Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.7, No.2, 2017

Assessment of IL-6 Cytokine Level in Rheumatoid Arthritis Patients Associated with Anemia

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Abstract
The role of interleukin-6 (IL-6) in rheumatoid arthritis patients associated with anemia stilled unclear because of the limited researches. Therefore we evaluated the levels of IL-6 in rheumatoid arthritis patients associated with anemia.30 patients with rheumatoid arthritis and anemia (23 males and 7 females), 30 patients with rheumatoid arthritis only (18 males and 12 females) and 30 healthy control groups (14 males and 16 females) were enrolled in our study, the levels of IL-6 cytokine were measured by enzyme-linked immunoabsorbent assays (ELISA), rheumatoid factor test (RF), Anti-CCP antibody test, Hematocrit %, Hb level g/dL for all cases also was assessed and statistical analysis of the data was then processed. The levels of IL-6 in patients with rheumatoid arthritis and anemia was ranged between 78 and 122 pg/ml also the second group, patients with rheumatoid arthritis only was ranged between 20 and 59 pg/ml, and control free disease was ranged between 1 and 10 pg/ml. Also our results showed the statistical significance between patients with rheumatoid arthritis and anemia and patients with rheumatoid arthritis only with (CI 95%)at (-43.355- -36.112), T-test (0.000), patients with rheumatoid arthritis and anemia and control free disease with (CI 95%)at (-100.364- -89.636), T-test (0.000) and patients with rheumatoid arthritis only and control free disease with (CI 95%)at (-62.241- -48.293), T-test (0.000) respectively. We concluded that IL-6 cytokine play important role in rheumatoid arthritis with anemia.
Keywords: Rheumatoid arthritis, Anemia, IL-6

Introduction

Rheumatoid arthritis (RA) is the greatest mutual autoimmune inflammatory arthritis in adults (Helmick CG *et al*., 2008). Rheumatoid arthritis (RA) primarily touches the synovial joints, resulting in pain, deformity and eventual functional limitation, causing substantial morbidity and accelerated mortality (Cush *et al*., 2007). The condition also has widespread extra articular manifestations including vacuities, inflammation in the heart and lungs and peripheral neuropathy. The disease confers an increased risk of many other diseases including cardiovascular disease, pulmonary dysfunction, renal disorders and intestinal pathologies, along with a significantly increased risk of premature death (NCCCC, 2009). Anemia of chronic disease is immune driven; cytokines and cells of the reticuloendothelial system induce changes in iron homeostasis, the proliferation of erythroid progenitor cells, the production of erythropoietin, and the life span of red cells, all of which contribute to the pathogenesis of anemia . Recent studies have demonstrated serum inhibition of in vitro erythropoiesis in adult and juvenile patients with chronic arthritis. (Yap and Stevenson, 1994: Gordeuk *et al.,* 2001). Moreover, tumor cells can produce proinflammatory cytokines and free radicals that damage erythroid progenitor cells (Weiss, 2002: Means 2003: Sullivan *et al*., 1998). Bleeding episodes, vitamin deficiencies (e.g., of cobalamin and folic acid), hypersplenism, autoimmune hemolysis, renal dysfunction, and radio- and chemotherapeutic interventions themselves can also aggravate anemia (Rodriguez et al., 2001: Groopman and Itri, 1999). Anemia is frequently seen in patients with active rheumatoid arthritis (RA) (Mowat, 1971). Many causes of anemia are associated with RA, such as deficiencies of iron (Hansen *et al*., 1983). Interleukin-6 (IL-6) is a monokine with biological activities related to inflammatory responses (Geiger, 1988: Nijsten *et al*., 1987). IL-6 levels were elevated in serum and synovial fluid of patients with active RA (Swaak et al.,1988: Houssian *et al*.,1988). About the effects of IL-6 on bone marrow only few data exist (Leafy *et al.,* 1988). In this study, we measured the serum IL-6 levels in patients diagnosed with both rheumatoid arthritis and anemia and correlated with patients diagnosed with rheumatoid arthritis only.

Materials and Methods

In this study, 30 patients with rheumatoid arthritis and anemia (23 males and 7 females) with an age mean ± SD (68.43± 8.516) family history (6 positive/24 negative), rheumatoid factor test (RF) (13 positive/17 negative), Anti-CCP antibody test (30 positive/ 0 negative), Hematocrit % mean ± SD (27.73 ± 3.005), Hb level g/dL mean ± SD (8.910± 1.0008), 30 patients with rheumatoid arthritis only (18 males and 12 females) with an age mean ± SD (66.57± 13.206) family history (2 positive/28 negative), rheumatoid factor test (RF) (10 positive/ 20 negative), Anti-CCP antibody test (30 positive/ 0 negative), Hematocrit % mean ± SD (45.70 ± 3.239), Hb level g/dL mean ± SD (14.897± 1.0826), and 30 healthy control groups (14 males and 16 females) with an age mean ± SD (1.53± 0.507) ,were recruited at Alkindy teaching hospital, Baghdad, Iraq. Patients with RA [diagnosed based on American Rheumatism Association (ACR) 1987 Criteria (Arnett et al. 1988). The study subjects were informed about the purpose of the study, and written consent was taken from each of them. Ethical approval was

obtained from the Local Ethics Committee.

Peripheral blood samples

Peripheral blood samples for laboratory measurements were drawn immediately. Venous blood was collected into sterile EDTA tubes and analyzed the same day for hemoglobin measurement. Anemia was defined as the reduction in Hematocrit levels below 35% and the reduction of hemoglobin below 12.0 g/dL. Peripheral blood was also drawn into sterile tubes, allowed to clot for 30 minutes, and spun at room temperature for 20 minutes at 2500 rpm; sera were aliquoted and stored at -70°C for interleukin 6 (IL-6) quantification by means of enzyme-linked immunosorbent assay (ELISA; Quantikine; R & D Systems Europe, Abingdon, England), IL-6 ELISA Kit was (AviBion Human IL-6 ELISA Kit, Orgenium Laboratories, Finland). Serum concentration of IL-6 was determined based on a standard concentration curve.

Statistical analysis

A computer statistical program was used (SPSS vs. 19) to analyze the data. Patients and control group parameters and the relations between the parameters were compared using "Paired Samples T" confidence interval (95% CIs) were calculated for different studied parameters. The confidence interval (CI) at 95% was used to describe the amount of uncertainty associated with the samples. Mean values and standard deviations were presented. P value less than 0.05 was considered as statistically significant.

Results

The three groups were compared to assess the level of IL-6 cytokine as described, the first group (patients with rheumatoid arthritis and anemia) with IL-6 concentrations ranged between 78 and 122 pg/ml and mean ± SD (99.47± 13.910) as shown in table 1, also the second group (patients with rheumatoid arthritis only) with IL-6 concentrations ranged between 20 and 59 pg/ml and mean ± SD (44.20± 10.015) as shown in table 2, and the last group was (control free disease) with IL-6 concentrations ranged between 1 and 10 pg/ml and mean ± SD (4.47± 2.501) as shown in table 3. Also the Paired Samples T test shown statistical significance between patients with rheumatoid arthritis and anemia and patients with rheumatoid arthritis only with (CI 95%)at (-43.355- -36.112), T-test (.000), patients with rheumatoid arthritis and anemia and control free disease with (CI 95%)at (-100.364- -89.636), T-test (.000) and patients with rheumatoid arthritis only and control free disease with (CI 95%)at (-62.241- -48.293), T-test (.000) respectively as shown in table 4.

Table 1: Showed the frequency and Percent of IL- 6 cytokine levels among patients with rheumatoid arthritis and anemia

IL- 6 cytokine level pg/ml	Frequency	Percent
78	3	10.0
79	1	3.3
87	3	10.0
89	2	6.7
91	2	6.7
93	2	6.7
94	1	3.3
99	2	6.7
103	3	10.0
109	1	3.3
110	2	6.7
111	1	3.3
112	1	3.3
113	1	3.3
117	1	3.3
118	1	3.3
120	1	3.3
121	1	3.3
122	1	3.3
Total	30	100.0

Table 2: Showed the frequency and Percent of IL- 6 cytokine levels among patients with rheumatoid arthritis only

IL- 6 cytokine level pg/ml	Frequency	Percent
20	1	3.3
23	1	3.3
32	1	3.3
33	2	6.7
34	1	3.3
37	1	3.3
39	2	6.7
40	1	3.3
42	2	6.7
43	3	10.0
44	1	3.3
47	1	3.3
48	3	10.0
51	1	3.3
52	1	3.3
53	2	6.7
55	2	6.7
56	1	3.3
57	2	6.7
59	1	3.3
Total	30	100.0

Table 3: Showed the frequency and Percent of IL- 6 cytokine levels among control

IL- 6 cytokine level pg/ml	Frequency	Percent
1	2	6.7
2	4	13.3
3	9	30.0
4	3	10.0
5	2	6.7
6	3	10.0
7	3	10.0
8	2	6.7
10	2	6.7
Total	30	100.0

Table 4: Showed the Paired Samples T test for IL- 6 cytokine levels among the three groups

		Paired Differences		t	Sig. (2-tailed)
		95% Confidence Interval Of the Difference			
		Lower	Upper		
Pair 1	patients with rheumatoid arthritis and anemia - patients with rheumatoid arthritis only	-43.355	-36.112	-22.439	.000
Pair 2	patients with rheumatoid arthritis and anemia - control free disease	-100.364	-89.636	-36.223	.000
Pair 3	patients with rheumatoid arthritis only - control free disease	-62.241	-48.293	-16.208	.000

***significant at P≤0.05**

Discussion

Cytokines are small, biologically highly active proteins that regulate the growth, function, and differentiation of cells and help steer the immune response and inflammation (Thomson A, 1998). IL-6 is a pleiotropic, immunomodulatory cytokine produced by a variety of cell types, including fibroblasts, endothelial c ells, monocytes and both benign and malignant lymphocyte s of B and T cell origin (Akira et al., 1993). It is a multifunctional cytokine, which plays a key role in the differentiation and growth of haematopoietic cells, B-cells, T-c ells, keratinocytes, neuronal cells osteoclasts and endothelial cells (Baue and He rrmann, 1991). The role of IL-6 cytokine in the pathogenesis of RA and their significance in clinical monitoring of the disease already mentioned in many studies (Koch et al., 1995: Brennan et al., 1995). In this disease the articular synovial membrane is infiltrated w ith inflammatory cells diffusing into the synovial fluid. The inflammatory process spreads from the synovial membrane to the cartilage and bone tissue causing their damage (Koch et al., 1995). Otherwise the pathogenesis of the anaemia associated with rheumatoid disease is unclear (Cartwright and Lee, 1979: Vreugdenhil et al., 1990). Recent studies have demonstrated serum inhibition of in vitro erythropoiesis in adult and juvenile patients with chronic arthritis (Dainiak et al., 1980: Prouse et al., 1987). Inhibition of erythropoiesis corresponded with the severity of the anaemia (Prouse et al., 1987). And with inflammatory activity (Dainiak et al., 1980: Prouse et al., 1987). According our results that a proved gradually elevation of IL-6 cytokine level among control and patients with rheumatoid arthritis and patients with both rheumatoid arthritis and anemia as mentioned previously, (Vreugdenhil et al.) Noticed that the serum IL-6 was elevated in anemia of chronic disease ACD and it correlated well with parameters of disease activity such as erythrocyte sedimentation rate and C-reactive protein. IL-6 addition to bone marrow cultures had inconsistent effects while anti-IL-6 addition resulted in impaired erythroid colony growth, suggesting stimulatory effects of IL-6 produced in the medium, which may be masked by simultaneous production of cytokines with suppressive effects. It was concluded that elevated serum IL-6 in ACD reflects disease activity. It probably plays no pathogenetic role in ACD. Its stimulatory effects on erythroid growth might counteract suppressive effects of other interleukins (Vreugdenhil et al., 1990). Some potent inhibitors of erythropoiesis have been described by(Panay, G.S), suggested that several cytokines like TNFα, IL-1, IL-6, IL- 8, IFN β and IFNy which mediate chronic inflammation in rheumatoid arthritis (RA) (Panay,1993). In conclusion, according to the mechanism and the role of IL-6 in rheumatoid arthritis and anemia we suggested that, there are strong associations between the levels of IL-6 with rheumatoid arthritis and anemia.

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