Original article

RELATIONSHIP OF INFECTION AND GAMMA INTERFERON (IFNγ) OF PLASMA AND LYMPHOCYTE CULTURE SUPERNATANT IN IRON DEFICIENCY ANEMIA PATIENTS WITH INFECTION

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ABSTRACT

Iron is an essential nutrient for every living cells because of it role as molecule for transport of oxygen, as well as DNA synthesis through synthesis of ribonucleotide reductase. Although the underlying mechanism of immune defect in iron deficiency anemia (IDA) is not clearly understood, multifactor events considered play their contributing roles such as impairment of T-cell proliferation and activities, altered cytokine production of Interleukin-2 (IL-2) and Interferon gamma (IFNγ).

Cross sectional study was done to asses the relationship of gamma IFN with infection in IDA patients on plasma and lymphocyte culture supernatant of IDA patients. Sixty-four IDA patients treated in Sanglah General Teaching Hospital were recruited, and 31 (48.4%) out of 64 IDA patients were man and 33 (51.6%) women, have been selected for the study. This study found 17 (26.7%) IDA patients with infection, aged 38 ± 14.48 years and 47 (73.3%) IDA patients without infection, with age average of 40.5 ± 14.4 years. The study revealed that there were no differences of cytokine level observed between older and younger age (upper and below 44.5 years) in IDA patients. Furthermore, no differences of cytokine level were found based on gender between IDA male 10.9 pg/l (8.60 – 12.65) patients and IDA female patients 10.6 pg/l (7.50 – 13.43) with Z -0.490, p = 0.624. Nevertheless, significant differences were noted between plasma and supernatant of IFNγ in IDA patients with infection when compared to IDA patients without infection (Z = - 2.638, p = 0.008 for plasma IFNγ; and Z = -2.569, p = 0.010 for supernatant IFNγ).

The study conclusion is that level of IFNγ from plasma and lymphocyte culture supernatant of patient suffered from IDA with infection is significantly lower when compared to IDA patient without infection.

Keywords: IDA with infection, plasma and supernatant IFNγ

INTRODUCTION

Iron deficiency anemia (IDA) is anemia caused by decreased level of iron in the body resulting in depleted iron reserve that is not sufficient for normal erythropoesis process. The most common cause of IDA is deficiency iron loss due to chronic bleeding.

Study of etiology of iron deficiency anemia for 3.5 years on 122 IDA patients revealed that ankylostomiasis, hemorrhoid, peptic ulcer and malignancy as a prominent causes. The study carried out in 2003 at Sanglah Hospital/Department of Internal Medicine, 14% of IDA cases were found with infections.[1] Iron deficiency can lead to increase morbidity especially for babies and pregnant woman due to impairment of immune response such as decreased killing activities of neutrophil, defect in T cell proliferation.[2,3]

The relation of iron deficiency with infections is difficult to study in humans, either by observational or non-interventional studies. As such, studies on the relation of IDA with infections tend to give inconsistent results. Several observational studies done on human subjects showed increase of infection cases in the subjects with IDA. On the contrary, other studies have found opposite results in which iron deficiency was even protective against infections.[4,5]

Many other studies on humans and experimental animals have shown significant decrease of the levels of certain cytokines in iron deficiency state. The study by Bergman *et al*.[6] on adult subjects with IDA showed a lower level of IL-2 than in normal subjects, but the levels of other cytokines such as IL-6, IL-10, and TNFα were not significantly different from those of normal subjects. A study in Malawi, Africa found IDA was associated with lower production of IFNγ and IL-8 as compared with subjects having normal iron state. In this study a strong correlation was also found between IDA and the level of IL6-producing lymphocytes.[7] A study on mice with IDA carried out by Kuvibidilla *et al*.[8] showed a decrease of IFNγ and IL-12 by 64% and 66% respectively, in which the level of these cytokines had positive correlation with iron status indicator ($r = 0.68$). Another researcher proved that suppressed production of IFNγ in mice with lipo-polysaccharide tolerance caused macrophages dysfunction that subsequently impaired cellular immunity.[9]

According to the above findings, it is understandable that the relationship between pro-inflammation interleukin and iron deficiency remains unclear. The production of IFNγ as pro-inflammation interleukin and CMI parameters is assumed to decrease in IDA cases. Based on that, it is necessary to carry out a study to assess the level of IFNγ in human IDA patients.

MATERIALS AND METHODS

This study was carried out using descriptive analytical cross sectional study to find out the mean difference of IFNγ levels in both IDA patients with infection and those without infection. The accessible population was all IDA patients who visited the Sanglah Hospital and several doctor's private practices in Denpasar. The study subjects were patients with IDA (intended samples) selected from the accessible population that met the inclusion and exclusion criteria by using consecutive sampling technique. The actual study subjects were patients with IDA who had confirmed to involve in this study by signing an informed consent. The criteria for IDA using combination of hypochromic microcyter anemia with level of ferritin serum less than 20 ug/l. Exclusion criteria of study were: patients who suffered from protein energy malnutrition, patients who received immunosuppressive medicine, such as steroid and chemotherapy, minimum in the last one month, patients with renal failure, genetic disorders, and malignancies, patients who did not consent to involve in the study. Patients who finally consent to join the study were informed about the study aims. Materials of the study consisted of plasma and supernatant of lymphocytes culture for IFNγ concentrations examination as well as list of questionnaires for obtaining data on age, gender, body weight, infections suffered, use of immunosuppressive medicines, malnutrition, malignancy and genetic disorder, and IDA diagnosis.

All collected data were first assessed before taken for analysis. The normality test of Kolmogorov-Smirnov (Shapiro-Wilk for small samples) was done to assess whether the data were normally distributed or not. Descriptive statistics was used to illustrate patient's characteristics and frequency distribution of various variables. U Mann-Whitney test was used to evaluate the average difference of IFNγ concentrations in IDA with and without infection. The above statistic analysis used $p < 0.05$ as the significance standard, by means of statistic software SPSS for window version 15.0.

RESULTS

There were 64 IDA patients in this study, 31 patients males (48.4%) and 33 females (51.6%) with age average of 40.5 ± 14.4 years. A number of 17 (26.7%) were IDA patients with infection and the remainder 47 (73.3%) were without infection. Type of infection mostly lung infection then followed by urinary infection, gastroenteritis and viral disease. All variables of data characteristics examined did not indicate any statistical significant difference between group of IDA patients with infection and those without infection. The average level of hemoglobin between the two groups did not differ statistically. Similar result was obtained if samples were differentiated into severe (Hb < 7 g/dl) and mild anemia. Basic characteristics of IDA patients according to presence of infection are described in Table 1.

Table 1. Basic characteristics of IDA Patient

Characteristics	IDA with Infection Means ± SD	IDA without Infection Means ± SD	P value
Age (years)	38±14.48	41±14.54	0.59
Sex (M/F)	8/9	23/24	0.89
Leukocyte (K/µl)	9.7 ± 3.91	8.0 ± 3.66	0.17
Hemoglobine (g/dl)	6.5 ± 1.82	6.6 ± 1.91	0.81
MCV (fl)	65.8 ± 7.81	81.9 ± 8.65	0.95
MCH (pg)	19.3 ± 2.57	19.2 ± 4.06	0.88
MCHC (%)	29.1 ± 1.78	28.0 ± 4.10	0.58
Thrombocyte (K/µl)	400.7 ± 132.40	418.5 ± 178.6	0.73
SI (µg/l)	12.1 ± 3.95	13.5 ± 7.18	0.34
Transferin Saturation (%)	4.28 ± 1.90	3.51 ± 2.03	0.22
Ferritin (µg/l)	10.5 ± 8.26	8.6 ± 7.50	0.39
AST (IU/l)	22.2 ± 10.10	23.14 ± 8.23	0.81
ALT (IU/l)	16.0 ± 11.10	18.6 ± 10.3	0.59
BUN (mg/dl)	10.7 ± 4.84	16.2 ± 6.46	0.05
SC (mg/dl)	0.99 ± 0.20	0.78 ± 0.31	0.07

Based on gender there were no significant differences showed between IDA male patients and IDA female patients as shown on Table 2.

Tabel 2. Profile of cytokine of IDA patients based on gender

Cytokine	Iron Deficiency anemia		Z value	P value
	Male (n=31) Median (Interquartile)	Female (n=33) Median (Interquartile)		
Plasma IFNγ (pg/l)	9.2 (8.50 – 27.50)	11.9 (9.20 – 83.60)	-1.834	0.067
Supernatan IFNγ (pg/l)	26.3 (14.50 – 43.00)	31.3 (17.50 – 43.50)	-0.336	0.707

As we aware of, old ages influence immune response as well as cytokine production. But on table 3 can be seen that there were no differences of cytokine level observed between older and younger age (upper and below 44.5 years).

Table 3. Profile of cytokine of IDA patients based on median ages

Cytokine	Iron Deficiency anemia		Z value	P value
	Age < 44.5 (n=39) Median Interquartile	Age > 44.5 (n=25) Median Interquartile		
Plasma IFNγ (pg/l)	11.9 (9.1 – 77.2)	9.2 (8.5 – 23.6)	-1.597	0.11
Supernatant IFNγ (pg/l)	39.0 (21.3 – 48.3)	19.8 (2.2 – 40.3)	-2.408	0.06

Statistic test applied was the non-parametric one because the non-normal distribution of IFNγ considering that Kolmogorov Smirnov test gave insignificant results (IFNγ $Z = 2.857$; $p < 0.001$) and still did not distribute normally even though a logarithm transformation has been applied to both variables. Hence, non parametric test of Mann Whitney U was used since this test was accurate as a substitute for other inadequate parametric tests that did not meet analysis requirements due to efficiency capacity close to 95% with an increase of n value.

The cytokine concentration difference examined from plasma and supernatant lymphocytes culture (Mann Whitney U test) of IDA group without infection

is shown on Table 4. It showed that IFNγ concentrations of the two groups were statistically significant difference with Z = -2.638 ; p = 0.008 and Z = -2.569 ; p = 0.010 respectively.

Table 4. Difference of median interquartile of cytokine of supernatant between IDA patient with infection and IDA patient without infection

Cytokine	Iron Deficiency anemia		Z value	P value
	With Infection (n=17) Median Interquartile	Without Infection (n=47) Median Interquartile		
Plasma IFNγ (pg/l)	9.1 (7.65 – 11.60)	14.5 (9.00 – 26.20)	- 2.638	0.008
Supernatant IFNγ (pg/l)	20.4 (11.20 – 30.45)	39.2 (18.80 – 48.30)	- 2.569	0.010

DISCUSSION

In this study the diagnosis of iron deficiency anemia was based on finding of hypochromic, microcytic red blood cells and ferritin serum level < 20 ug/l. According to the National Health and Nutrition Evaluation Survey II, the use of two or more indicators of the iron status is highly recommended for the diagnosis of iron deficiency anemia. This study also noted that single low serum ferritin level comprised one important indicator for the diagnosis of iron deficiency anemia, besides iron depleted state.[14]

Among other causes of acquired disturbances of cellular immunity is aging. In the elderly, the decline in the quality of the first line of defense (ie., atrophy and dryness of skin and mucous membrane), reduced vitality, and increased risk for trauma, together with retardation of the response process, should probably be regarded as the major causes of increased susceptibility to infections. But in this study can be seen that there was no differences of IFNγ level between younger and older age as well as no differences was found based on gender. Other study of 41 IDA patients were also found no differences of cytokine result either based on age or gender.[11]

Iron Deficiency Anemia and Infections

In this study iron deficiency anemia with concomitant infections was found in 17 subjects (26.7%) of 64 patients with confirmed IDA. In 2006, according to patient records in the Division of Hematology and Medical Oncology of the Department of Internal Medicine, Sanglah Hospital, 21 of 78 IDA patients were with infections. In a previous study carried out in 2003 at Sanglah Hospital/Department of Internal Medicine, 14% of IDA cases were found with infections.[1] In Malawi, Africa 26% of IDA cases was found with HIV, 24% with bacterial infections, and 20% with malaria.[7] Another study done in Israel on 680 IDA patients found a total of 5644 episodes of acute ear infections with an average of 8.3 episodes per patient; and 77.6% among 528 patients had simultaneous infections in both ears.[12]

Results of our study seem to agree with the theory that patients with IDA are prone to infections, as indicated by the finding of significant lower level of plasma and supernatant IL-2 and IFNγ in IDA patients with concomitant infections as compared with those without infections ($p < 0.05$). As a response to infection, the body must activate its immunity mechanism, either natural or adaptive ones. For developing an optimal immunity response, especially the cellular immunity response, the body produces and activates the cytokines IL-2 and IFNγ.

Our study results also agree with that of a study done in China where 63 IDA patients with recurrent upper respiratory tract infections were examined for their levels of IL-2 and sIL-2R as well as for their subset lymphocyte T cells. In the latter study it was found that activation of IL-2 and percentage of CD3 and CD4 were significantly lower in children suffering from IDA with repeated upper respiratory infections as compared with a healthy control group.[13]

The relation of iron deficiency with infections is difficult to study in humans, either by observational or non-interventional studies. In fact, iron deficiency can be regarded as a form of malnourishment that exists as

intertwined social and health problems resulted from poverty. Moreover, it is against ethical consideration to delay administration of iron preparation to patients with IDA because this may worsen the patients' condition. As such, studies on the relation of IDA with infections tend to give inconsistent results.[5]

Several observational studies have done on human subjects showed increase of infection cases in the subjects with IDA. A study, considered a pioneer of its kind until now, was done in London in 1928 to observe the effects of IDA on infection episodes. In this study it was found that about half of 541 subjects studied had significant lower episodes of infections in the group given iron preparation. Another study involving a greater number of subjects was done in Chicago in 1966 where more than 1000 babies with IDA were studied; the result showed a significant lower number of upper respiratory tract infections in those babies given iron preparation.[14] Higgs and Wells[15] studied 31 patients with chronic musculocutaneous candidiasis, of which 23 had IDA, and found that 9 among 11 patients got better outcome after given iron preparation. A prospective clinical study on post surgery patients showed a significant greater number of infection cases among those having low level of serum ferritin (228 cases) as compared with those (220 cases) who had normal level of serum ferritin.[5]

A study in Malawi, Africa found IDA was associated with lower production of IFNγ and IL-8 as compared with subjects having normal iron state. In this study a strong correlation was also found between IDA and the level of IL6-producing lymphocytes.[7] Similarly, another study done in Paris on 53 patients with IDA and 28 controls with normal iron state found significant different levels of IL-2 in the two groups. An experimental study on iron deficient made animals also showed a lower level of IL-2.[16] Kuvibidila in his study on mice that were made iron deficient found a lower level of IL-12p40 and IFNγ in the group of mice with iron deficiency as compared with those mice given rations with normal iron supplement ($p < 0.05$). There was a positive correlation between the levels of cytokines and indicators of iron state (r 0.688, = < 0.05).[8] A similar result was also found in a controlled study on children in which a lower secretion of IL-2 was noted in children with iron deficiency as compared with the healthy control group.[17]

The mechanism of how iron deficiency can disturb cellular and nonspecific immunity responses is not yet clearly understood, but it is thought to be due to multifactorial features. These include decrease of activity of enzymes containing iron such as ribonucleotide reductase, myeloperoxidase, decrease of cytokines production, decrease of competent T cells, and probably disturbance of signals transduction. The exact phase of disturbance of signal transduction where iron plays a part is yet to be clarified, although previous studies have confirmed the influence of the disturbance in the activity of protein kinase C and its translocation on the plasma cells membrane and T cells of the spleen. These findings were seen in both human and animal study subjects. It is also known that binding of iron causes decreased production of mRNA needed for development of protein kinase C.[18] In the initial stage of T cells activation, disturbance occurs in the hydrolysis of *phosphatidylinositol 4,5-bisphosphate* (PIP2) by phospolipase C (an enzyme containing zinc), with its final result as *inositol 1,3,5-triphophate* (IP3) and *diacylglycerol* (DAG), which regulate activity of protein kinase C. Both protein kinase C and hydrolysis of phospholipid membrane are important for the initial process of transduction signal that can cause proliferation of T cells and activation of other functions. Thus, disturbance of protein kinase C and hydrolysis of phospholipid membrane result in disturbance of immune response in subjects with iron deficiency.[19]

Many studies on humans and animals have shown the presence of disorders of the cellular immunity and other nonspecific immunities in subjects with iron deficiency, but the relation of iron deficiency with infection remains unclear. Susceptibility to infections

comprises a complex mechanism; it does not depend on one's iron status alone but also on other factors such as body condition, types of parasites/microorganisms, and environment. Among the other influencing factors are exposure to microorganisms, other nutritional deficiency, types of population (babies, children, females, males, elderly), duration and severity of the deficiency, type, dosage, and duration of iron therapy, and existence of other precondition factors. It is clear that these concomitant factors greatly influence the degree of susceptibility to and severity of infections, regardless of the level of iron status. Iron deficiency influences susceptibility to certain types of infection, and duration and severity of the infections depend on the body condition and types of parasites, either extra or intra cellular microorganisms.[20]

CONCLUSIONS

From results and discussion described above, it can be concluded that concentration of IFNγ in plasma and supernatant of lymphocytes culture was significantly lower in IDA patients with infection than those without infection. Therefore IDA patients were more susceptible to certain infection because of impaired immune response due to low level of IFNγ.

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