the mean values mitotic index for lymphocytes of immunized animals were 11.56 ±3.22 whereas, mean values for lymphocytes exposed to phytohemagglutinine (PHA) were 41.49±26.298 comparable to the mean values were 1.18 ± 0.516 in control (non immunized) group (Table-6) .

TABLE 6: lymphocytes mitotic indices (MI) in immunized against *Shigella dysenteriae* type1 A gand in PHA stimulated lymphocytes

Mitotic index %			No
PHA 5mg /ml	Ag 0.1mg/ml	Control	
20.28	11.88	1.50	1
36.35	16.22	1.80	2
75.80	9.60	0.70	3
14.27	12.45	1.30	4
60.78	7.68	0.60	5
41.49 ± 26.298	11.56 ± 3.22	1.18 ± 0.516	Mean ± S.D

DISCUSSION

Shigella dysenteriae type 1 had a potent shigatoxin which important for induction both humoral and cell mediated immunity which were more evident in this study[11]. Elisa test gave good results in estimation the level of antibodies against *Shigella dysenteriae* type 1 toxins. In this study we used living *Shigella dysenteriae* type1 organism instead of dead bacteria because the live bacteria proliferated continuously and gave Ags and toxins, so enhance good immune response, whereas, dead bacteria need recurrent

doses because did not proliferated and may destroyed by the macrophages and the neutrophils, in addition to, loss of some Ags and toxins through their killing[12] comparable to live organisms contain complete Ag and toxins and following their proliferation in the living body, which was evident in this study in white mice .This study revealed high level of cell mediated immunity detected by DTH skin test, this test depended on secretion of lymphokines from sensitized lymphocytes lead to aggregation of neutrophils, lymphocytes and macrophages together with edema and congestion detected by erythema diameter and skin thickness area at the inoculation site of Ag[10,13], this study also revealed high level of cellular immunity detected by macrophage migration inhibition test and lymphocytes transformation test. The MMIT revealed that inhibition of macrophage migration in immunized animal under the effect of Ag comparable to complete migration of these cells in control group. The sensitized lymphocytes in immunized animals induced the lymphokines against *Shigella dysenteriae* type 1 lead to aggregate the macrophages and lymphocytes and inhibit their migration under the effect of macrophage inhibitory factor (MIF) which was more evident in this study[14,15]] similarly sensitized lymphocytes proliferated continuously and gave clones of these cells detected by high level of mitotic index in immunized animals comparable to non-immunized (control) a similar findings reported by [13] that the mitotic index increased with the state of immunization of lymphocytes by Ag and mitogen.

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