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Appendix -1- (A)

Lymphocyte transformation for Female for thalassemia patients

patients .No	With 20µl PHA									
	Sample 1 (mean of three slides)			**Sample 2 (mean of three slides)**			**Sample 3 (mean of three slides)**			**Mean of Trans. Cells %**
	Transformed Cells No.	%	Nor. Cells No.	Transformed Cells No.	%	Nor. Cells No.	Transformed Cells No.	%	Nor. Cells No.	
1	14.3	14.3	85.7	12.6	12.6	87.4	18	18	82	15.6
2	12.3	12.3	87.7	14.6	14.6	85.4	13.3	13.3	86.7	13.4
3	26.3	26.3	73.7	14.3	14.3	85.7	16.3	16.3	83.7	19
4	22	22	78	15.6	15.6	84.4	22	22	78	19.8
5	24.3	24.3	75.7	17.6	17.6	82.4	26.3	26.3	73.7	22.7
6	27	27	73	29.6	29.6	70.4	16.3	16.3	83.7	24.3
7	19	19	81	24.6	24.6	75.4	34.3	34.3	65.7	26
8	23	23	77	16.6	16.6	83.4	24	24	76	21.2
9	27	27	73	18	18	82	25	25	75	23.3
10	20.3	20.3	79.7	13.3	13.3	84.7	23	23	77	19.5
11	23	23	77	21.6	21.6	78.4	30	30	70	25
12	10.3	10.3	89.7	12.6	12.6	87.4	15.6	15.6	84.4	13
13	20.3	20.3	79.7	17	17	83	22.3	22.3	77.7	20
14	31.3	31.3	68.7	26.3	26.3	73.7	31	31	69	29.6
15	26	26	74	17	17	83	23.6	23.6	76.4	22.2
16	29.3	29.3	70.7	28.3	28.3	71.7	18.3	18.3	81.7	25.3
17	21	21	79	17	17	83	25.6	25.6	74.4	21.2
18	27	27	73	26.6	26.6	73.4	31.6	31.6	68.4	28.4
19	17	17	83	23.3	23.3	76.7	24.6	24.6	75.4	21.8
20	28.6	28.6	71.4	23.3	23.3	76.7	17	17	83	23
21	18.3	18.3	81.7	27.3	27.3	72.3	29.3	29.3	78.7	25
22	21	21	79	16.6	16.6	83.4	21.6	21.6	78.4	20
23	30.6	30.6	69.4	34	34	66	34.6	34.6	65.4	33
24	13	13	87	12	12	88	15.6	15.6	84.4	13.5
25	26	26	74	25.3	25.3	74.7	27	27	73	26
26	14	14	86	10	10	90	15	15	85	13
Mean SD±	**22 5.936**	**22% 5.936**	**77.9 5.936**	**31.4 6.405**	**31.4% 6.405**	**80 6.354**	**23.1 6.242**	**23.1% 6.242**	**77 6.121**	**21.7% 5.160**

Appendix -1- (B)

Lymphocyte transformed for Male of thalassemia patients

patients .No	With 20μl PHA									
	Sample 1 (mean ofthree slides)			Sample 2 (mean of three slides)			Sample 3 (mean of three slides)			Mean of Trans. Cells %
	Transformed Cells No.	%	Nor. Cells	Transformed Cells no.	%	Nor Cells No.	Transformed Cells No.	%	Nor. Cells No.	
1	15	15	85	14	14	86	17.3	17.3	82.7	15.4
2	7.6	7.6	92.4	11.3	11.3	88.7	4.6	4.6	95.4	7.8
3	14	14	86	17.3	17.3	82.7	15	15	85	15.4
4	15.3	15.3	84.7	10	10	90	12.3	12.3	87.7	12.5
5	17.3	17.3	82.7	14	14	86	15	15	85	15.5
6	18.6	18.6	81.4	14	14	86	19.6	19.6	80.4	17.4
7	10	10	90	12.3	12.3	87.7	14.3	14.3	85.7	12.2
8	29	29	71	29	29	71	19	19	81	25.6
9	28.6	28.6	71.4	27.3	27.3	72.7	33.3	33.3	66.7	30
10	26	26	74	23.3	23.3	76.7	29.6	29.6	78.4	26.3
11	27	27	73	17.6	17.6	82.4	24	24	76	23
12	20.6	20.6	79.4	22.6	22.6	77.4	32	32	68	25
13	24.3	24.3	76.7	32.6	32.6	67.4	23.6	23.6	76.4	27
14	23	23	77	16.3	16.3	83.7	21.6	21.6	78.4	20
15	20.6	20.6	79.4	22.3	22.3	77.7	29	29	71	24
16	27	27	73	19.6	19.6	80.4	27	27	73	24.5
17	11	11	89	14	14	86	18.3	18.3	81.7	14.4
18	27	27	73	24.3	24.3	75.7	34.3	34.3	65.7	28.5
19	21	21	79	18.3	18.3	81.7	24.6	24.6	75.4	21
20	19.3	19.3	80.7	24.3	24.3	75.7	16.3	16.3	83.7	20
21	29	29	71	20.6	20.6	79.4	26.3	26.3	73.7	25.3
22	22.3	22.3	77.7	22	22	78	14.6	14.6	85.4	20
23	27	27	73	19.6	19.6	80.4	25.3	25.3	74.7	24
24	22	22	78	25.3	25.3	74.7	27.6	27.6	72.4	25
25	13	13	87	20.3	20.3	79.7	9.6	9.6	90.4	14.3
26	28.8	28.8	71.2	26.3	26.3	73.7	29	29	71	28
27	14.3	14.3	85.7	14.6	14.6	85.4	20.3	20.3	79.7	16.5
28	23	23	77	23.6	23.6	76.4	28.6	28.6	71.4	25
29	14.6	14.6	85.4	16.3	16.3	73.7	22.6	22.6	77.4	17.8
30	16.3	16.3	83.7	17	17	73	23.3	23.3	76.7	19
31	12	12	88	25.6	25.6	74.4	16.3	16.3	83.7	18
32	29	29	71	23.3	23.3	76.7	17	17	83	23
33	33.6	33.6	66.4	27.3	27.3	72.7	36.3	36.3	73.7	32.5
34	29.6	29.6	70.4	22.6	22.6	71.4	30.3	30.3	69.7	27.5
35	26.3	26.3	69.7	17.3	17.3	82.7	23	23	77	22.2
36	20	20	80	18.6	18.6	81.4	24.3	24.3	75.7	21
37	17.3	17.3	82.7	15.3	15.3	84.7	21.3	21.3	78.7	18
38	15.3	15.3	84.7	11.3	11.3	88.7	12.3	12.3	81.7	13

39	17	17	83	18.6	18.6	81.4	24.6	24.6	75.5	20
40	13	13	87	9	9	91	13.3	13.3	82.7	11.7
41	12	12	88	17.6	17.6	82.4	16.3	16.3	83.7	15.3
42	18.6	18.6	81.4	15.3	15.3	84.7	18.6	18.6	81.4	17.5
43	14.3	14.3	85.5	13.3	13.3	82.7	15.6	15.6	84.4	14.5
44	14	14	86	21	21	79	19.6	19.6	80.4	18
Mean SD±	**20.1 6.520**	**20.1% 6.520**	**79.8 6.619**	**19.2 5.454**	**19.2% 5.454**	**80 5.706**	**21.5 7.026**	**21.5% 7.026**	**78.6 6.353**	**20.2% 5.605**

Appendix -1- (c)

Lymphocyte transformation of Male (control)

.No	**With 20μl PHA**									
	Sample 1 (mean ofthree slides)			**Sample 2 (mean of three slides)**			**Sample 3 (mean of three slides)**			**Mean of Trans. Cells %**
	Transformed Cells No.	%	Nor. Cells No.	Transformed Cells No.	%	Nor. Cells No.	Transformed Cells No.	%	Nor. Cells No.	
1	56	56	44	53	53	47	47	47	53	52
2	58.6	58.6	41.4	57	57	43	63	63	37	60
3	61	61	39	58	58	42	67	67	33	62
4	56	56	44	56.3	56.3	45.7	62.3	62.3	37.7	58
5	46	46	54	50	50	51	53	53	47	49
Mean SD±	**55.5 5.714**	**55.5% 5.714**	**44.48 5.714**	**54.86 3.301**	**54.86% 3.301**	**45.74 3.562**	**58.4 8.207**	**58.4% 8.207**	**41.54 8.207**	**56.2 5.495**

Appendix -1- (D)

Lymphocyte transformation of Female (control)

.No	With 20µl PHA									
	Sample 1 (mean of three slides)			**Sample 2 (mean of three slides)**			**Sample 3 (mean of three slides)**			**Mean of Trans. Cells %**
	Transformed Cells No.	%	Nor. Cells No.	Transformed Cells No.	%	Nor. Cells No.	Transformed Cells No.	%	Nor. Cells No.	
1	52.6	52.6	47.4	58.3	58.3	41.7	57	57	43	56
2	45	45	55	43.3	43.3	56.7	53	53	47	47
3	42.3	42.3	57.7	42.3	42.3	57.3	47	47	53	43
4	54.6	54.6	45.4	48.3	48.3	51.7	50	50	50	51
5	48.2	48.2	51.8	43.3	43.3	56.7	43.5	43.5	56.5	45
Mean SD±	**48.54 5.118**	**48.5% 5.118**	**51.4 5.118**	**47.1 6.685**	**47.1 6.685**	**52.9 6.685**	**50.1 5.22**	**50.1 5.22**	**49.9 5.22**	**48.4 5.176**

Appendix -2- (A)

Lymphocyte transformation of thalassemia patients in age between 3-7 years

patients .No	With 20µl PHA									
	Sample 1 (mean of three slides)			**Sample 2 (mean of three slides)**			**Sample 3 (mean of three slides)**			**Mean of Trans. Cells %**
	Transformed Cells No.	%	Nor. Cells No.	Transformed Cells No.	%	Nor. Cells No.	Transformed Cells No.	%	Nor. Cells No.	
1	14.3	14.3	85.6	12.6	12.6	87.4	20.3	20.3	79.7	15.7
2	15	15	85	14	14	86	17.3	17.3	82.7	15.4
3	17.6	17.6	82.4	11.3	11.3	88.7	4.6	4.6	95.4	12
4	14	14	86	17.3	17.3	88.7	15	15	85	15.4
5	17.3	17.3	82.7	14	14	86	15	15	85	15.5
6	27	27	73	29.6	29.6	70.4	16.3	16.3	83.7	24.3
7	27	27	73	18	18	82	25	25	75	23.3
8	20.6	20.6	79.4	22.6	22.6	77.4	32	32	68	25
9	20.6	20.6	79.4	22.3	22.3	77.7	29	29	71	24
10	27	27	73	24.3	24.3	75.7	33	33	67	28
11	27	27	73	19.6	19.6	80.4	25.3	25.3	74.7	24
12	28.6	28.6	71.4	26.3	26.3	73.7	29	29	71	28
13	14.3	14.3	85.7	14.6	14.6	85.4	20.3	20.3	79.7	16.5
14	24	24	76	23.6	23.6	76.4	28.6	28.6	71.4	25.5
Mean SD±	**31.3 5.623**	**31.3% 5.623**	**80 5.614**	**26.3 5.626**	**26.3% 5.626**	**81 6.005**	**31 8.053**	**31% 8.053**	**77.8 8.053**	**20.9% 5.481**

Appendix -2- (B)

Lymphocytes transformation of thalassemia patient in age between 8 - 12 years

patients .No	With 20µl PHA									
	Sample 1 (mean of three slides)			**Sample 2 (mean of three slides)**			**Sample 3 (mean of three slides)**			**Mean of Trans. Cells %**
	Transformed Cells No.	%	Nor. Cells No.	Transformed Cells No	%	Nor. Cells No.	Transformed Cells No	%	Nor. Cells No.	
1	12.3	12.3	87.7	14.6	14.6	85.4	13.3	13.3	86.7	13.4
2	26.3	26.3	83.7	14.3	14.3	85.7	16.3	16.3	83.7	19
3	22	22	88	15.6	15.6	84.4	22	22	78	19.8
4	24.3	24.3	85.7	17.6	17.6	82.4	26.3	26.3	73.7	22.7
5	19	19	81	24.6	24.6	75.4	34.3	34.3	65.7	26
6	18.6	18.6	81.4	14	14	86	19.6	19.6	80.4	17.4
7	23	23	77	16.6	16.6	83.4	24	24	76	21.2
8	10	10	90	12.3	12.3	81.7	14.3	14.3	85.7	12.2
9	23	23	77	21.6	21.6	78.4	30	30	70	25
10	26	26	74	27.3	27.3	72.7	33.3	33.3	66.7	30
11	10.3	10.3	89.7	12.6	12.6	81.4	15.6	15.6	84.4	13
12	26	26	74	23.3	23.3	76.7	29.6	29.6	70.4	26.3
13	26	26	74	17	17	83	23.6	23.6	76.4	22.2
14	11	11	89	14	14	86	18.3	18.3	81.7	14.4
15	19.3	19.3	80.7	24.3	24.3	75.8	16.3	16.3	83.7	20
16	29	29	71	20.6	20.6	79.4	26.3	26.3	73.7	25.3
17	13	13	87	20.3	20.3	79.7	9.6	9.6	90.4	14.3
18	27	27	73	26.6	26.6	73.4	31.6	31.6	68.4	28.4
19	21	21	79	18.3	18.3	81.7	24.6	24.6	75.4	21
20	16.3	16.3	83.7	17	17	83	23.3	23.3	76.7	19
21	12	12	88	25.6	25.6	74.4	16.3	16.3	83.7	18
22	28.6	28.6	71.4	23.3	23.3	76.7	17	17	83	23
23	26.3	26.3	73.7	17.3	17.3	82.7	23	23	77	22.2
24	13	13	87	12	12	88	15.6	15.6	84.4	13.5
25	26	26	74	25.3	25.3	74.7	27	27	73	26
26	14	14	86	10	10	90	15	15	85	13
27	18.6	18.6	81.4	15.3	15.3	84.7	18.6	18.6	81.4	17.5
28	14	14	86	21	21	79	19.6	19.6	81.4	18
Mean SD±	19.8 6.253	19.8% 6.253	81.2 6.274	18.6 4.980	18.6% 4.980	80.9 4.661	21.5 6.512	21.5% 6.512	78.4 6.526	20% 5.074

Appendix -2- (C)

Lymphocytes transformation of thalassemia patients in age between13 - 17 years

patients .No	With 20μl PHA									
	Sample 1 (mean of three slides)			**Sample 2 (mean of three slides)**			**Sample 3 (mean of three slides)**			**Mean of Trans. Cells %**
	Transformed Cells No.	%	Nor. Cells No.	Transformed Cells no	%	Nor. Cells No.	Transformed Cells No.	%	Nor. Cells No.	
1	20.3	20.3	79.7	17	17	83	22.3	22.3	77.7	20
2	27	27	73	17.6	17.6	82.4	24	24	76	23
3	29.3	29.3	70.7	28.3	28.3	71.7	18.3	18.3	81.7	25.5
4	24.3	24.3	75.7	32.6	32.6	67.4	23.6	23.6	76.4	27
5	23	23	77	16.3	16.3	83.7	21.6	21.6	78.4	20
6	27	27	73	19.6	19.6	80.4	27	27	73	24.5
7	21	21	79	17	17	83	25.6	25.6	74.4	21.2
8	22	22	78	25.3	25.3	74.7	27.6	27.6	72.4	25
9	14.6	14.6	83.4	16.3	16.3	73.7	22.6	22.6	77.4	17.8
10	28.6	28.6	71.4	23.3	23.3	76.7	17	17	83	23
11	33.6	33.6	66.4	27.3	27.3	72.7	36.3	36.3	63.7	32.5
12	18.3	18.3	81.7	27.3	27.3	72.7	29.3	29.3	70.7	25
13	29.6	29.6	70.4	22.6	22.6	77.4	30.3	30.3	69.7	27.5
14	21	21	79	16.6	16.6	83.4	18.6	18.6	81.4	19
15	15.3	15.3	84.7	11.3	11.3	88.7	12.3	12.3	87.7	13
16	34.6	34.6	65.4	34	34	66	34.6	34.6	65.4	34
17	12	12	88	17.6	17.6	82.4	17	17	83	15.5
18	16	16	84	12.6	12.6	87.4	16	16	84	14.8
19	22.3	22.3	77.7	22	22	78	14.6	14.6	85.4	20
Mean SD±	**23 6.384**	**23% 6.384**	**76.7 6.250**	**21.2 6.443**	**21.2% 6.443**	**78 6.421**	**23 6.421**	**23 % 6.665**	**76.9 6.665**	**22.5% 5.562**

Appendix -2- (D)

Lymphocytes transformation of thalassemia patients in age between18 - 22 years

patients .No	With 20μl PHA									
	Sample 1 (mean of three slides)			**Sample 2 (mean of three slides)**			**Sample 3 (mean of three slides)**			**Mean of Trans. Cells %**
	Transformed Cells No.	%	Nor. Cells No.	Transformed Cells No.	%	Nor. Cells No,	Transformed Cells No.	%	Nor. Cells No.	
1	15.3	15.3	84.7	10	10	90	12.3	12.3	87.7	12.6
2	21	21	79	15.3	15.3	84.7	23	23	77	19.7
3	32.3	32.3	67.7	28	28	72	19	19	89	25.7
4	31.3	31.3	69.7	26.3	26.3	73.7	31	31	61	29.5
5	21	21	79	18.3	18.3	81.7	24.6	24.6	75.4	21.5
6	20	20	80	18.6	18.6	81.4	24.3	24.3	75.7	21
7	17.3	17.3	82.7	15.3	15.3	84.7	21.3	21.3	78.7	18
8	17	17	83	18.6	18.6	81.4	24.6	24.6	75.4	20
9	13	13	87	9	9	91	13.3	13.3	86.7	11.7
Mean SD±	**20.9 3.241**	**20.9% 3.241**	**79.2 3.24**	**17.7 4.21**	**17.7% 4.21**	**82.2 4.21**	**21.4 2.67**	**21.4% 2.67**	**78.52 2.67**	**19.9% 5.612**

Appendix -3- (A)

Lymphocyte transformation of normal humane with using Phytic acid

.No	With 0.15 µg/µl Phytic acid									
	Sample 1 (mean or three slides)			**Sample 2 (mean of three slides)**			**Sample 3 (mean of three slides)**			**Mean of Trans. Cells %**
	Transformed Cells No.	%	Nor. Cells No.	Transformed Cells No	%	Nor. Cells No.	Transformed Cells No	%	Nor. Cells No.	
1	17	17	83	17.6	17.6	82.4	13.3	13.3	86.7	16
2	15.6	15.6	84.4	13.3	13.3	86.7	15.3	15.3	84.7	15
3	20.6	20.6	79.4	17	17	83	16.3	16.3	83.7	18
4	24	24	76	18.6	18.6	81.4	17.3	17.3	82.7	20
5	24	24	76	19.3	19.3	80.7	22.6	22.6	77.4	22
6	14.3	14.3	85.7	16.3	16.3	83.7	14.3	14.3	85.7	15
7	20.3	20.3	79.7	17	17	83	19.3	19.3	80.7	19
8	27.3	27.3	72.7	22.6	22.6	77.4	25	25	75	25
9	17.3	17.3	82.7	14.3	14.3	85.7	17.3	17.3	82.7	15
10	22.3	22.3	77.7	19.6	19.6	80.4	21	21	79	21
Mean SD±	**60.9 5.211**	**20.2% 5.211**	**79.7 5.211**	**52.2 5.65**	**17.5% 5.65**	**82.4 5.65**	**54.6 3.33**	**18% 3.33**	**81.8 3.33**	**18.6% 5.017**

Appendix -3- (D)

Lymphocyte transformation of Control without Phytic acid

.No	Without Phytic acid									
	Sample 1 (mean or three slides)			**Sample 2 (mean of three slides)**			**Sample 3 (mean of three slides)**			**Mean of Trans. Cells %**
	Transformed Cells No.	%	Nor. Cells No.	Transformed Cells No.	%	Nor. Cells No.	Transformed Cells No.	%	Nor. Cells No.	
1	12.6	12.6	87.4	9	9	91	11.3	11.3	88.7	11
2	10.6	10.6	89.4	8	8	92	9.3	9.3	90.7	9
3	13	13	87	10.6	10.6	89.4	14	14	86	12.5
4	15.3	15.3	84.7	11.3	11.3	88.7	15.3	15.3	84.7	14
5	16.6	16.6	83.4	17	17	83	17.3	17.3	82.7	17
6	13	13	87	9.6	9.6	90.4	13.3	13.3	86.7	12
7	14	14	86	11	11	89	15.6	15.6	84.4	13.3
8	22	22	78	17.6	17.6	82.4	17.3	17.3	82.7	19
9	9.3	9.3	90.7	11.6	11.6	88.4	9	9	91	10
10	17	17	83	12.3	12.3	87.7	15.6	15.6	84.4	15
Mean SD±	**14.3 3.619**	**14.3% 3.619**	**85.6 3.619**	**11.8 3.170**	**11.8% 3.170**	**88.2 3.170**	**13.8 3.038**	**13.8% 3.038**	**86.2 3.038**	**13.2% 3.101**

Appendix -4- (A)

Phagocytosis test for Female of thalassemia patients

patients No.	Sample 1 (mean of three slides)		Sample 2 (mean of three slides)		Sample 3 (mean of three slides)		Mean of Pha.
	Pha.	Non Pha.	Pha	Non Pha.	Pha	Non Pha.	
1	40.6	59.4	35.3	64.7	36.3	63.7	37.5
2	28.3	71.7	20	80	25	75	24.5
3	42.6	57.4	35.3	64.7	40.6	59.4	39.5
4	30.3	69.7	30	70	30.3	69.7	30.2
5	32	68	27.6	62.4	29.6	70.4	29.8
6	41.3	58.7	47.3	52.7	50.6	49.4	47
7	57	43	48.3	51.7	54.3	45.7	53
8	39	61	41.3	58.7	49.6	50.4	43
9	43	57	46	54	50.6	49.4	46.5
10	38.6	61.4	36.3	64.7	41	59	38.3
11	56.6	43.4	52.3	47.7	60.3	39.7	56.4
12	26.3	73.7	22.3	77.7	25	75	24.5
13	34	66	32.6	67.4	40.6	59.4	35.7
14	56	44	41.6	53.4	50.3	49.7	51
15	33.6	66.4	27.6	72.4	30.3	69.7	30.5
16	53	47	56.6	45.4	63.6	36.4	57.7
17	17.3	82.7	19.3	80.7	20	80	19
18	43.6	56.4	35	65	43	57	40.5
19	13.3	82.7	13.3	86.7	17	83	14.5
20	41.6	58.4	33.6	66.4	38.6	61.4	38
21	45.3	54.7	35	65	42.6	57.4	41
22	38	62	30.6	69.4	35.6	64.4	34.7
23	42.3	57.7	27.6	72.4	35	65	35
24	16	84	18.6	81.4	25.6	74.4	20
25	39	61	43.6	46.4	38.3	61.7	40
26	29.3	70.7	33	67	31.6	68.4	31
Mean SD±	**37.5 11.652**	**62.2 11.340**	**34.3 10.866**	**64.8 11.310**	**38.4 11.973**	**61.5 11.973**	**36.8% 11.222**

Appendix-4-(B)

Phagocytosis test for Male of thalassemia patients

patients No.	Sample 1 (mean of three slides)		Sample 2 (mean of three slides)		Sample 3 (mean of three slides)		Mean of Pha. %
	Pha.	Non Pha.	Pha	Non Pha.	Pha	Non Pha.	
1	55	45	59.3	40.7	58	42	57
2	16.6	83.4	11.6	88.4	10	90	12.4
3	33.6	66.4	29.3	70.7	32.6	67.4	32
4	56	44	48	52	52	48	52
5	19	81	18.3	81.7	21	79	19.5
6	36.3	65.7	40.3	59.7	36	64	37.5
7	37.3	62.7	24.6	75.4	33	67	31.5
8	42.6	57.4	45	55	43	57	43.5
9	47.3	52.7	43.3	56.7	49.6	50.4	46.7
10	50	50	44	56	44	56	46
11	35.6	54.4	38.6	61.6	37	63	37
12	59.3	40.7	52.3	47.7	62	38	57.8
13	54.3	45.7	48	52	54.3	45.7	52
14	46	54	36	64	44.3	55.7	42
15	38	67	34.6	65.4	32.6	67.4	35
16	24	76	23.3	76.7	24.6	75.4	24
17	31	69	24.6	75.4	34	66	30
18	44.3	55.7	45.3	54.7	49.3	50.7	46
19	48	52	53.3	46.7	53.3	46.7	51.5
20	63.3	32.7	58.3	41.7	55.3	44.7	60
21	20.3	79.7	16.3	83.7	20.3	79.7	19
22	37	63	31	69	37	63	35
23	26	74	21	79	25	75	24
24	31.3	68.7	25.6	74.4	27.6	72.4	28
25	45	55	47.3	52.7	48.6	51.4	47
26	17.6	82.4	14.6	85.4	17	83	16.4
27	43.3	56.7	40.6	59.4	42	58	42
28	58	42	48.6	51.4	55.3	44.7	54
29	27	73	32.6	61.4	38.6	61.4	33
30	30.3	69.7	27.6	72.4	30.3	69.7	29.5
31	39.6	60.4	30	70	35.6	64.4	35
32	37.3	62.7	34.3	65.7	37.3	68.7	36.3
33	22.3	77.7	18.3	81.7	25	75	22
34	34	66	33	77	37.6	62.4	35
35	34.6	65.4	41.6	58.4	38	62	38
36	31	69	28	72	31	69	30
37	38.6	61.4	39.3	60.7	36	64	38
38	26.6	73.4	28.3	81.3	35	65	30
39	23.6	76.4	23.3	76.7	31.3	68.7	26

40 41 42 43 44	38.6 26.6 36.3 63.3 43.7	61.4 73.4 63.7 36.3 56.3	39.3 23.6 36.3 58.3 35	60.7 76.4 63.7 41.7 65	36 24.6 32 63 43	64 73.4 68 37 57	38 25 35 61.5 40.5
Mean SD±	**37.9 12.357**	**61.8 12.661**	**35.2 12.296**	**65 12.641**	**38 12.164**	**62 12.159**	**37% 12.045**

Appendix -4- (C)

Phagocytosis test for normal control of human (male)

No.	**Sample 1 (mean of three slides)**		**Sample 2 (mean of three slides)**		**Sample 3 (mean of three slides)**		**Mean of Pha.**
	Pha.	Non Pha.	Pha	Non Pha.	Pha	Non Pha.	
1 2 3 4 5	69.7 68 62.4 81.4 64.7	29.3 32 27.6 18.6 35.3	74.4 62.4 70.4 81 60	25.6 37.6 29.6 19 40	77 67.7 73 87 67.7	23 32.3 27 13 32.3	80 66 72 83 64
Mean SD±	**69.3 9.235**	**29.4 9.235**	**68.8 7.358**	**31 7.358**	**72.7 8.269**	**27.2 8.454**	**71.2% 8.007**

Appendix -4- (D)

Phagocytosis test for normal control of human (Femal)

No.	Sample 1 (mean of three slides)		Sample 2 (mean of three slides)		Sample 3 (mean of three slides)		Mean of Pha.
	Pha.	Non Pha.	Pha	Non Pha.	Pha	Non Pha.	
1	62.4	37.6	54.7	45.3	58.3	41.3	58
2	73.7	26.3	70.7	29.3	77.4	22.6	74
3	81	19	76.7	23.3	79.7	20.3	79
4	70.4	29.6	75.7	24.3	73	27	73
5	59.4	39.6	62.4	37.6	66.7	33.3	63
Mean	**69.3**	**29.4**	**68.8**	**31**	**72.7**	**27.2**	**71.2%**
SD±	**9.235**	**9.235**	**7.358**	**7.358**	**8.269**	**8.454**	**8.007**

Appendix -5- (B)

Phagocytosis test for thalassemia patients in age between 8 -12

patients No.	Sample 1 (mean or three slides)		Sample 2 (mean or three slides)		Sample 3 (mean or three slides)		Mean of Pha. %
	Pha.	Non Pha.	Pha	Non Pha.	Pha	Non Pha.	
1	28.3	71.7	20	80	25	75	24.4
2	42.6	57.4	35.3	64.7	40.6	59.4	39.5
3	30.3	69.7	30	70	30.3	69.7	30.2
4	32	68	27.6	72.4	29.6	70.4	29.8
5	57	43	48.3	51.7	54.3	45.7	53
6	36.3	63.7	40.3	59.7	36	64	37.5
7	39	61	41.3	58.7	49.6	50.4	43.2
8	37.3	62.7	24.6	75.4	33	66	31.6
9	57.3	42.7	52.3	47.7	60.3	39.7	56.6
10	47.3	52.7	43.3	56.7	49.6	50.4	46.6
11	26.3	73.7	22.3	77.7	25	75	24.5
12	33.6	66.4	27.6	72.4	30.3	69.7	30.5
13	31	69	24.6	75.4	34	66	29.8
14	63.3	36.7	58.3	41.7	63	37	61.5
15	20.3	79.7	16.3	83.7	20.3	79.7	19
16	31.3	68.7	25.6	74.4	27.6	72.4	28
17	13.3	86.7	13.3	86.7	17	83	14.5
18	41.6	58.4	33.6	66.4	38.6	61.4	38
19	27	73	32.6	67.4	38.6	61.4	33
20	30.3	69.7	27.6	32.4	30.3	69.7	29.4
21	37.6	62.4	30	76	35.6	64.4	35
22	34	66	33	67	37.6	62.4	35
23	29.3	70.7	34.6	65.4	31.6	68.4	32
24	36.6	64.4	36.3	63.7	32	68	35
25	18.6	81.4	18.6	81.4	25.6	74.4	21
26	34.6	65.4	41.6	58.4	38	62	38
27	26.6	73.4	39.6	60.4	36	64	38
28	50	56	44	56	44	56	46
Mean SD±	**35.4 11.572**	**64.7 11.344**	**32.9 10.873**	**65.8 12.757**	**36 11.128**	**63.7 11.119**	**35% 10.829**

Appendix -5- (C)

Phagocytosis test for thalassemia patients in age between 13 -18 years

patients No.	Sample 1 (mean or three slides)		Sample 2 (mean or three slides)		Sample 3 (mean or three slides)		Mean of Pha.%
	Pha.	Non Pha.	Pha	Non Pha.	Pha	Non Pha.	
1	43.7	56.3	35	65	43	57	40.5
2	24	76	23.3	76.7	24.6	75.4	24
3	17.3	82.7	19.3	80.7	20	80	19
4	43.6	56.4	35	65	43	57	40.3
5	26	74	21	79	25	75	24
6	58	42	48.6	51.4	55.3	44.7	54
7	45.3	54.7	35	65	42.6	57.4	41
8	37.3	62.7	34.3	65.7	37.3	62.7	36.3
9	38	62	30.6	69.4	35.6	64.4	34.7
10	22.3	77.7	18.3	81.7	25	75	22
11	27	73	32.6	67.4	38.6	61.4	33
12	36	64	34.6	65.4	40.6	59.4	37
13	53	47	56.6	45.4	63.6	36.6	57.7
14	52.3	47.7	48	52	54.3	45.7	52
15	31	69	28	72	31	69	30
16	28	72	30.3	69.7	27	73	28.6
17	43	57	43.6	56.4	42.3	57.7	43
18	34.6	65.4	31.3	69.7	33	67	33
19	35.6	64.4	38.6	61.4	37	63	37
Mean SD±	**36.6 11.163**	**63.3 11.163**	**33.8 10.146**	**66.2 9.922**	**37.8 11.451**	**62 11.42636**	**36.1% 10.668**

Appendix -5- (D)

Phagocytosis test for thalassemia patients in age between 18-22 years

patients No.	Sample 1 (mean of three slides)		Sample 2 (mean of three slides)		Sample 3 (mean of three slides)		Mean of Pha. %
	Pha.	Non Pha.	Pha	Non Pha.	Pha	Non Pha.	
1	56	44	48	52	52	48	52
2	38.3	61.7	35.3	64.7	41	59	38.2
3	42.6	57.4	45	55	43	57	43.5
4	56	44	46.6	53.4	50.3	49.7	51
5	48	52	45.3	54.7	53.3	46.7	49
6	26.6	73.4	28.3	71.7	35	65	30
7	24.6	75.4	23.3	76.7	31.3	68.7	26
8	38.6	61.4	39.3	60.7	36	64	38
9	26.6	73.4	23.6	76.4	24.6	75.6	25
Mean SD±	**39.7 12.161**	**60.3 12.161**	**37 9.980**	**62.8 9.980**	**40.7 9.913**	**59.3 9.954**	**39.1% 10.491**

Appendix -6- (B)

Phagocytosis test of normal human with Phytic acid 0.15µg/µl

patients No.	Sample 1 (mean of three slides)		Sample 2 (mean of three slides)		Sample 3 (mean of three slides)		Mean Of Pha.
	Pha.	Non Pha.	Pha.	Non Pha.	Pha.	Non Pha.	
1	71	29	68.4	31.6	70.7	29.3	70
2	59.7	40.3	59	41	58.7	41.3	59
3	63.7	36.3	66.7	33.3	65	35	65
4	79	21	74.7	25.3	79	21	77
5	63.7	36.3	57.7	42.3	58.7	41.3	60
6	48	52	50	50	53	47	50.4
7	70.7	29.3	64.7	35.3	65.7	34.3	67
8	75	25	67.4	32.6	76.7	23.3	73
9	66.7	33.3	62.4	37.6	72	28	67
10	56	44	54.4	45.6	67.4	39	57
Mean SD±	**65.35 12.161**	**34.65 12.161**	**62.54 9.980**	**37.46 9.980**	**66.69 9.913**	**33.95 9.954**	**64.5% 10.491**

Appendix -3 - (B)

Lymphocyte transformation assay with using Phytic acid 0.05µg/µl

patients .No	With 0.05µg/µl Phytic acid									
	Sample 1 (mean ofthree slides)			Sample 2 (mean of three slides)			Sample 3 (mean of three slides)			Mean of Trans. Cells %
	Transformed Cells no	%	Nor. Cells No.	Transformed Cells No.	%	Nor. Cells no	Transformed Cells No.	%	Nor. Cells No.	
1	13	13	87	11.3	11.3	88.7	9	9	91	11.1
2	11	11	89	9.3	9.3	91.7	8	8	92	9.7
3	14	14	86	14	14	86	10.6	10.6	89.4	12.8
4	15	15	85	15.3	15.3	84.7	11.3	11.3	88.7	13.8
5	17	17	83	17.3	17.3	82.3	17	17	83	17.1
Mean SD±	**١٤ 2.253**	**14% 2.253**	**86 2.253**	**13.4 2.263**	**13.4% 2.263**	**86.6 2.263**	**11.1 2.825**	**11.1% 2.825**	**89.9 2.825**	**12.9% 2.825**

Appendix -3 - (C)

Lymphocyte transformation assay with using phytic acid 0.1µg/µl

patients .No	With 0.1µg/µl Phytic acid									
	Sample 1 (mean of three slidea)			Sample 2 (mean of three slides)			Sample 3 (mean of three slides)			Mean of Trans. Cells %
	Transformed Cells No.	%	Nor. Cells No.	Transformed Cells No.	%	Nor. Cells No.	Transformed Cells No.	%	Nor. Cells No.	
1	18	18	82	10	10	90	13	13	87	13.6
2	10	10	90	11	11	89	17	17	83	14.6
3	22	22	78	17	17	83	10	10	90	16.3
4	14	14	86	12	12	88	15.5	15.5	٨٤,٥	13.8
5	12	12	88	10	10	90	15.6	15.6	٨٤,٤	12.5
Mean SD±	**15.2 4.816**	**15.2% 4.816**	**84.8 4.816**	**12 2.915**	**12 % 2.915**	**88 2.915**	**14.2 2.764**	**14.2 % 2.764**	**85.8 2.764**	**14.1% 1.411**

Appendix -5- (A)

Phagocytosis test of thalassemia patients in age between3 – 7 year

No.	Sample 1 (mean of three slides)		Sample 2 (mean of three slides)		Sample 3 (mean of three slides)		Mean of Pha. %
	Pha.	Non Pha.	Pha	Non Pha.	Pha	Non Pha.	
1	40.6	59.4	35.3	64.7	36.3	63.7	37.5
2	53	47	59.3	40.7	58	42	57
3	16.6	83.4	11.6	88.4	9	91	12.4
4	35.3	64.7	29.3	70.7	32.6	71.4	32.5
5	19	81	18.3	81.7	21	79	19.5
6	41.3	58.7	51.3	49.7	50.6	49.4	48
7	43	57	46	54	50.6	49.4	47
8	62.6	37.4	54.3	43.7	62	38	59.7
9	38	62	34.6	63.4	32.6	61.4	35
10	44.3	53.7	45.3	54.7	49.3	50.7	46.3
11	37	63	31	79	37	63	35
12	45	55	47.3	52.7	48.6	51.4	47
13	17.6	82.4	14.6	85.4	17	83	16.4
14	43.3	52.7	40.5	59.4	42	58	42
Mean SD±	**38.3 13.102**	**61.2 13.338**	**37 14.858**	**63.4 15.511**	**39 15.610**	**60.8 15.673**	**38.2% 14.378**

Appendix-6-(C)

Phagocytosisi assay using Phytic acid 0.05µg/µl

No.	Sample 1 (mean of three slides)		Sample 2 (mean of three slides)		Sample 3 (mean of three slides)		Mean Of Pha.
	Pha.	Non Pha.	Pha.	Non Pha.	Pha.	Non Pha.	
1	71	29	68.4	31.6	70.7	29.3	70
2	59.7	40.3	59	41	58.7	41.3	59
3	63.7	36.3	66.7	33.3	65	35	65
4	79	21	74.7	25.3	79	21	77
5	63.7	36.3	57.7	42.3	58.7	41.3	60
Mean SD±	**65.35 12.161**	**34.65 12.161**	**62.54 9.980**	**37.46 9.980**	**66.69 9.913**	**33.95 9.954**	**64.5% 10.491**

Appendix-6-(D)

Phagocytosis assay using 0.1µg/µl phytic acid

No.	Sample 1 (mean of three slides)		Sample 2 (mean of three slides)		Sample 3 (mean of three slides)		Mean Of Pha.
	Pha.	Non Pha.	Pha.	Non Pha.	Pha.	Non Pha.	
1	48	52	50	50	53	47	50.4
2	70.7	29.3	64.7	35.3	65.7	34.3	67
3	75	25	67.4	32.6	76.7	23.3	73
4	66.7	33.3	62.4	37.6	72	28	67
5	56	44	54.4	45.6	67.4	39	57
Mean SD±	**65.35 12.161**	**34.65 12.161**	**62.54 9.980**	**37.46 9.980**	**66.69 9.913**	**33.95 9.954**	**65.3% 10.491**

Phagocytosis test for normal control of human

No.	Sample 1 (mean of three slides)		Sample 2 (mean of three slides)		Sample 3 (mean of three slides)		Mean of Pha.
	Pha.	Non Pha.	Pha	Non Pha.	Pha	Non Pha.	
1	69.7	29.3	74.4	25.6	77	23	80
2	68	32	62.4	37.6	67.7	32.3	66
3	62.4	27.6	70.4	29.6	73	27	72
4	81.4	18.6	81	19	87	13	83
5	64.7	35.3	60	40	67.7	32.3	64
6	62.4	37.6	54.7	45.3	58.3	41.3	58
7	73.7	26.3	70.7	29.3	77.4	22.6	74
8	81	19	76.7	23.3	79.7	20.3	79
9	70.4	29.6	75.7	24.3	73	27	73
10	59.4	39.6	62.4	37.6	66.7	33.3	63
Mean SD±	**69.3 9.235**	**29.4 9.235**	**68.8 7.358**	**31 7.358**	**72.7 8.269**	**27.2 8.454**	**71.2% 8.007**

Lymphocyte transformation of Control without Phytic acid

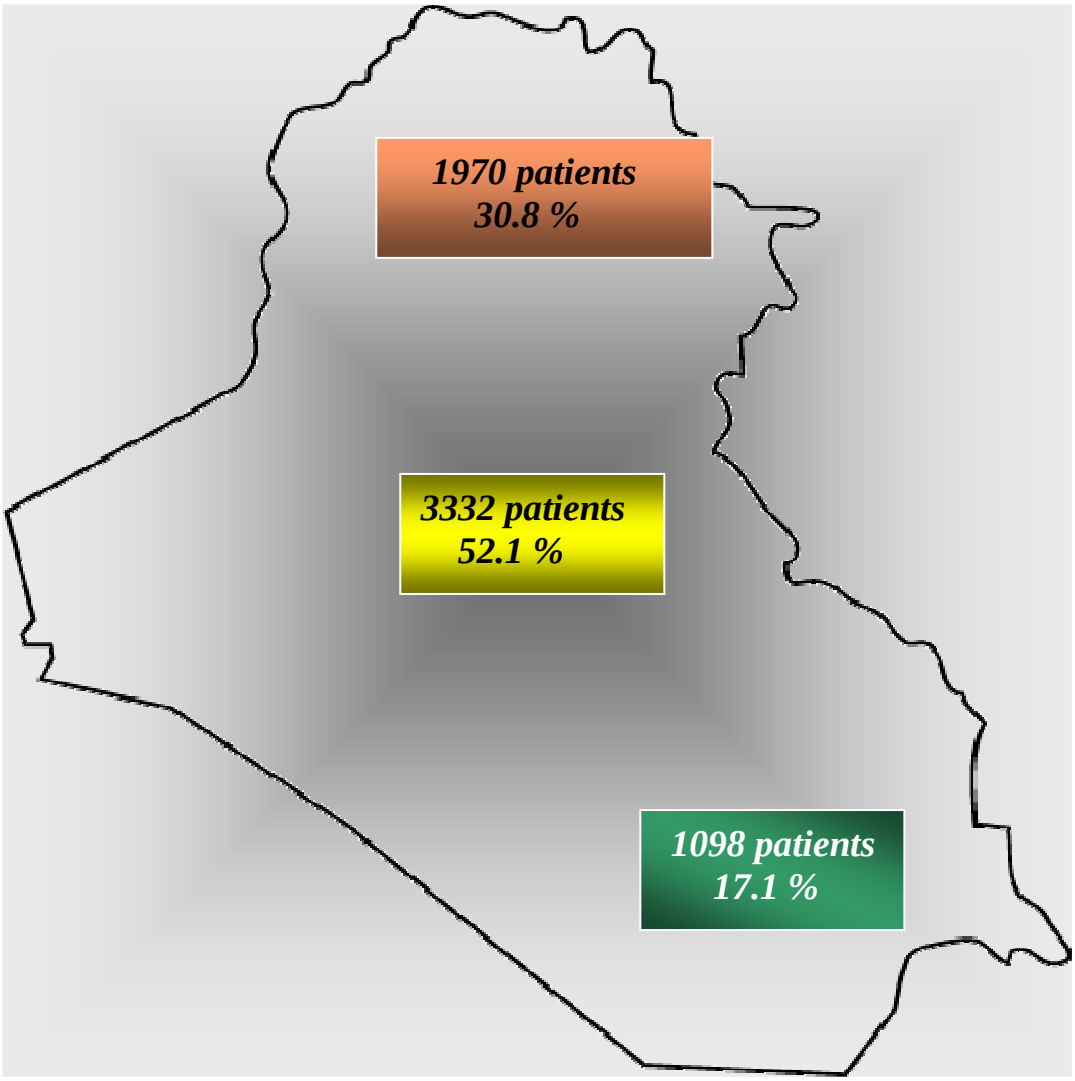
.No	**Without Phytic acid**									
	Sample 1 (mean or three slides)			**Sample 2 (mean of three slides)**			**Sample 3 (mean of three slides)**			**Mean of Trans. Cells %**
	Transformed Cells	%	Nor. Cells	Transformed Cells	%	Nor. Cells	Transformed Cells	%	Nor. Cells	
1	12.6	12.6	87.4	9	9	91	11.3	11.3	88.7	11
2	10.6	10.6	89.4	8	8	92	9.3	9.3	90.7	9
3	13	13	87	10.6	10.6	89.4	14	14	86	12.5
4	15.3	15.3	84.7	11.3	11.3	88.7	15.3	15.3	84.7	14
5	16.6	16.6	83.4	17	17	83	17.3	17.3	82.7	17
6	13	13	87	9.6	9.6	90.4	13.3	13.3	86.7	12
7	14	14	86	11	11	89	15.6	15.6	84.4	13.3
8	22	22	78	17.6	17.6	82.4	17.3	17.3	82.7	19
9	9.3	9.3	90.7	11.6	11.6	88.4	9	9	91	10
10	17	17	83	12.3	12.3	87.7	15.6	15.6	84.4	15
Mean SD±	**14.3 3.619**	**14.3% 3.619**	**85.6 3.619**	**11.8 3.170**	**11.8% 3.170**	**88.2 3.170**	**13.8 3.038**	**13.8% 3.038**	**86.2 3.038**	**13.2% 3.101**

Appendix -7- (A)

Thalassemia distribution in Iraq states (2005) According to Ministry of health / thalassemia program).

The directorate	Major thalassemia	Intermediate thalassemia	Minor thalassemia	Others HbD1 and HbC1 and alpha thalassemia	Total
Baghdad	1726	497	29	59	2457
Basra	454	-	-	12	1192
Ninava	631	-	-	-	631
Babel	243	74	4	15	357
Kirkuk	140	93	72	6	314
Dialah	188	36	5	1	237
Wasit	123	38	-	14	180
Theqar	139	16	6	-	169
Anbar	73	-	-	-	73
Diawanyia	103	26	-	2	136
Miasan	181	-	-	-	182
Kerbalaa	154	-	-	1	155
Muthana	73	-	-	-	73
Salah aldeen	34	8	10	-	52
Arbel	414	5	-	-	424
Sulyeimanya	450	-	-	-	450
Diahok	147	1	4	7	230
Najaf	86	-	-	-	86
Total	5359 (72.4%)	794 (10.8%)	130 (1.7%)	117 (1.6%)	7398

Appandix -7- (B)



Geographical distribution of thalassemia among Iraqi populations (2005) ***According to Ministry of health / thalassemia program).***

Appendix -7-(C)

According to (Ministry of health / thalassemia program , 2005)

App.7-C-1*: Marital relationship between the parents*

Marital relationship between the parents	number	%
First and second cousin marriage	4058	70.7
Un related parents	1684	29.3
Total	5742	100

App.7-C- 2:*Distribution according to thalassemia patients` age*

< 1 year	1-5 year	6-15 year	16-25 year	>25 year	Total
187 (3.1%)	1384 (22.9%)	3064 (50.7%)	990 (16.4%)	412 (6.9%)	6038 (100%)

App.7-C- 3 : Study *of the social state in Iraqi patient*

App.7-C- 3-1 - Number of affected persons in the family

Affected persons	1 person	2 persons	3 persons	4 persons	5 persons	Total
No.	4647	1124	190	61	4	6026
%	77.12%	18.64%	3.16	1.02	0.06	100

App.7-C-3-*2 - The educational status according to social standard*

Educational status	<5 years	Primary school	Secondary school	High school	Out of school	total
Urban area	1071 38.2%	830 29.6%	783 27.95%	26 0.92%	93 3.33%	2803
Rural area	243 27.2%	257 28.84%	68 7.63%	3 0.33%	320 35.9%	891

App.7-C-3- ***3 - The martial statue of the patients***

The marital status	Married	Unmarried	Total
Number %	151 2.6	5610 97.4	5761 100

App.7-C- 4*: Management and follow up of the patients*

App.7-C- 4 -1 -*Regularities of visits to thalassemia centers*

Regulation of visite	Regular	Irregular	Total
Urbon areas	2257 (80.3%)	552 (19.7%)	2809 100%
Rural areas	746 (75.1%)	247 (24.9%)	993 100%
Total	3003 (79%)	799 (21%)	3802 100%

App.7-C- 4 - *2 - Mean hemoglobin for the patients according to specified areas.*

Men Hb for the patient	<=79g/l	80-99 g/l	>=100g/l	Total
Urban areas	1213 50.1%	998 41.2%	212 8.7%	2423
Rural areas	612 66.1%	264 28.5%	50 5.4%	926
Total	1825 54.4	1262 37.6%	262 7.8%	3349

App.7-C- 4 - 3 - *Frequent of blood transfusion / month*

Frequency / month	once	twice	Three X	Four X	total
No.	2990	972	323	116	4401
%	67.9	22.1	7.3	2.7	100

App.7-C- 4 - 4 - Operative history of the patients

Type of the operation	Splenectomy	Cholecydectomy	Total
No.	861	54	5096
%	16.9	1.1	100

App.7-C- 5 - The *complications*

Blood borne viral infection	Delayed puberty	Hypocalcemia	Cardiomyopathy	Osteoparosis
1244 23.14%	489 9.1%	395 7.35%	318 5.9%	132 2.46%

D M	Others	Hypothyroidism	Total no. of analyzed patients
111 2%	105 2%	13 0.24%	5376

App.7-C- 5 - Viral *screening*

Viral screening	HBs Ag	HCV Ab	HIV	Total no. of patients
No. %	1221 0.5	23 24	0 0	5707

App.7-C- 5 -Vaccination

	HBS		Meningococcal		Pneumococcal		Total no. of analyzed patients
	Regular	Irregular	Regular	Irregular	Regular	Irregular	
No.	1799 67.5%	868 32.5%	1172 84.7%	212 15.3%	1300 70.2%	553 29.8%	4132 100%
Total	2667 64.5%		1384 33.5%		1853 44.8%		

Committee certification

We the examining committee, certify that we have read this thesis and examined the student in its contents and that, according to our opinion, is accepted as a thesis for the degree of Master of Science in Biotechnology.

Signature:
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Supervision certificate

***We** certify that this thesis was prepared under our supervision in Al-Nahrain University / College of Science as a partial requirement for the degree of Master of Science in Biotechnology.*

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In review of the available recommendation I forward the thesis for debate by the examining committee.

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2:1: Thalassemia

Thalassemia describing a group of inherited disorders characterized by reduction or absence of hemoglobin amounts (Bojanawski, 2002).

This is lead to anemia and inability of the body to deliver needed oxygen to maintain normal body function. The genetic characteristic of disease was fully appreciated in 1940s following eradication of malaria in Cyprus. The disease found mostly in area sorrowed Mediterranean sea, Africa, Malaysia, southeast Asia, southern China, southeast China (Hollestein, 2005).

It is called Mediterranean anemia or Cooley's anemia because it's meditercesty first described homozygous state firstly by Cooley and Lee in 1925 (Rice, 1996).

Thalassemia is among the most common genetic disorder world wide, 4.83% of the world populations carrying globins variants, including 1.67% of them are heterozygous for homozygous alpha and beta thalassemia (Rund and Rachmilewitz, 2005).

World wide, 15 million people have clinically apparent thalassemia disorder. Both sexes are equally affected with thalassemia (Yaish , 2005).

Galacteros (2002) in his research was estimated that 100,000 babies a year born with sever form of thalassemia, about 10000 in India alone. Geographically thalassemia belt include the Mediterranean which is make it most common in Africa, Greek, Italian, Middle Eastern and southern Asian population. also passing through west in direction andin other direction the central Asian countries like turkey, Iran, Afghanistan on to Pakistan and India passing to the south East Asian countries like Indonesia, Burma and Thailand, Vietnam and Cambodia..

Some studies have focused on the prevalence of thalassemia gene in some Middle Esten countries ,but none were from Iraq , in spite of evidence suggesting that thalassemia are not uncommon among Iraqies (Yahya *et.al*,1996)

Genetic counseling is important for families that carry the thalassemia gene because some one with the trait has a 25 %(1 in 4) chance of having a child with the disease if his or her partner also carries the trait. People with different form of thalassemia show wide range of illness from the disease ,some people only have mild anemia with little or no effects, where as others require frequent blood transfusions (Greenbery,2005).

In patient with beta thalassemia major a high incidence of cardiac involvement still exists despite improved prognosis with chelating therapy. Development of sever right heart failure is commune and has been attributed to pulmonary hypertension secondary it lung homochromatic.

The possibility of direct right ventricular myocardial involvement in the absence of significant pulmonary hypertension has not been adequately investigated (Hahalis *et al*, 2001).

2:2: Hemoglobin

Hemoglobin is a protein carried by red blood cells, picked up oxygen in the lungs and delivers it to the peripheral tissue to maintain viability of cells (Bridges, 2002).

Hemoglobin actually gives the red cell its color, oxygenated blood brighter than the depleted blood. Fresh hemoglobin produced in the bone marrow. Its develops a hunger for oxygen molecules , where blood is carried in to the lung the iron of hemoglobin attract available oxygen ,then travel to the entire blood stream releasing oxygen to muscles and organs (Pollick , 2006).

2:2:1: Hemoglobin structure

Globulin made from two alphas (α) and two other beta (β) protein chain (Figure 2 – 1).In infant because fetus obtain their oxygenated blood from their mother and not their own lungs ,two subset they had, alpha and gamma

globins with several nitrogen atoms and one iron atom (Rice, 1996). Pollick ,2006)

The normal human adult hemoglobin Hb A (figure 2 -2) oxygenates in solution or insider blood cells is cooperative. i.e.: the binding of the first oxygen legend to Hb subset, enhance the binding of subsequent oxygen molecules to the remaining sub units (Lukin *et. al*, 2002)

Like all other protein the genetic code of them exist in the DNA and it's identical in all people consist from four genes code for alpha protein and two other genes code for the beta chain. The **α** and **β** chain are made in precisely equal amounts, despite the differing number of genes. The protein chains join in developing red blood cells and remain together for the life of the red cell (Bridges, 2002).

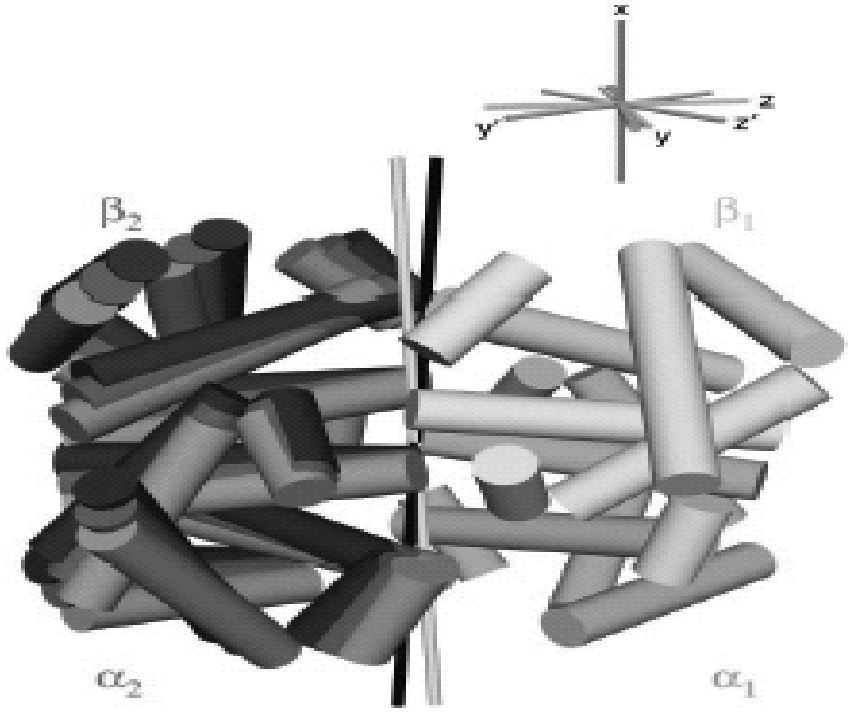

Figure (2 - 1): Quarterly structure of hemoglobin (Lukin *et.al,* 2002).

α 1 and α 2 represent the alpha globin chains

β 1 and β 2 represent the Beta globin chains .

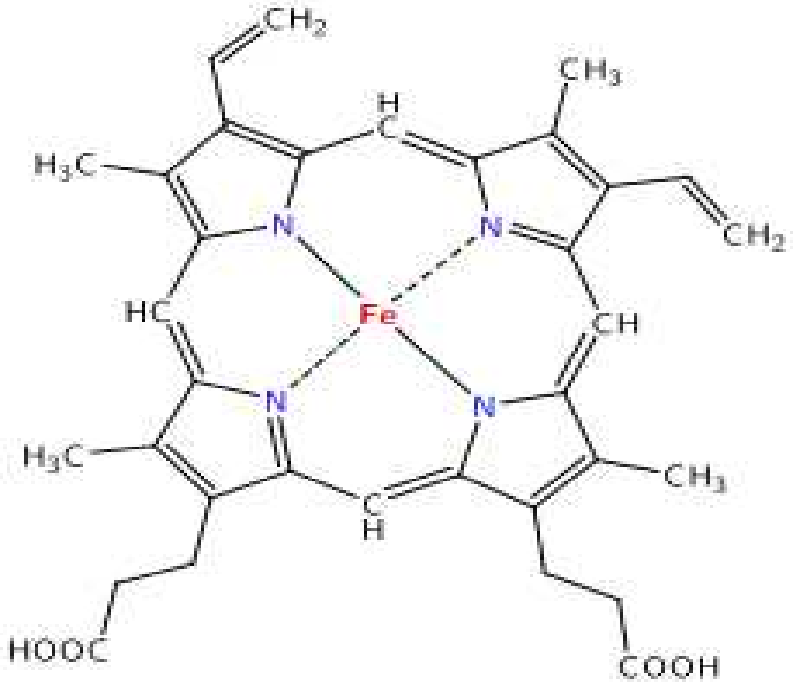

Figure (2–2): Chemical structure of hemoglobin (Jakubowski, 2006).

2:3: Thalassemia disease

In thalassemia there is genetic failure in the production of globin gene (Rice, 1996). This failure caused by alteration in the genes because of mutation.

Since genes are inherited the abnormal hemoglobin gene will pass to the children, the children will produce defected hemoglobin identical to parents (Bridges, 2002).

Hollenstein (2005) estimate that if the person inherits one gene from one parents, he will be carrier to disease but not have symptoms and if inherited from two parents the disease will develop.The defect in globins cause very low level of hemoglobin or not at all, in the red blood cell which represent the carrier of

oxygen to body organs which lead to decrease production and increase destruction of red blood cell and increase iron load .

Cunningham *et .al*, (2000) show that blood transfusion therapy is used to maintain nearly normal hemoglobin level and partially suppress the increased but ineffective erythropoiesis.

Multiple blood transfusions associated with alloimmunization and erythrocyte autoimmune, risk of exposure to infectious pathogens, immune deficiency, splenectomy and heart failure (singer *et.al*, 2000).

Hendricks, L.K. and Kutlar (2003) found that many patients and their families find it difficult to sustain compliance with chronic treatment regimens for other life treating disease that arise during childhood or adolescence, such as bone marrow or renal transportation. Hyperparathyroidism (HPT) secondary to siderosis in thalassemia patients was first described by Gabriele in 1971.It was later detected in more patients and more reports appeared ; it is now a well-recognized complication of blood transfusion therapy , secondary to iron deposition in paranthyroid glands. It was possibly more common in patients born or treated before the era of intensive chelating therapy. It has also been documented that asymptomatic hypocalcemia is much more common, and can be missed for some time unless specifically looked for (Aleem *et . al*, 2000).

Early trails of allogenic bone marrow transplantation (BMT) of homozygous beta thalassemia represent a kind of treatment to this patient (Lucarelli *et.al* , 1999).

Douglas (2004) shown that in mean time a continuing challenge in thalassemia research is to find a new way to reduce the disease complication by investigation naturally present phenotype of beta thalassemia , like increase fetal gamma globins chain or decrease synthesis of alpha globins or beta globins .

2:4: Thalassemia types

Thalassemia divided in to two main types and these types divided to classes depending on the type of protein defected (Bojanowski , 2002).

2:4: Beta thalassemia

The most common known type of thalassemia and it is also called Cooley's anemia, It is characterized by a genetic deficiency in the synthesis of beta-globins chains (Takeshita, 2005) .This type lead to accumulation of unpaired alpha-globins chain in erythrocyte precursors and RBC ,that alter cell membrane function , result in early cell destruction and ineffective erythropoiesis of sever hemolytic anemia (Bohl *et.al*,2000).

As Rice (1996) show that the two gene controlled beta chain located on chromosome 11. The homozygous state, beta thalassemia major cause sever transfusion-dependant anemia. In heterozygous state the beta thalassemia minor cause mild to moderate anemia.

I: Beta thalassemia major:

It's a homozygous state because of both beta globins genes are mutated and the production of β –globins chain is severely impaired (Takeshita, 2005).

Rice (1996) had shown in his research that these patients are well at birth but develop a life treating anemia by one or two months. They must support with blood transfusion which result in iron over load unless treated, patients will die.

Hollestien (2005)show symptoms within first tow years of life , they become pale listless and have poor appetite , they grow slowly and often develop jaundice .

II: Beta thalassemia intermediate

Represent a moderate anemia .its required target or occasional transfusion and iron over load may be severing (Galacteros, 2000).

Thalassemia intermediate is a clinical term used to describe patients with phenotypes that are more severe than transfusion dependent thalassemia major (Camaschella and Capellini , 1995).

Miller (2005) in his research mentions that it's diagnosed in the first year of a child's life. Doctors may be prompted to test for it when a child has chronic anemia or a family history of the condition, the disorder can be successfully treated and managed.

III: Beta thalassemia minor

This is the most common of the thalassemia .It is called beta thalassemia trait or heterozygous (carrier - type).one of the beta - globins gene is defective. The effect can be a complete absence of the b-globins protein or reduce synthesis of the protein. The genetic defect either missense or nonsense mutation in the b-globins gene (Takeshita, 2005).

The alpha chain combine with the available β-chain resulting in decreased level of hemoglobin A .there ,still remain excess alpha chain and this stimulate the production of delta chain.

Alpha and delta chain combine to form increase amount of hemoglobin A2. the excess of alpha chain switch off gamma chain production and this lead to become normal in adult (Rice ,1996).

2:5: Alpha thalassemia

The most prevalent of all thalassemia the deficient or absent production of alpha – globins synthesis (Hendricks and Kutlar, 2003).

There are four genes coding for alpha –chain production, these gene are located on chromosome 16 (Douglas,2004).

Hendricks and Kutlar (2003) show that intracellular precipitation of unmatched β- chains from inclusion bodies, cause damage in red blood cell precursor in the marrow and ineffective erythropoiesis.

Alpha thalassemia can be a trait or disease. The trait condition does not cause any health problem, in other sight anemia that is not correctable with iron supplementation (Bojanowski, 2004).

Bojanowski (2002) was show that alpha thalassemias have two main types, hemoglobin H disease and alpha- thalassemia major. In hemoglobin H disease events of hemolytic anemia caused by the rapid break down of the red blood cells .its thought to be triggered by various environmental causes such as infection and exposure to certain chemicals.

Alpha thalassemia major is more sever disease that most often lead to miscarriage or still birth in affected fetus. Skeletal changes due to expanded erythrocytes in the marrow affect one third of patients .most affected babies don't survive to be born or die shortly after birth .these babies most often needs special treatment before birth in order to survive .After birth individual with alpha-thalassemia are depend on blood transfusion every few weeks (Hendricks and Kutlar , 2003).

2:6: Symptoms of thalassemia

The hereditary nature of the disease, physical deformities, growth retardation, puberty and demands of regular blood transfusion, iron over load and chelating therapy are example of challenges faced by patients (Bush *et .al,* 1998). The symptoms range from mild anemia to moderate or sever yellowish of skin, fatigue, listlessness and reduce appetite (Hollenstein, 2005). Takeshita (2005) mentioned that patient also have skeletal abnormalities observed in the patient include an expanded bone cortex .Bone changes also can be observed in the long bones vertebrae and pelvis .Heart is a major organ that is

affected by iron over load and anemia .enlargement of spleen ,liver problems and gallstone also founded.

2:7: Complication of thalassemia

2:7:1: Iron overload

Regular red blood cell transfusion eliminates the complication of anemia and compendnsatory bone marrow expansion, permit normal development through out childhood and extend survival (Oliviri and Brittenham, 1997).

Rund and Rachmilewtiz (2005) display the cause of the accumulation as the break down of transfused erythrocytes is retained iron accumulated in body and deposed in visceral organ (manly in the heart ,liver and endocrine glands)cause tissue damage , organ dysfunction and failure. These phenomena called iron over load case.

Olivieri et .al(1995) show that iron over load may be prevented or treated with chelating agents to completing with iron and promoting excretion outside the body .

2:7:2: Opportunistic bacteria

The major complication of blood transfusion are those related to transfusion of infectious agents while the underlying cause of increased susceptibility to bacterial infection is not completely understood evidence suggests that iron overload alter the chemostatic and phagocytic properties of neutrophil , there by reducing their ability to kill invading pathogens (Hoen,1999).

Olivieri and Britenham (1997) show that iron also induce hepatic damage is exacerbated by a second complication of transfusion infection, with hepatitis C virus , the most frequent cause of hepatitis in thalassemia children.

At few years ago 25% of transfused patient more exposed to hepatitis B (Yaish, 2005).

Bacterial infection is the second commonest cause of death in thalassemia major .two main factors predispose to bacterial infection in thalassemia syndromes. These are iron over load and removal of spleen. splenectomy predisposes to infection with capsulated organisms such as *pneumococcuse* , *hemphilus* and *meningococcus* (Porter , 1996).

Peng *et. al*,(2000) study 39 patient with b-thalassemia who received frequent blood transfusion and found among these patient , 13 developed 22 opisodes of infection and bacteremia accounted for 16/22 of all infection .

Three patient develop meningitis , two patients had liver abscesses , three patients had soft tissue infection and one patient had lobar pneumonia and large number of infected with G- ve bacteria as iron over load develop free irons found in plasma .Bacterial make low molecular weight , molecules enable them to a quire iron necessary for there growth, bacteria unable to make these molecules a quire iron from other species or may liver intra – cellular .one of the most bacteria is *Yersinia* species which affected a bout 10 %of thalassemia patient (Porter , 1996).

Plasmodiums species is parasitizing red blood cell and proliferate at their cost are inherited by these abnormal red blood cells.

2:7:3: Immunological defect

The human immune system is truly a amazing constellation of response to attack the antigen (bacteria, virus, parasite and cancer). There are two specific defense mechanism, humeral and cell-mediated immunity (Carter, 2001).

Iron is an essential micronutrient for immune response and specific effects have been suggesting that iron may be a cofactor in the regulation of immune function (Cunningham, 2000).

Iron have immunoregulatory properties , any shifting in iron , increase or deficiency may produce sever deleterious physiological effects , such as decreased antibody –mediated and mitogen stimulated phagocytosis by monocytes and macrophages, alteration in T-lymphocyte subsets and modification of lymphocyte distribution in different compartment of the immune system (Walker .and Walker,2000) .

Iron has specific effect on both adaptive and the innate immune system. Iron may favor the growth of intracellular pathogens by reducing phagocytic function. It may promote the growth of hepatitis C virus (Cumingham *et. al* , 2000).

Shaiegan *et. al, (*2002) found a defect observed in patient with thalassemia make them susceptible to different kind of infection, one of this is the immunosuppressive effect of blood transfusion, also as abnormalities in humeral immunity such defect in alterative complement pathway, immunological level and in cell mediated immunity such as decreased natural killer cell activity, defect neutrophil function , decreased T-(CD4)/ T-(CD8) ratio and cell subset abnormalities .

This abnormality include agreater number and activity of suppressor T-cells , reduce proliferation capacity , number and level of (CD4) leading to decreased CD4/CD8 ratio as well aw defective activity of natural killer cell.

The B-lymphocytes are characterized by increased number, high activities and impaired differentiation. Neutrophil and macrophages are associated with defective chemotaxis and phagocytosis (Dimitrios , 2003).

The development of anti RBC antibodies (alloantibodies /outoantibodies) can complicate transfusion therapy; some alloantibodies cause transfusion reaction and limit the availability of further safe transfusion (Singer, 2000).

2:7:4: Heart failure

Most death in patient with thalassemia are due to cardiac failure involvement. The complication range from constrictive pericarditis to heart failure and arrhythmias (Yaish, 2005).

Congestive heart failure is the main cause of death in patient with blood transfusion (Dimitrios, 1995).

Jessup and Manno (1998) show that iron over load and its deposition in the tissue is the main cause of heart failure, chelating therapy clearly benefits many patients, Cardiac dysfunction and enhances survival but is not uniformly successful.

Unbound iron may generate reactive harmful oxygen metabolites and toxicity. Chelating therapy has imported proved prognosis in b – thalassemia by reducing the incidence of heart failure and by reversing cardiomyopathy (Hahalis *et. al*, 2005).

2:7:5: Splenectomy

In patient with thalassemia in whom yearly transfusion requirement exceed 200 ml packed cell per kg blood weight, splenectomy should significantly diminish RBC requirements and iron accumulations (Olivieri and Brittenham, 1995).

Coovking (2003) explain that spleen may be removed if it enlarged and painful. Its not do on people under 11 year old. Surgically removing the spleen may help reduce the number of blood transfusion that is needed.

2:7:6: Other complications

There is many other complication associated with thalassemia disease, some of these are:-

2:7:6:1: liver disease

Is a common cause of death after age 15 years in patient with thalassemia iron-induce hepatic damage exacerbated by a second complication of transfusion infection with hepatitis C virus, the most frequent cause of hepatitis in thalassemia children (Olivieri and Brittenham , 1997).

Pardit *et .al* (1998) present the risk of transfusion – transmitted hepatitis to those who depend on transfusion and still develop liver disease due to viral infection.

Also regular blood transfusion for some time develop liver enlargement due to swelling of the phagocytic and psrenchymal cells from the deposition of hemosider (Yaish, 2005).

2:7:6:2: Endocrine complication

Impairment of growth and endocrineopathies, particularly hypogonadism are commune feature of thalassemia (Rund and Rachmilewitz , 2005).

Yaish (2005) show other feature frequently exist with thalassemia like diabetes mellitus,50%or more exhibits clinical or sub clinical diabetes, this due to defective pancreatic production of insulin .

2:8: Clinical diagnosis of thalassemia

Thalassemia is always inherited anemia ,passed from parents to children through their genes .many families have thalassemia carriers ,but the trait often goes undiagnosed because the trait produce few or no symptom ,there the thalassemia is not diagnosed until a baby is born with the disease .

Because there are different kind of thalassemia and cause different kinds of health problems resulting from this orders (Miller, 2005) this is the most common ways to detect thalassemia :

1- Study the hemoglobin level if its out of reference range , reticulocyte count , studding blood film ,complete blood cell count, mean corpuscular volume and study Hb electrophoresis ,these compared with the normal range (Hendricks and Kutlar,2003).

2- The iron level testing is a very important because in thalassemia patient there is an increase in iron level compare to normal human (Hollenstein,2005).

3- Molecular diagnostic test can be determined if a mutation is present in suggestive family history.. Recent technology play an important role in the diagnosis like DNA recommendation , gene mapping and polymerase chain reaction endoneucleases (Takeshita , 2005).

2:9: Evaluation of immunological competent of thalassemia patient:-

Two important tests used to study the activity of the immune system.

First lymphocyte transformation assay examines the ability of lymphocytes (T and B) to respond to polyclonal stimuli (PHA, CON A, PWM, IL-2, and Anti-CD3). A normal response suggests that the patient's T and B lymphocytes have a normal capacity to proliferate upon encountering an appropriate stimulus. For a global assessment of lymphocyte function, order 'Lymphocyte Transformation, Spontaneous' and 'Lymphocyte Transformation, Mitogen' or 'Lymphocyte

Transformation . second think is the phagocytosis study the ability of phagocytic cell to ingest antigen (Kumaratilaks and Ferrante,2000).

2:91: Cell- mediated immune response

Specific acquired immunity against infectious disease may be mediated by antibodies and/or T-lymphocytes (Linnemeyer,, 1993).

Lymphocytes are small cells about 8 – 10 µm in diameter, with scanty cytoplasm and spherical nucleus occupying almost the entire cell. The nucleus has condensed chromatin that is strongly basophilic on routine histological sections (Talaro and Talaro, 1996).

Nowell,(1980)was the first scientist who used the lymphocyte transformation assay , wich could be defined as, "apecific chain of morphological and biological changes that occur in the lymphocytes when activated by specific antigen or nonspecific mitogen ".

Lymphocyte transfusion tests to assess the ability of the lymphocyte to proliferate, to recognize and respond to antigens. Two types of lymphocyte transformation test, mitogens assay and antigen assay .The mitogen assay performed using nonspecific plant lectins, evaluate the mitotic response of T and B lymphocytes to foreign antigen (Jacobs *et.al*, 1996).

T-lymphocytes are blastogenic response to phytohemagglutinin PHA and Concanavalin (coA) where Pokeweed PWM is to T and B-lymphocyte and lipopolysaccharid (LPS) actvate only B- cells.

This difference referred to marker selectivity for T-lymphocyte compound to B-lymphocyte (Peterson *et.al*, 1981).

Activation occur through four phases , that begin with resting or silent phase (G0),,the first growth phase (G1), protein and nucleic synthesis phase (S- phase) , and finally the second growth phase (G2). The resting phase begin when the specific antigen or mitogen bind through specific ligand with a receptor on the surface of the lymphocyte cell (lymphocyte), which lead to the activation of the

enzyme that responsible for the activity of the cell such as Cyclic – Guanidin Monophosphate , (CGMP) and whose percentage increased during the first minute of activation and thus lead to the appearance of the morphological changes as a result of the transformation of rest , small cells (lymphocytes) to blast cell called (lymphoblast) where there is an increase in the size of lymphatic cells, increase in the number of vacuoles , and the nuclei become more visible inside the cell as a result of the accumulation of the nucleus's proteins(Stites,1994) .

On the other hand, biological changes involve increase in the cell membrane permeability , increasing in the percentage of penetration of positive ions such as K+ to the inside of the cells , at this point the cells inter the (G1) phase, in wich there is a continuous penetration of both glucose and Ca+2 ions, that are essential for the synthesis of other enzymes in the nucleus , this phase characterized by the production of proteins that are essential for the cell, ribose nucleic acid (RNA) and lymphokinase , this phase occur during 12 – 24 hours . After that the cell inter the (S- phase); which characterized by the synthesis of Deoxiribose nucleic acid (DNA) and duplication of the cellular chromatin , and it is reach it's maximum point during 48 hours , and finally the activated cells inter (G2- phase) after 72 hours of activation with the specific antigen or mitogen (Beahr et.al,2000) .

The lymphocyte transformation percentage can be measured either by :

1. Determination of the percentage of formation of lymphoblast cells or determination of (mitotic index).
2. Using methods for the determination of the cellular uptake of thymidine – H3 enzymatic activity (fluoremetric assay) (Dotsik and Sanderson, 1987).

2:9:2: Humeral immunity in thalassemia patients

Abnormalities in humeral immunity such as defects in alternative complement pathways and abnormal immunoglobulin levels (Shaiegan , 2002).

Motalebnejad *et.al* (2002) in there study on the immunoglobulin thalassemia patients of gingivitis and the rat of salivary immounoglobulin of thalassemia patients, that T- cells affect on the activity of B cells. With regard to reduction of activity of T-cells lymphocyte (CD4) in thalassemia and the effect of these cells on promotion of B- lymphocyte function, B- cells can not response to gingivitis by synthesis of immunoglobulin. Therefore there was no significant difference between the rate of IgA, IgG and IgM and ,there was not relationship between severity of gingivitis and the rate of salivary immunoglobulin.

2:9:3: Non specific immune response

Phagocytosis one of innate immunity refers to antigen nonspecific defense mechanisms that host uses immediately or within several hours after exposure to antigen and removing free microorganism in the blood and tissues fluid (Kaiser, 2002).

There are two types of phagocytosis include:

Nonimmune phagocytosis ,in which macrophages are able to phagocytose forign particle matter , microorganisms and the debris of cellular injury directly , without evoking the immune response. However, microbial phagocytosis killing by macrophages are greatly facillated by the presence of specific immunoglobulins, complement and lymphokines.

Immune phagocytosis , macrophage have surface receptors for C3b and for Fc fragment of immunoglobulin . Particle that coated with immunoglobulin or complement is phagocytiosed more readly than naked particles(Tailor and Parakama,1995).

Phagocytosis describing the engulfment and destruction of extra cellular derived material by phagocytic cells, such as macrophages and neutrophil .five steps in the phagocytosis process, attachment of bacteria by Pseudopodia, Ingestion of the bacterium forming a phagosome which more toward the

lysosome and fusion to phagosome releasing lysosomal enzymes to it, digest the ingestited material and release from the cell (Sears, 1997).

Cantinieaux *et.al*, 1990 study the effect of iron in serum of thalassemia patient on the ability of phagocytic cells and compare it with its ability when using chelating therapy, which increasing its defense.

2:10: Treatment of thalassemia

Thalassemia is a very dangerous disease because of its complication, therefore currying of disease is impossible but we can prevent and treat its complication, the most important ways:-

2:10:1: Blood transfusion

Rund and Rachmilewitz (2005) mention the most important therapy of thalassemia is regular transfusion therapy to maintain hemoglobin levels of at least 9 – 10 g per deciliter allows for improved growth , development and also reduce hepatosplenomegaly due to extramedullary hemoatpoesis as well as bone deformities .

Olivieri and Brittenham (1997) were appear that transfusion regimes itself appears critical in the control of body iron loading.

The transfused blood always should be 5 ml/kg/h every 3-5 weeks to maintain Hb level .consider administration of acetaminophen and diphenl dramine hydrochloride before each transfusion to minimize febrile or allergic reaction (Yaish, 2005).

The donors' blood should be tested for infectious agent and immunization of donors to decrease the incidence significantly. 25% of transfused patient were

exposed to hepatitis B virus, hepatitis C is the common cause of hepatitis in thalassemia older than 15 years (Mercola, 2005).

2:10:2: Bone marrow transplantation (BMT)

Bone marrow transplantation from genotypically are option for homozygous b –thalassemia (Giorgio *et.al,* 2002).

The correct use of altodenic stem cells, make the immunological acceptable to the patient from non allogenic which it's nearly impossible to use (Sodani *et.al*, 2004).

Lucarelli *et. al* (1999) analyst results of transplantation , allowed them to identity three classes of risk using the criteria of degree of heptomegaly , the degree of portal fibrosis and the quality of chelating treatment given before the transplant .

The incidence of fulminates espies and growth impairment was significantly higher in transplanted patients (Piga *et.al,* 1998).

2:10:3: Using chelating agent

Chelating agents are small molecules that bind very tightly to metal ions, some chelators are simple molecules that are early manufactured (Ethylene diamine tetra acetic acid EDTA) and other is complex protein made by living organism (e.g.: transferring). The property shared by all chelators is that the metal ion bound to the chelator is chemically inert. The main roles of chelators are to detoxify ions and prevent poisoning (Kberle, 1964).

Olivieri and Brittenham (1995) study the patient with thalassemia regular progress of transfusion sustain growth and development during childhood but without chelating therapy. Iron within the transfused red cells accumulates in exorable and will damage the liver, heart and endocrine organs and may be fatal by adolescence.

2:10:3:1: Iron chelators

Iron has six electrochemical coordination sites that should be tightly bound to block the ability of iron ion to catalyze redox reaction and allow efficient transport of excretion without iron redistribution (Cohen et.al, 2004).

Takeshita (2005) study the most chelators used was deferoxamine (Desferal) see (figure 2- 3) administered as slow subcutaneous infusion through portable pump. Freely soluble in water, approximately 8 mg of iron bounded by 100 mg of defroxamine. The agent is excreted in bile and urine resulting in red discoloration. Adult does 20 – 40 mg/kg/dsc infused over 8 – 12 h may be administered IV/IM if necessary.

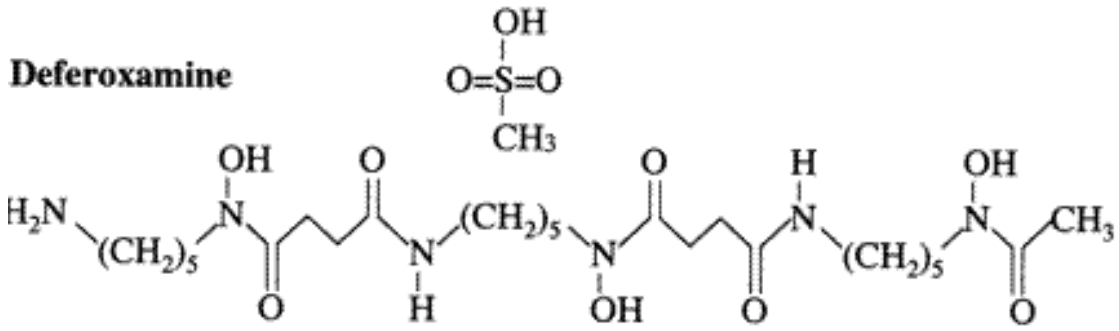

Figure (2 - 3): Chemical structure of Deferoxamine (Rahko *et.al*, 1986)

As shown in figure (2-3) the drug has multiple carbonyls and hydroxyl group provide electrons to coordinate with those in Fe++ (Rahko *et.al*, 1986).

Cohen *et. al* (2004) mention new iron chelators like deferiprone , desferrithiocin, hydroxybenzyl – etgylene diamine diacetic acid ,pyridoxal , isonicotinayl hydro(zone), GT56-252 , 40(CHF1540), ICL670 .

Yaish (2005) show deferoxamin toxicity local reaction at the site of injection is reported in many patient and occasionally can be sever .loss of hearing has been reported in 30-40% of patient ,color and visual feel toss .those complication more commonly when not enough iron is available for chelators .

2:11: Phytic acid

Phytic acid also called phytate, myo-inositol and {Inositol hexaphosphate (IP6)} is naturally occurring polyphosphorylated carbohydrate that is present in substantial amount is almost all plant and mammalian cells .Its was recently recognized to possess multiple biological functions (Vacenik and Shamsuddin, 2003).

Inositol is widely distributed in pants, foods and constituents 0.4-6.4% of most seeds and nuts, usually dietary intakes are range from 1 -1.5 g in a day. (Baten *et. al,* 1989). Show the structure of inositol hexaphosphate (Graf and Eaton, 1990).

Inositol hexaphosphate is central inositol ring structure surrounded by six phosphate groups (Vaceik *et.al,* 1998).

Jessup and Manno (1998) show the roles of phytic acid that it’s the main phosphorus of cereals; it's known to bind essential divalent cation such as calcium, magnesium, iron, zinc and manganese, forming their bioavailability in human and other monogastric animals.

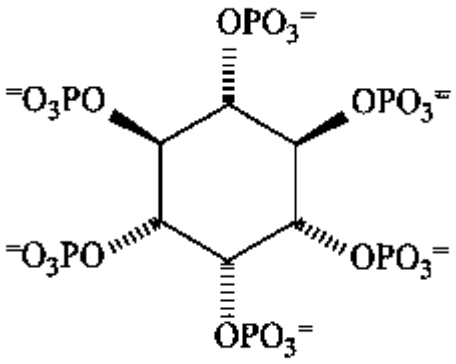

Figure (2 – 4): Chemical structure of inositol hexaphosphate (Graf and Eaton, 1990).

2:11: Phytic acid effect

There are many benefit of Phytic acid ranging from well knows activity to under discovering and researches.

2:11:1: Phytic acid as chelating agents

IP6 form cheaters with divalent cations such as calcium, magnesium, manganese, zinc, copper and iron, found in food, if taken with foods as nutritional supplements containing these elements (Graf and Eaten, 1990). Porres *et.al*, (1999) in his study as antioxidant protection of IP6 through chelating is believed to fight cancer because they prevent free radical damage to DNA. Unbounded iron can be the catalyst for the relatively harmless hydrogen peroxide and forms the highly reactive hydroxyl radical. IP6 chelate for iron and preventing the hydroxyl radical production before it starts.

IP6 has been used against a wide variety of cancers like blood based cancers such as leukemia, liver cancer (Tsang, 1999).

The potential effect of IP6 to induce differential ion and maturation of malignant cells often resulting in reversion th the normal phenotype. IP6 show increase differentiation of human colon carcinoma cell, prostate cancer, breast cancer and rhabdomyosarcoma cell (Vacenik and Shamsuddin, 2003).

2:11:2: Antimicrobial agent

Iron has been known to be associated with infection ,iron chelatore have great potential to become an important tool for fighting bacterial and viral infection also excess iron level promote the development of tuberculosis (Loher ,2002). Metal binding chelators very affective against *Yersinia pestis* , botulism ,small box and anthrax (Emery, 1991). IP6 have very effective form of iron chelation over 15 years ago, it was used to treat malaria (Mercol, 2005).

IP6 a very strong antibiotic and antioxidant, it has been found to have similar iron-chelating properties of desferioxamine a drug been found to have commonly used to kill germ and undesirable mineral from the body (Sardi, 2001).

2:11:3: Immunological effect

It has been found that Ip6 stimulate the immune system by acting antioxidant and enhancing natural killer cells (Dalzell, 2005).

Research suggests that Ip6 supports normal cell growth and development through its roe in cellular signaling and supports of natural cell defense. IP6 also exerts an indirect antioxidant effect through the chelating of free transition metals. (Baten *et.al,* 1989).

Shamsuddin *et .al*, (1997) were found that IP6 has dramatic increase in the activity of natural killer cells in animal treated within , this increase corresponds

nearly with decreased tumor incidence in treated animals , since natural killer cells main function in the body is to seek out and destroy cancerous and virus infected cells .

IP6 is anti- inflammatory, given to rats have lung inflammation and fibrosis, it was reduced this by 6-30 fold (Tsang, 1999).

2:12: Thalassemia in Iraq

Thalassemia disorders are widely distributed throughout the world , but none were from Iraq , in spite of evidence suggesting that thalassemia are not uncommon among Iraqis (Yahya et.al , 1996).

Al – Karagoli (2002) study the molecular diagnosis and hematological analysis of beta thalassemia patients within Iraqi population.

Ministry of health in Iraq have special program for thalassemia , which make a survey every year on the thalassemia center in Iraq, patient no. and the services given to them. This program managed by department of technical affaire, supporting branch , department of treatment , ministry of health.

Its show that a national survey was carrued out In 1999 reveled that crrier raye in Iraq 4.8%. 6400 patients suffering from hereditary anemia , resisted in 29 health center distributed allover the country . It was estimated that the number of patient was 3778 in 2002 all over country (except the northern part). 6034 in 2003 all over country and 6400 in 2005 as shown in the appendixes (7).

3:1: Sample groups

3:1:1: Patients group

A total of 70 β- thalassemia patients from inherited blood diseases in Al –Karama teaching hospital in Baghdad. There ages range from 3 to 22 years old and from both sex. they comprises 26 female patients and 44 male patients, the patients ages between 3 – 7 years 14 patents , between 8 – 12 years 28 patients , between 13- 17 years 19 patients, and between 18 -22 years 9 patients.

3:1:2: Phytic acid tested group

The studying of Phytic acid activity on the immune system represents 10 healthy normal human, and the study done in the laboratory of biotechnology department, Al – Nahrain University.

3:1:3: Control group

A control group of 10 normal (5 Female and 5 Male)individuals were included in this study ,with age ranging from 20 to 30 years old.

3:2: Blood samples

Three ml of peripheral blood was collected from each patient by venipuncture and put in heparinized tube in concentratiom 50 Iu / ml (Sigma Chemical company / England) .Blood was separated into two parts have the same labeling.

3:2:1: Hematological Tests

The WBC count test was done by using 20µl of heparinized β – thalassemia patient, the test was done in the laboratories of Al – Karama Teaching Hospital. The PCV and Hb tests were done by the laboratories of Al – Karama Teaching Hospital.

3:3: Apparatus

Apparatus	**company / country**
Autoclave	Tomy /Japan
Balance	Ohaus / France
Glass Pasteur pipettes	John poulten England
Hemocytometer	Fine – optic /GDR
Heparinized tube	Sigma /Germany
Incubator	Sanyo/Japan
Laminar flow hood	Heraeus / Germany
Light microscope	Olympus /Japan
Micropipette	Oxford /USA
Microscope camera	Olympus / Japan
Millipore filter unit 0.22 µm	Millipore and What
Oven	Sanyo / Japan
pH meter	WTW / Germany
Water bath	Memmert / Germany

3:4: Material and solutions

3:4:1: Stock solution for cell culture

3:4:1:1: Antibiotics (Holden/Germany). .

Benzyl penicillin (1000000u) and Streptomycin (1g) dissolved in Distilled water according to **(Freshney, 2000)**. The solution were Sterilized by filtration in 0.22 µm filter, stored at -20° C.

3:4:1: ٢: Plasma preparation

Human plasma was taken from ***(blood bank /Al-Karama hospital)*** type AB+ used after heat inactivation. Plasma thwarted at 37 C°, after that heated inactivation at 56° C for 30 min. pH = 7.2.

3:4:1:3: Tissue culture media pH = 7.2

DulBecco`s modified Eagles powder media produced by **(Sigma chemicals company / Germany) according to method described by (*Further et.al, 1985*).**

Media powder	13.22 g
Sodium bicarbonate (analar) ***(BDH chemicals, Ltd / England)***	2 g
Heps **(laboratories limited Irvine,Scotland)**	4 g
Antibiotics	10 ml
D.D.W	1000 ml
Plasma	100ml

Mixed, sterilized by filtration through 0.22 µm Millipore filter. Dispensed in to 20 ml aliquots, store at -20 °C.

3:4:1:4: Hypotonic solution KCl

KCl solution prepared according to (**Addhiah, 1990**) the solution prepared.

A (0.5587 g) of KCL dissolved in (100 ml) D.W.. The hypotonic solution molarities was 0.075 M , stored at 4° C .

3:4:1:5: Fixative solution

this solution will freshly made according to **Patten (1967)** method in which 3 volume of methanol 99% mixed with 1 volume of Glacial acetic acid .The two solution were mixed together in ratio of (3- 1 v/v).

3:4:1:6: Giemsa stains (BDH chemicals Ltd/England).

The stain prepared according to **(Allen *et.al* ,1977).** 2 g of Giemsa Stain powder was added to100ml absolute methanol 99%, stirring 2 h. at 50 C°, incubated at 37° C for 24 h, filtered before use.

3:4:1:7: Sorenson's buffer

$Na_2 HPO_4$ (9.47 g) and $KH_2 PO_4$ (9.08 g).dissolved together in (1000 ml D.D.) water, stored at 4 C°.

One ml of filtered stain diluted in 4 ml of Sorenson's buffer immediately used in staining for 2-5 min pH = 7.

Wash the stain slide by the same buffer.

3:4:2: Stock solution for lymphocytes transformation assay.

- Phytohemaglutinine solution

Crude PHA was obtained from **(Biotechnology center/al-Nahrain University)**in a concentration (0.1 g /ml) sterilized by 0.22 µm Millipore filter and dispense in to 1 ml aliquots in botoles, stored in -20 °C.

3:4:3: Stock solution for phagocytes test:

- ***Staphylococcus aureus*** **suspension. (Atlas *et. al,* 1996).** Pure bacteria culture obtained from **(the Health laboratory center in Baghdad)** its ATCC25923, were harvested with sterile saline and make a bacterial suspension in concentration of $1X10^6$ bacterial cell per ml by total viable count method and chose the dilution no. 5 of 10^{-5} which give the count ($12\ X10^5$ cell/ml) Dispensed it to 5 ml aliquots, store in 4 °C .

3:4:4 Stock solutions for leukocyte count: (John and Lewis, 1984).

(2 g) of Gentian violet mixed with (2 ml) Acetic acid in (1000 ml) distal water and store in a dark bottle.

3:4:5: Phytic acid soltusion:

Dissolve Phytic acid powder **(Sigma chemicals company / Germany)** (0.05 µg , 0.1µg and 0.15 µg) in l µl solutionof dulbeco`s media prepared before to make concentrations and pH = 7.

Sterilized by 0.22 µm, filter and store at -20 °C.

3:4:7: Normal saline

(0.85 g) of NaCl Dissolved in distilled water, Adjust pH at7, autoclaved. **(Atlas *et. al,* 1996).**

3:3:8: Sterilization

3:3:8:1: Moist Heat sterilization

Autoclave was used to sterilize buffer, solutions, pastor pipit, tips and test tubes and filter units, at 121 °C. for 20 min.

3:3:8:2: Filtration (membrane sterilization)

Millipore filter 0.22 µm was used to sterilize the blood culture media , Phytic acid solution and antibiotics

3:4: Methods

3:4:1: Lymphocyte transformation assay

This assay was done according to method described by **(Further *et.al,* 1985).**

3:4:1:1: Blood culturing

A set of three tubes were prepared from each patient's blood sample.

(0.25 ml) of heparinized blood of β thalassemia patient was added to (2.5 ml) complete tissue culture media in each sterile tube and 20µl PHA was added to each tube. Another set were used (0.25 ml)of heparinized blood of normal human as a control was added to (2.5 ml) complete tissue culture media in each sterile tube and (20µl PHA) was added to each tube. heparinized blood .

The tubes incubated for 72 h. at 37 °C.

3:4:1:2: Harvesting

The cell suspension was mixed gently and then centrifuged at 1200 rpm for 10 min at room temperature.

The supernatant was discarding and the remaining cell pellet re-suspended with hypotonic solution KCl.

3:4:1:3: Hypotonic treatment

The cells were re suspended in 2 ml of 0.075 M KCl at 37 °C with continuous shaking, more KCl was added gradually until the volume became 8 ml. the cell suspension was incubated at 37°C for 90 min occasional shaking .

Cells were collected by centrifugation at 1500 rpm for 10 min .supernatant was discarded and cell pellets were treated with the fixative.

3:4:1:4: Fixation

A portion of (5 ml)of freshly made fixative was added drop wise to the tube with continuous agitation to the cells. Cell suspension was then centrifuged at 1200 rpm. For 10 min at room temperature.

The fixative was decanted and another (5 ml) fixative was added and the cells were collected by centrifugation. Fixative was changed 3 times before spreading the cells on the slides.

3:4:1:5: Slide preparation

The cell suspension was pleated by centrifugation at 1200 rpm. for 10 min , the suspension was discarded and cells re suspended in 1 ml amount of fixative **(3:4:1:4)**and by pasture pipette ,2 -3 drops of cell suspension were dropped from 30 cm on to wet , grease free slide and allow to air dry at room temperature for staining . The remaining of cell suspension was stored at – 20 °C.

3:4:1:6: Staining

Slide were stained with freshly made Giemsa stain (1 part of Giemsa stain to 4 parted of Sorenson's buffer) , for 2-5 min min. slides were washed then by the same buffer, allowed to air dry at room temperature .studied under light microscope by oil emersion lens .

3:4:1:7: Slide study

Measuring the number of lymphocyte and lymphoblast for every slide then mean to every sample (100 cell in every slide).

The percentages were obtained according to following equation:

%Lymphocyte transformed = lymphoblast cell / total X100
(lymp.+lymphoblast)

3:4:2: Lymphocyte transformation assay with Phytic acid

(0.25 ml) heparinized normal human blood was added to 2.5 ml tissue culture media.

Three concentration of Phytic acid were used (50µg/µl, 100µg/ µl, 150µg/µl). Each concentration was added to every set of tube but without PHA. Other three were as a control without neither PHA nor Phytic acid.

The same method was done to the other concentration.

The harvesting, slides preparation, staining of slides, study as the same a above.

3:4:3: Phagocytosis assay by (Further et.al, **1985).**

1 ml of heparinized β – thalassemia patient blood was mixed with 1 ml of bacterial suspension ($1x10^6$ cell /ml), a set of three tubes were made. Other set contain 1 ml heparinized normal human blood added to 1 ml of bacteria suspension as a control.

The tubes were incubated for 30 min with slow shake. A blood film had been made, waited until dried, fixed with methanol 99%, after dried the slides were stained by Giemsa stain and they were studied under oil emersion of light microscope. Also three slides to every tube. The slides were studied under light microscope (100 cells in every slide).

Phagocytic cells (neutrophil and basophiles which engulfing bacterial cells.

The phagocytosis calculated according to the following equation. .

%phagocytosis = phagocytic cells /total (phago+ non phago) X100

3:4:4: Phagocytosis assay with Phytic acid

Serial concentration (0.05µg/µl, 0.1 µg/µl, 0.15 µg/ µl) were added to each set of tubes contain 1 ml of normal human blood and 1 ml of bacterial suspension (1 $X10^6$ cell/ ml) . Another set Serial of concentration (0.05µg/µl, 0.1 µg/µl, 0.15 µg/ µl) were added to each set of tubes contain 1 ml of normal human blood and 1 ml of bacterial suspension (1 $X10^6$ cell/ ml) to phagocytosis assay were made but without Phytic acid, as a control.

Incubated for 30 min. at 37 °C. blood film had been made, fixed and studied under light microscope.

3:4:5: Leukocyte count according to (John and Lewis, 1984).

20 µl of blood were diluted by 380 µl leukocyte solution in clean glass tube. The suspension mixed for at lest 1 min. (Neubauer hemocytometer) as filled of suspension by pastor pipette.

Light microscope was used to calculate the cell under (10 X lens) and use the following equation:

Leukocyte count/L = no. of cell counted/volume X dilution

= no. of cell counted/volumeX50

4:1: Evaluation of Immunological competent of thalassemia patients:-

4:1:1: Cell – mediated immune response

The study include 70 β- thalassemia patients and the statistical analysis of the mean values for lymphocyte transformation response to PHA of 70 β-thalassemia patient and 10 normal healthy volunteers (control) presented in figures (4 – 2, 4 – 3) and appendixes (1) and (2) according to sex and ages.

Lymphocyte transformation to PHA was show significant decrease of β-thalassemia patients (Female) (21.7%- SD ±5.160**)** in compare to normal control (Female) (48.4% - SD± 5.176) (P <0.05).

Lymphocyte transformation to PHA was show significant decrease of β-thalassemia patients (Male) (20.2%- SD ±5.605) in compared to normal control (Male) (56.2% - SD± 5.495) (P <0.05) and show in figure (4 – 1).

Lymphocyte transformation among different ages (3 -7, 8-12, 13-17, 18 -22 years) alternatively show no significant difference (20.9%- SD±5.4, 20%- SD±5.0, 22.5%- SD ±5.5, 19.0% - SD ±5.6).

The information of difference between sex (male and female) and among ages for lymphocyte transformation of β – thalassemia patients very little or nil but the results in comparison to normal control had agreement with Wanachiwanawin (1996); Walker and walker (2000) in which they found that Lymphocyte transformation index with various mitogens were lower than in normal human, the observation were more obvious in patients with sever disease (sever anemia) and those who had infected frequency.

Pathogenesis, iron over load and transfusion therapy all this effect on the immune competence in beta thalassemia which have reveled numerous quantitative and functional defect involving T and B lymphocytes which reflect the defect in the transformation cells. (Cunningham *et.al,* 2000).

T lymphocyte play an important role in cellular immunity due to there ability to produce many interleukins, which have specific regulatory effect on other cells,

the defect in there response ,Therefore any disorder or decrease in the function and number of T CD4 lead to an obvious immune suppression (Villacres and Bergmani, 1999).

Iron over load of thalassemia patients affect the balance between helper and T CD8 cell and impair proliferation response. The change in T lymphocyte subsets include a greater number and activity of T – cell (CD8), reduced proliferate capacity, number and level of activity of helper T-cell (CD4) leading to decrease CD4/CD8 ratio (Farmakis *et.al*, 2003).

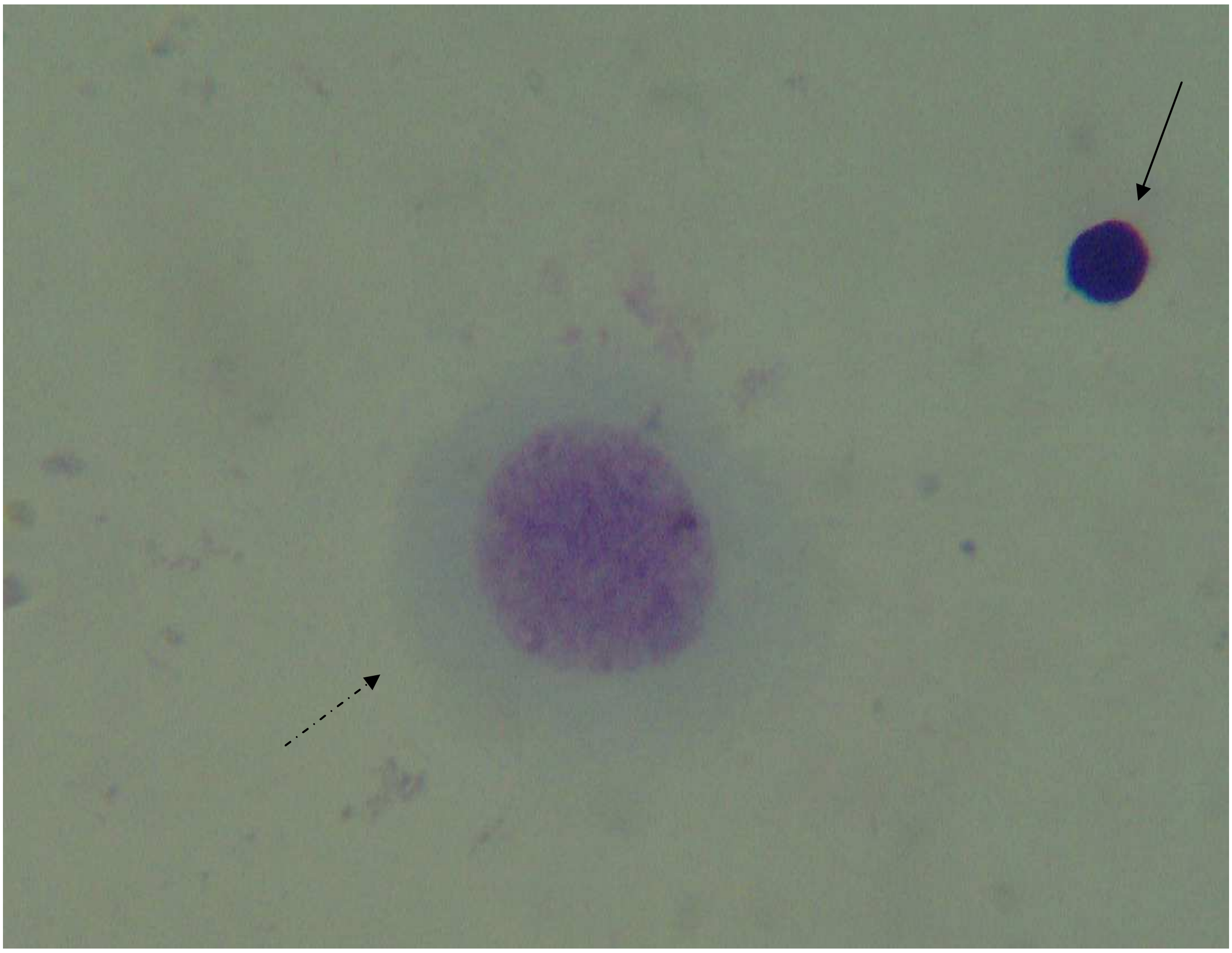

Figure (4- 1): lymphocyte and lymphoblast transformed using PHA.

——→ Poited towred lymphocyte , -·-·-·-→ pointed towred lymphoblaste, Oil lencesX1000 .

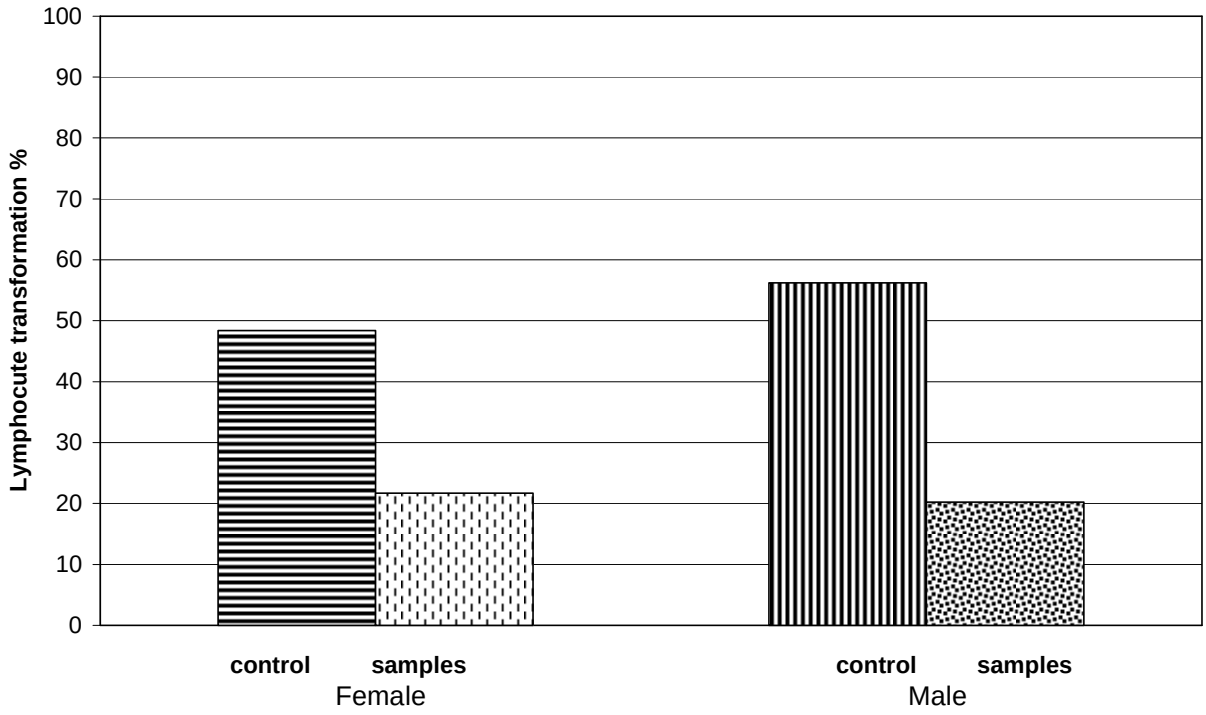

Figure (4 – 2): lymphocyte transformation according to sex (70 patients).

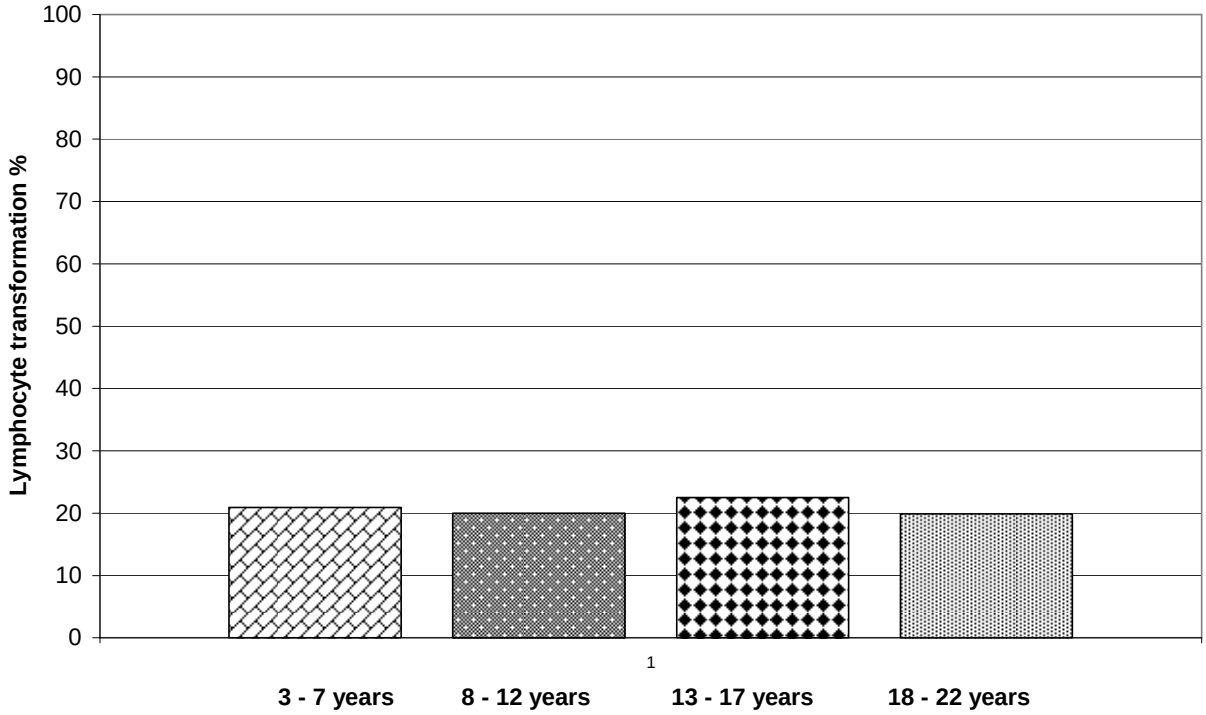

Figure (4 – 3): lymphocyte transformation according to ages (70 patients).

4:1:2: Cell mediated immune response with Phytic acid

The analysis of the mean values of lymphocyte transformation assay with Phytic acid were made, 10 normal volunteers with Phytic acid compared to 10 control without Phytic acid which presented in figure (4 - 4)using concentration 0.15µg/µl .(see Appendix (3)).

The lymphocyte transformation assay with using Phytic acid in a concentration of (0.05µg/ µl and 0.1 µg/ µl) alternatively (12.9% - SD± 2.825, 14.1%-SD±1.411) show no significant differences in comparison with control without Phytic acids (18.6 % - SD± 5.017) ($p<0.05$).

Lymphocyte transformation assay with Phytic acid after adding (0.15µg/µl) was show significant differences (18.6%-±3.4) in compared with control without Phytic acid (13.2% - SD ±3.1).

The presences of Phytic acid may affect the mineral bioavailability in the blood; on the other hand the immune system response has been recognized as an adequate index for the evaluation of the nutritional values of the diet which studied the effect of legume consumption on humeral and cellular immune response. (Larraled and Martinez, 1991).

Recent study showed that Phytic acid increased natural killer cell function by 49% above the baseline, also there activity increased in mice (Baten,1989; Shamsuddin, 1995).

Phytic acid had been found to stimulate the immune system by acting as enhancing natural killer cell which is responsible for attacking and destroying foreign antigen (Dalzell, 2005).

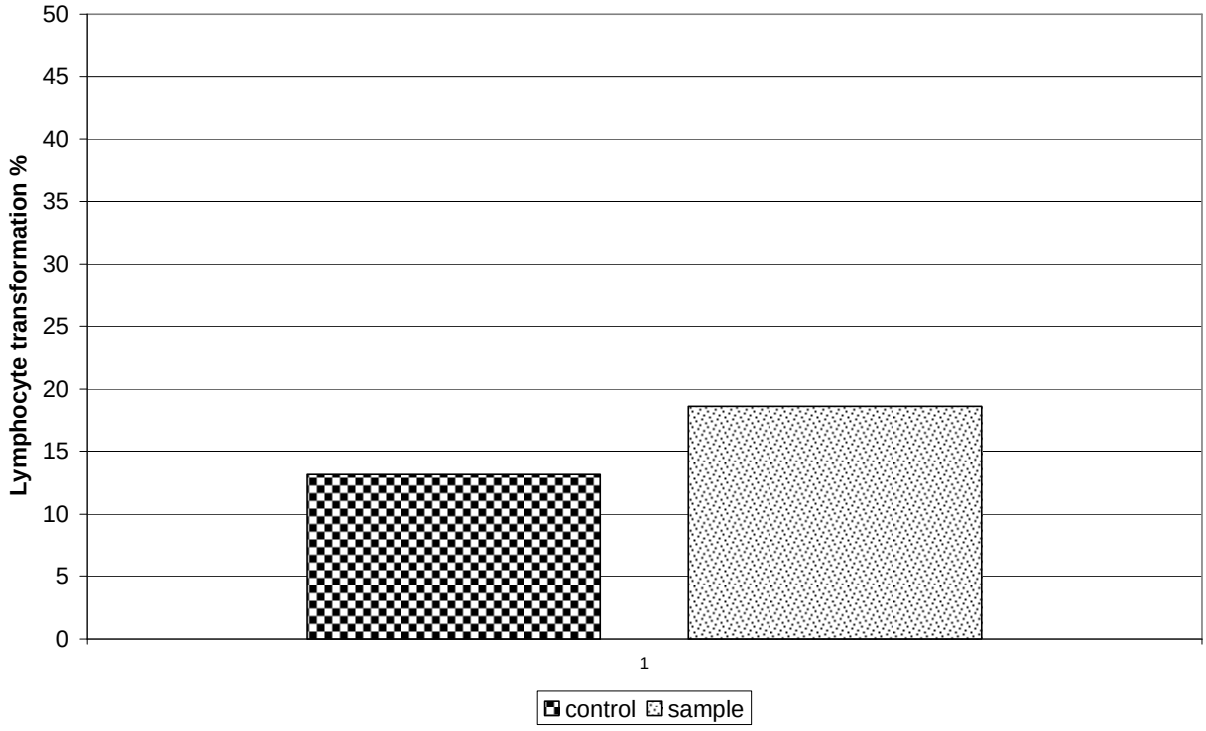

Figure (4 – 4): lymphocyte transformation with using Phytic acid in concentration 0.15µg/µg 10 samples with 10 normal controls).

4:2: Non specific immune response

4:2:1: Non specific immune response without Phytic acid

The mean values of the analysis of phagocytic activity percentage for 70 β-thalassemia patients and compare to values of normal volunteers were presented in Figure (4 – 6) (4 -7){ see Appendix (4) and (5)}.

The phagocytic activity of β thalassemia patients (Female) (36.8%- SD ±11.0) show significant decrease when compared with control (Female) (71.2% - SD± 8.001) ($P<0.05$).

The phagocytic activity of β thalassemia patients (Male) (37.0%-±12.0), in compared with control (Male) (71.2%-±8.2) ($p<0.05$).

The phagocytic activity percentage of different ages (3-7, 8-12,13-17,18-22 years) alternatively (38.2%-±14.3, 35.0%-±10.8, 36%-±10.6, 39.1%-±10.4) show no significant differences (see figure 4 – 5).

Studies (Hoen, 1999; Cunningham *et.al*, 2000) were done using *Candida albicans* for thalassemia patients and they show reduction in phagocytic activity toward *Candida albicans*. Iron over load alters phagocytic properties of neutrophil by reducing their ability to kill invading pathogens.

Neutrophil function test of thalassemia patients show that neutrophil is unable to kill target bacteria or migrate to the infection site chemotaxis (Shaiegaen *et.al*, 2002; Framakis *et.al*, 2003).

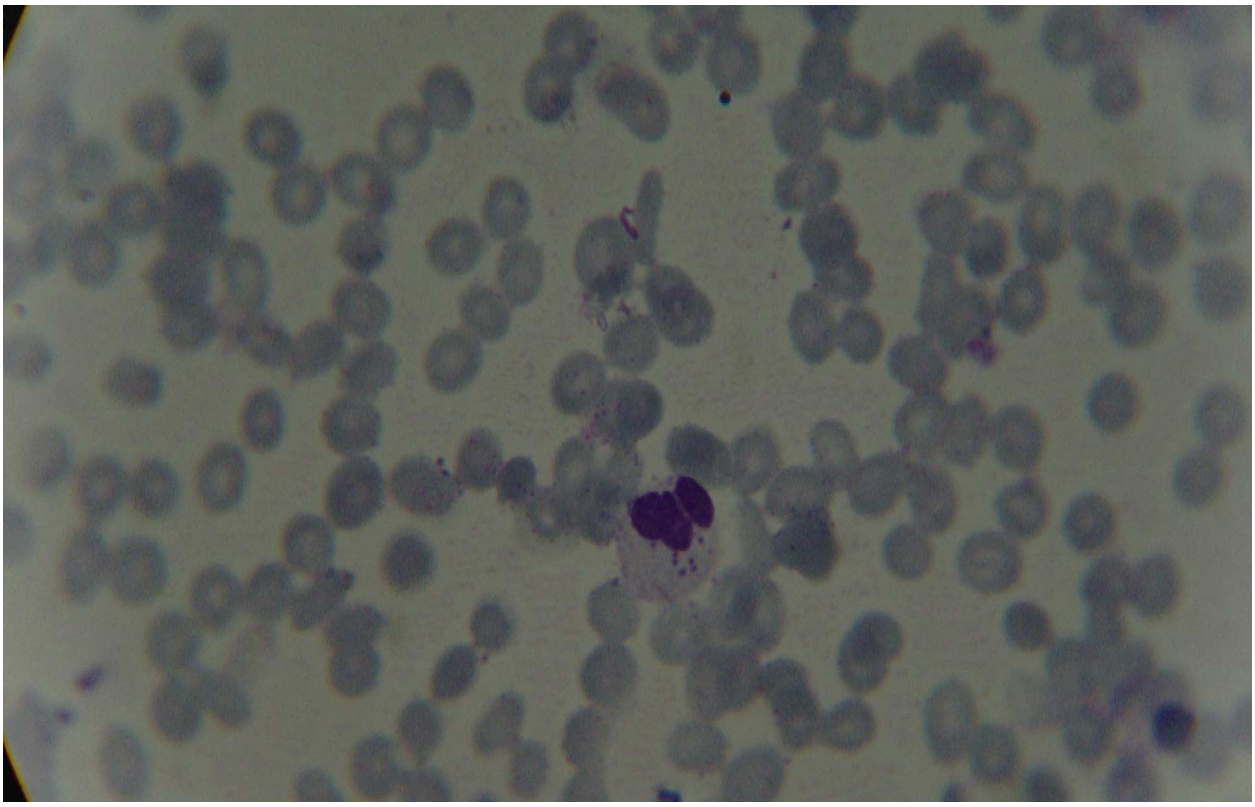

Figure (4 – 5): picture show the phagocytic cell (neutrophil) phagocyte *Staphylococcus aureaus* . Oil lencesX1000.

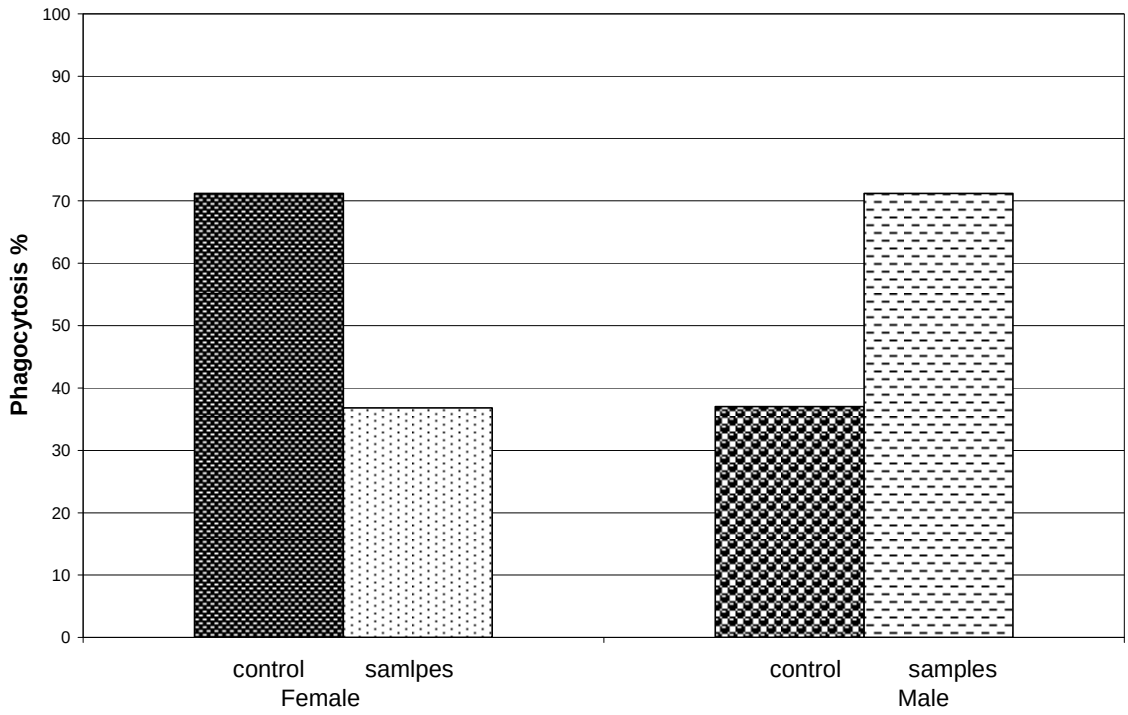

Figure (4 – 6): Phagocytosis percentage study according to sex (male and female) 70 patients.

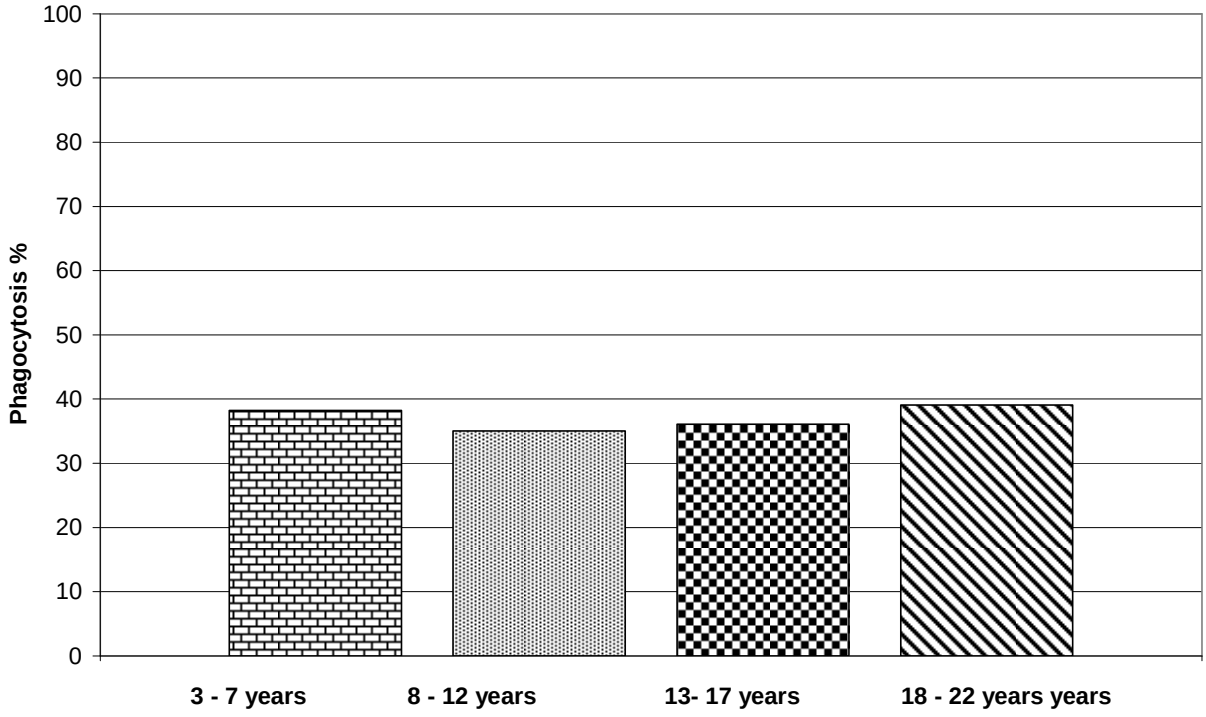

Figure (4 – 7): Phagocytosis percentage study according to ages 70 patients .

4:2:2: Non specific immune response with Phytic acid.

The analyses of the mean values for the percentage of phagocytic activity of 10 normal compare to 10 normal control volunteers presented in figures (4-8) see Appendix (6).

The phagocytic percentage using Phytic acid is in a concentration of (0.05µg/ µl and 0.1 µg/ µl) alternatively (64.5% - SD± 10.491, 65.3%- SD±10.511) show no significant differences between them.

Lymphocyte transformation assay with Phytic acid after adding (0.15µg/µl) was show also no significant differences (64.5% - SD ±10.491) in compared with control without Phytic acid (71.2% - SD ± 8.007).

The analysis of the mean value of phagocytic percentage to 10 healthy with Phytic acid (71.2%-±8.2) after adding the three volumes of Phytic acid, show no significant differs with 10 controls without Phytic acid (64.5%-±8.0).

All patients had been subjected to a desferroxamin treatment (which has chelating function like Phytic acid and it the world wide treatment medicine to thalassemia) and high blood transfusion regimes, neutrophil function show normal activity when compare to control (Speer *et .a l*, 1990).

Phytic acid is a natural glyconeutrient from plants that supports the innate immune system (Shamsuddin , 1995).

Graf and Eton (1990) were showed that Phytic acid stimulate neutrophil activity and play a rule in immune defense while neutrophil are capable of surrounding , engulfing and digesting foreign mater

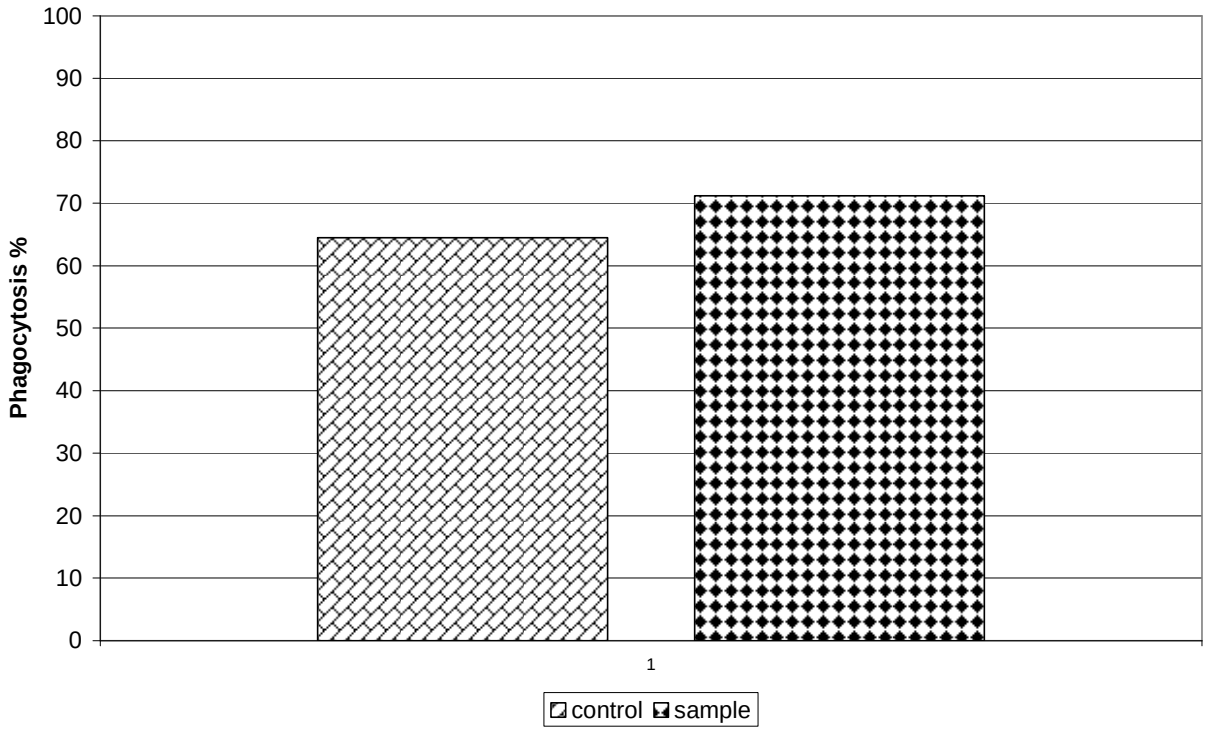

Figure (4 –8):Phagocytosis assay with using Phytic acid to10 normal concentration 0.15 µg/µl with 10 controls without Phytic acid.

4:3: Hematological study

The analyses of hematological study of thalassemia patient were presented in figure (4 -9).

The hemoglobin level of 20 thalassemia patients is (8.3g/dl -±1.6) show significant decrease as compare to 20 normal control (13.7g/dl – ± 1.1) ($p<0.005$). The mean values of hemoglobin (Hb) concentration in thalassemia patients were significantly lower than normal controls, because thalassemia is a hereditary defect in globin chain lead to impair link to heam group and this affect hemoglobin synthesis and cause anemia (Han *et.al*, 1992; Al- Karagoli, 2002; Hendrick, 2003; Takeshita, 2005; Yaish, 2005).

The analysis of packed cell volume of 20 thalassemia patient (26.0 -±5.0) appeared significant decrease in compare with 20 normal control (42.1 -±3.6) ($p<0.005$).

When the PCV reflect the percentage of red blood cells in a sample, its low percentage as compare to control reflect the hemolytic properties in RBC as a result of the disease (Han *et.al,* 1992; Moyle, 2002; Al- Karagoli, 2002).

The analysis of White cells count of 20 thalassemia patient (8.3 X1000 - ±5.2) show no significant differences from normal control (6.2X1000 –± 1.6). There is no difference between the number of WBC of thalassemia patients and control which reflect the defect in the immune system cells itself and not in the number of cells .Thalassemia patient are more susceptible to infection by transfusion blood infection, spleenectomy and progressive iron overload (lopez et.al, 1996; Cunningham *et.al*, 2000).

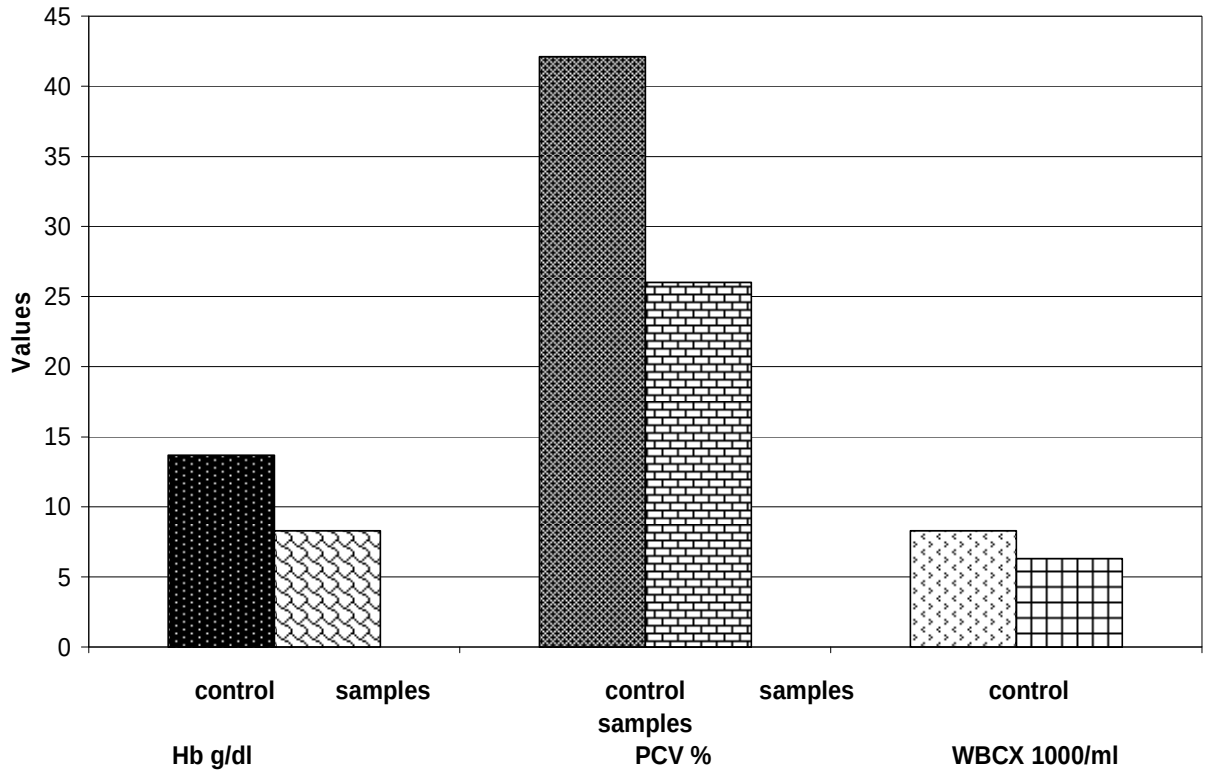

Figure (4 – 9): Hematological analysis 20 thalassemia patient with 20 healthy control.

Chapter one

Chapter two

Literature review

Chapter three

Materials and Methods

Chapter four

Results and discusion

Referances

Conclusion

1. The immune responses were studied by lymphocyte transformation assay and phagocytosis assay there were an obvious immune defects in thalassemia patients.
2. The study concludes that immune response is not influenced among ages and sex.
3. There were decreases in Hb and PCV values while WBC count was normal.
4. Phytic acid has an immunoactivation property as studied by lymphocyte transformation assay and phagocytosis assay.
5. The active volume of Phytic acid (0.15µg/µl) was the minimal active volume for lymphocyte for normal human.

Recommendations

1. Advanced study in bacterial approach of thalassemia patients are recommended which need long time study and high population.
2. Future study is needed for Phytic acid chelating properties and immunoactivation for thalassemia patients.
3. *In vitro* and *In vivo* study to determine the effective dose of Phytic acid for thalassemia patients.

List of Contents

Summary	i
Contents	ii
List or figures	vi
List of abbreviation	viii
Chapter one Introduction	
1:1: introduction.	1
1:2: Aim of the study.	3
Chapter two Literature review	
2:1: Thalassemia	4
2:2:2: Hemoglobin	5
2:2:2: Hemoglobin structure	5
2:3: Thalassemia disease	7
2:4: Thalassemia types	9
2:4: Beta thalassemia	٩
2:5: Alpha thalassemia	10
2:6: Symptoms of thalassemia	11
2:7: Complication of thalassemia	12
2:71: Iron overload	12
2:72: Opportunistic bacteria	13
2:73: Immunological defect	14
2:74: Heart failure	15
2:75: Splenectomy	15
2:7:: Other complications	١٦
2:8: Clinical diagnosis of thalassemia	١٧

2:9: Evaluation of immunological competent of thalassemia patient	17
2:9:1:cell- mediated immune response	18
2:٩:2: Humeral immunity in thalassemia patients	19
2:9:3:non specific immune response	20
2:10:Treatment of thalassemia	٢١
2:10:1:Blood transfusion	21
2:10:2:Bone marrow transplantation (BMT)	22
2:10:3:Using chelating agent	22
2:11:Phytic acid	24
2:11:Phytic acid effect	25
2:11:1: Phytic acid as chelating agents	٢٥
2:11:2: Antimicrobial agent	٢٦
2:11:3: Immunological effect	٢٦
2:13: Thalassemia in Iraq	٢٧
Chapter three Materials and methods	
3:1: Sample groups	28
3:2: Blood samples	28
3:3: Apparatus	٢٩
3:2: Material and solutions	٣٠
3:2:12: Sterilization	32
3:3: Methods	٣٣
3:3:1: Lymphocyte transformation assay	٣٣
3:3:2: Lymphocyte transformation assay with Phytic acid	٣٥
3:3:3: Phagocytosis assay	٣٥
3:3:4: Phagocytosis assay with phytic acid	٣٦
3:3:5: Leukocyte count	٣٦

Chapter four Results and discussion	
4:1: Evaluation of Immunological competent of thalassemia patients:-	٣٧
4:1:1: Cell – mediated immune response	٣٧
4:1:2: Cell mediated immune response with phytic acid .	٤٠
4:2: Non specific immune response	42
4:2:1: Cell mediated immune response	٤٢
4:2:2: Cell mediated immune response with Phytic acid	٤٥
4:3: Hematological study	٤٧
Conclusion	٤٩
Recommendations	٥٠
References	٥١
Appendixes	٦٦

1:1: Introduction

Thalassemia is inherited disorders of hemoglobin (Hb) synthesis resulting from an alteration in the rate of globins chains production.

Decrease in the rate of production of two certain globins chains (alpha and beta) creates an imbalances in Hb synthesis (Yaish, 2005).

Thalassemia is named according to the amino acid chain defects in the type chain hemoglobin molecules (alpha thalassemia mean that alpha chain is affected and beta thalassemia refers to the affect of beta chain) (Hollenstein, 2005).

Alpha thalassemia genes are found in southest Asians, blacks and people of the Middle East. Alpha thalassemia minor is a carrier state with no anemia or symptoms, the second type has slightly abnormal red cells but still no anemia and the third type produce mild anemia but do not lead to serious complications (range from non to very sever) and it is wide spread in African and Mediterranean. While the beta thalassemia minor cause no symptoms, beta thalassemia intermediate is a mild form of Cooley's anemia, the disease is mild until adulthood. Cooley anemia or thalassemia major is the most sever type (Ferguson, 2002).

Thalassemia is among the most common genetic disorder world wide ;4.83% of world population carrying globin variations , while 2.4 in1000 birth babes have homozygous or compared heterozygous for alpha and beta thalassemia(Rund and Rachnilewitz,2005).

Regular blood cell transfusion are eliminates the complication of anemia and permit normal development throughout childhood and extend survival. In parallel transfusion result in exorable accumulation of tissue iron with time and is fatal in the second decade of life. The major normal adult hemoglobin designated Hb A. consists of two alpha and two beta chains. 95% of hemoglobin in normal individual over one years age with small amount (less than 2.5% of A2) and F accounting for the remainder (Wigges, 2006).

Iron and its binding proteins have immunoregulatory properties and shifting of immunoregulatory balance by excess or deficiency may produce sever, deleterious physiological effects on the immune response (Walker and Walker, 2000).

There are two important tests used to study the activity of the immune system. Lymphocyte transformation assay examines the ability of lymphocytes (T and B) to respond to polyclonal stimuli (PHA, CON A, PWM, IL-2, and Anti-CD3). Where is the phagocytosis study the ability of phagocytic cell to ingest antigen (Kumaratilaks and Ferrante, 2000).

Thalassemia patient have more episodes of infecting then normal healthy control includes mild infecting, sever infection therapy related infection.

These infection do not cause sever morbidity or mortality but may decrease sense of well – binding and working ability of the patients. Therapy related infection include transfusion transmitted disease and desferrioxamine – related infection. The majorities of the former are post – transferring hepatitis and human immunodeficiency virus, recently hepatitis C virus becomes major etiological agent (Wanachiwanawin, 2001).

Multiple blood transfusion and iron chelators are the most important protecting life ways especially with beta thalassemia major. deferoxamin is the most chelating agent used for thalassemia patient (Takeshita,2005).

Phytic acid the main phosphorus storage of cereals, legumes and oil seeds, its known to bind essential divalent caution such as calcium, magnesium, iron, zinc and manganese, forming largely insoluble complex and there by decreasing their availability in human and other mongastric animals (Phytic acid may exert anti carcinogen benefits, have similar iron – chelating properties as desferrioxamine which is a drug used to kill germ and chelating agent to thalassemia patients) (Rimbach and Pallauf, 1998).

1:2: Aim of the study

1. Evaluation of immune system for thalassemia patients by studding the specific immune response and non specific immune response.
2. Studding the activation properties of Phytic acid to the human immune system.
3. Hematological study for patients including PCV, Hb, total WBC count.

List of abbreviation

α globins	Alpha globins
α thalassemia	Alpha thalassemia
β globins	Beta globins
β thalassemia	Beta thalassemia
Hb	Hemoglobin
IP6	Inositol hexaphosphate
PCV	Packet cell volume
PHA	Phytohemagglutinine
T – CD4	T lymphocyte (cluster of differentiation type 4)
T – CD48	T lymphocyte (cluster of differentiation type 8)
WBC	White blood cells
IV	intra Venus injection
IM	intra muscular injection

List of figures

Figure number ***Page***

(2 - 1)	**Quarterly structure of hemoglobin**	٦
(2 – 2)	**Chemical structure of hemoglobin**	٧
(2 - 3)	**Chemical structure of Deferoxamine**	٢٣
(2 – 4)	**Chemical structure of inositol hexaphosphate**	٢٥
(4 - 1)	**Lymphocyte and lymphoblast transformed using PHA.**	٣٨
(4 – 2)	**Lymphocyte transformation according to sex (70 patients with 10 controls).**	٣٩
(4 – 3)	**Lymphocyte transformation according to ages (70 patients with 10 controls).**	٣٩
(4 – 4)	**Lymphocyte transformation with using Phytic acid 10 samples with 10 normal controls).**	٤١
(4 – 5)	**Picture show the phagocytic cell (neutrophil) phagocyte *Staphylococcus aureaus***	٤٣
(4 – 6)	**Phagocytosis percentage study according to sex (male and female) 70 patients with 10 controls.**	44
(4 – 7)	**Phagocytosis percentage study according to ages 70 patients with 10 controls.**	44
(4 –8)	**Phagocytosis assay with using Phytic acid to10 normal with 10 controls without Phytic acid.**	46
(4 – 9)	**Hematological study 20 thalassemia patient with 20 controls.**	48

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جمهورية العراق
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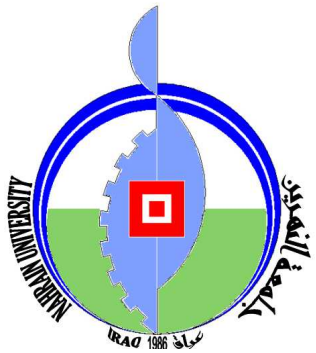
جامعة النهرين
كلية العلوم
قسم التقانة الاحيائية



بعض اوجه المناعة لمرضى الثلاسيميا في بغداد

رسالة مقدمة الى كلية العلوم

جامعة النهرين

وهي جزء من متطلبات نيل درجة الماجستير علوم

في

التقانة الاحيائية

من قبل

رفل حسام الدين عبد الله

بكلوريوس في التقانة الاحيائية ٢٠٠٣

٢٠٠٦ اب

شعبان ١٤٢٧

الاهداء

الى من غمرتني بحنانها وتعبت لتعبي ...وتحملت معي عنائي.

نورعيني .....

امي الحنون

الى القلب الكبير و الرحيم ..... من كان مني موضع فخر

واعتزاز .....

ابي الحبيب

الى من صبروا بعون الله على بلواهم .....من اجلهم اتممت هذا

البحث .....

المرضى و عوائلهم

بسم الله الرحمن الرحيم

أُوْلئكَ الذينَ هَدَى اللّهُ فَبِهُداهمُ إقْتَدِه قُّل لآ

أَسْأَلَكُمُ عَلَيهِ أَجراً إِنْ هُوَ إِلآ ذِكرىٰ للعالمينَ

صدق الله العظيم

سورة الانعام اية ٩٠

In the name of Allah Most Gracious, Most Merciful

Those were who received Allah guidance, therefore follow their guidance.

Say: "I do not ask you for any reward for it";

It is nothing but a reminder to the nations.

Al – An`am

Sura 6/90

الخلاصة:

تضمنت الدراسة سبعين حالة لمرضى فقر دم البحر الابيض المتوسط اخذت من مركز امراض الدم الوراثي في مستشفى الكرامة التعليمي في مدينة بغداد / العراق .

قيمت كفاءة الجهاز المناعي للمرضى من خلال قياس عدد الخلايا المتحولة (نوع T) عند معاملتها ب PHA و مقارنتها بالاشخاص الاصحاء (السيطرة) . حيث لوحظ ان هناك فروقات معنوية في النتائج اذ وجد نقصان في نسبة خلايا T المتحولة في مرضى الثلاسيميا عن الاشخاص الاصحاء .

دراست فعالية الخلايا البلعمية خارج الجسم الحي و مقارنتها بالاشخاص الاصحاء ولوحظ فروقا معنويا وبينت النتائج نقصان في نسبة الخلايا الملتهمة في المرضى عن الاصحاء .

و اظهرت النتائجالمتعلقة بنسبة خلايا T المتحولة عند اضافة Phytic acid لعشرة عينات دم لاشخاص اصحاء و مقارنتها مع السيطرة بدون Phytic acid وجد فروقا معنويا في نسبة الخلايا T المتحولة, حيث ازدادت الخلايا المتحولة بمقارنتها مع السيطر ،بينما لم تلاحظ فروقات معنوية عند دراسة الخلايا البلعمية خارج الجسم الحي عند اضافة Phytic acid .

تم قياس نسبة الهيموغلوبين وحجم تراص خلايا الدم و عدد كريات الدم البيضاء لعشرين حالة من مرضى الثلاسيميا و مقارنتها مع السيطرة و اظهرت النتائج الاتية : حدوث نقصان معنوي في نسبة الهيموغلوبين و نرسيب خلايا الدم للمرضى مقارنة مع السيطرة بينما لم يظهر عدد كريات الدم البيض اي فروقات معنوية .

Summary:

The present study includes 70 cases of thalassemia patients from inherited anemia center, Al-Karama hospital in Baghdad / Iraq.

Evaluation of the immune system function done for those patients by measuring the number of transformed cell (type T) using PHA and compared with healthy people (control). It was noted that there were significant decrease in the of transformed T cells in thalassemia patients compared to control.

When phagocyte cells function studied *in vitro* and compared with control also significant decrease in the percentage of phagocytic cells than control.

Ten samples studied for lymphocyte transformation (type T) of normal healthy people with using Phytic acid and compared with control without Phytic acid, significant increase in the percentage of lymphocyte transformed T cell in compared with control, but when phgocytosis studied *in vitro* and compared with control show no significant differs.

Hemoglobin (Hb), packed cell volume (PCV) and total white blood cell count (WBC) were measured for 20 thalassemia patients and compared with control. Hb and PCV show significant decrease than control while total WBC count show no significant different.