# Estimation levels of Interferon Gamma in Patients Infected with a *Entamoeba Histolytica*

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## Abstract

This research was carried out in Ramadi, Iraq, at the Ramadi Teaching Hospital Department of Biology laboratories at the University of Anbar's College of Education for Pure Sciences. Samples were collected from different age groups. The study was conducted includes the finding of the role of interferon gamma and granular white blood cells in prevention from *Entamoeba histolytica* parasite, where the quantitative estimate of interferon gamma was measured using the ELISA technique for the purpose of detecting the concentrations of this cytokine in infected people with amoebiasis and comparing with healthy people to prove the important role of this cytokine during infection with this parasite , The results indicated that there was a statistically significant difference, $p \leq 0.05$, in the concentration of INF-γ in samples of affected individuals (78.01 (±9.07)) compared with INF-concentration in control samples (59.18 (±3.937)), Also, the numbers of white blood cells and granulocytes in infected people with this parasite were detected and compared with healthy people using the complete blood count device to detect. The findings indicated a statistically significant distinction, $p \leq 0.05$, in the count of White Blood Cells (WBCs) in samples of infected individuals (9.136 (±1.249)) compared to the count of WBC in control samples (6.00 (±0.822)) and that there was a significant difference, $p \leq 0.05$, in the count of Granulocytes (Gran) in samples of infected individuals (6.885 (±1.575)) compared to the count of Gran in control samples (4.122 (±0.845)). The results obtained demonstrated the role of granular white blood cells and interferon gamma in combating and controlling this parasite.

***Keywords**: Interferon gamma; Granulocytes; Entamoeba histolytica.*

# تقدير مستويات الانترفيرون غاما في المرضى المصابين بطفيلي الاميبا الحالة للنسيج

**بلال جاسم محمد ، أ.د. ثائر عبد القادر صالح [2] وَ صفاء عبد لطيف[3]**

## الخلاصة

أجري هذا البحث في مدينة الرمادي ، العراق ، في مستشفى الرمادي التعليمي ومختبرات قسم علوم الحياة في كلية التربية للعلوم الصرفة ، جامعة الانبار . جمعت العينات من مختلف الفئات العمرية . اشتملت الدراسة على اكتشاف دور الانترفيرون غاما وخلايا الدم البيضاء الحبيبية في الوقاية من طفيلي الاميبا الحالة للنسيج ، اذ قدر الانترفيرون غاما كميا باستخدام تقنية الامتزاز المناعي



P-ISSN: 2664-0562
E-ISSN:2664-0554

(الاليزا) لغرض الكشف عن تركيز هذا السيتوكين في الاشخاص المصابين بداء الاميبات ومقارنته بالاصحاء غير المصابين . أشارت النتائج إلى وجود فروق معنوية إحصائيًة ، p≤0.05 ، في تركيز الانترفيرون غاما -في عينات الافراد المصابين (78.01 (± 9.07)) مقارنة بتركيزه في عينات السيطرة (59.18 (± 3.937)) . وقدرت اعداد خلايا الدم البيضاء والخلايا الحبيبية باستخدام جهاز فحص صورة الدم الكاملة (CBC) للكشف عن الفروق بين اعداد خلايا الدم الحبيبية ، أشارت النتائج إلى وجود فروق ذات دلالة إحصائية ، p≤0.05 ، في عدد خلايا الدم البيضاء (WBCs) في عينات الأفراد المصابين (9.136 (± 1.249)) مقارنة بعدد خلايا الدم البيضاء في عينات السيطرة (6.00 (± 0.822)) وأن هناك فرقًا معنويًا ، p≤0.05 ، في عدد الخلايا الحبيبية (Gran) في عينات الأفراد المصابين (6.885 (± 1.575)) مقارنة بعددها في عينات السيطرة (4.122 (± 0.845)) . أثبتت النتائج التي حصل عليها دور كريات الدم البيضاء الحبيبية والإنترفيرون غاما في مكافحة هذا الطفيلي والسيطرة عليه.

***الكلمات المفتاحية :*** *الانترفيرون غاما ، الخلايا الحبيبية ، الاميبا الحالة للنسيج.*

**Introduction**

This parasite is responsible for more deaths than any other parasitic disease, as infection with this parasite leads to deaths around the world ranging from (40-100,000) deaths in a year, as this parasite infects and colonizes the intestine [1], the parasite has two forms, the trophozoites and the cyst stage [2]. The parasite is transmitted through water and food sources contaminated with cysts of this parasite [ 3, 4]. In a mentioned earlier that in some rare cases there are no symptoms, but in 10% of cases the parasite begins to dissolve the intestinal tissue and moves with the blood circulation to the liver and causes abscesses in the liver [5]. The parasite also causes hemorrhagic colitis. This parasite infects the intestinal wall, where it causes severe intestinal symptoms with high temperatures with mucous and bloody stools [6]. The parasite causes lesions and ulcers in the digestive system, and inflammatory cells gather in these ulcers, which are directed by special signals secreted by infected cells, represented by epithelial cells and other infected cells. Neutrophil cells and macrophages migrate to the site of parasite infection by being recruited by some pro-inflammatory cytokines [1]. Where these immune cells stick to the membranes of the parasite and carry out the process of engulfment, where the neutrophils and macrophages carry out the process of phagocytosis [ 7، 8].

During infection with the *Entamoeba histolytica* parasite, many cytokines are stimulated, which regulate the immune response by activating white blood cells. The role of cytokines has been proven in previous studies in regulating the work of monocytes and activating amoebicides in [9]. The cytokine interferon gamma (IFN-γ) has a role in activating and regulating a wide range of immunological responses [10]. The role of interferon gamma is to activate white blood cells, specifically neutrophils, which are the first immune cells to attack the parasite after infection [11], Cellular immunity relies heavily from neutrophil blood cells, which is the first cellular barrier that is



P-ISSN: 2664-0562
E-ISSN:2664-0554

recruited for the purpose of combating and controlling the parasite [12]. The production of interferon-gamma is stimulated by the binding that occurs between epithelial cells and the carbohydrate receptors present in the membranes of the parasite, thus activating the production of NFkB, which cause production of interleukins and inflammatory cytokines, cytokines such as tumour necrosis factor-alpha and interferon-gamma [13, 14].

**Aim of study:** is to estimate the level of interferon gamma (IFN-γ) and blood granulocytes count in patients infected with *Entamoeba histolytica.*

**Materials and Methods**

**Collection of specimens**

Eighty blood samples were collected from people infected with the parasite from Al-Ramadi Teaching Hospital from the period from the 1st of August to the 1st of November for people ranging in age from 4 years to 70 years, consisting of males and females, in addition to collecting 20 control samples.

**Sample preparation**

After placing the samples in White tubes and EDTA tubes, using a centrifuge at a speed of 6,000 cycles for a period of 10 minutes, serum was obtained in preparation for the examination of Interferon – gamma on the ELISA Technique. Anticoagulant tubes EDTA were used for the purpose of conducting a complete blood Count examination for the purpose of conducting an examination of white blood cell.

**Assay Procedure by Elisa**

The kit for interferon gamma was supplied by SUNLONG company of Chinese origin. The absorbance reading was at a wavelength of 450 nm, while the normal ratios ranged from 15.6pg/ml-1000 pg/ml.

**Statistical analysis**

The statistical analysis was performed by using T test with a probability of 0.05. The results showed statistically significant differences [15].

**Results**

The quantitative assessment of Interferon – gamma was measured using the ELISA technique (direct ELISA) on 80 blood samples from people infected with the parasite, along with 20 control samples, and after conducting a statistical process on the results figure 1.



P-ISSN: 2664-0562
E-ISSN:2664-0554

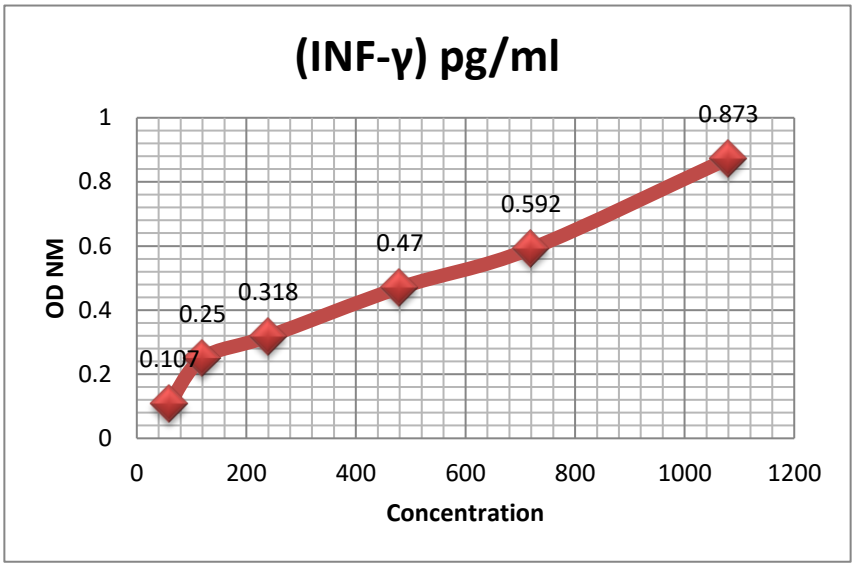


**Fig. (1):** Standard curve for interferon gamma.

The findings indicated a statistically significant distinction, p≤0.05, in the concentration of **INF-γ** in samples of infected individuals (78.01 (±9.07) compared to the concentration of **INF-γ** in control samples (59.18 (±3.937) shown in table.

**Table (1):** Interferon gamma concentrations in infected and healthy people.

| Parameter | Healthy (control) | | | Patients | | | Test statistic | p-value |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | SEM | Mean | SD | SEM | | |
| INF-γ | 59.18 | 3.937 | 1.607 | 78.01 | 9.07 | 2.516 | 4.82 | 0.0001** |

**WBCs count**

The granulocyte blood cells of the blood samples of the infected and healthy people preserved in the anticoagulant tubes (EDTA Tubes) were also measured using the complete blood count (CBC) examination device for the purpose of detecting the role of these cells and the extent of their influence and impact on infection with this parasite and revealing their role in combating the parasite table 2.

**Table (2):** White blood cells and granulocyte counts in patients and healthy people.

| Parameter | Healthy | | | Patients | | | Test statistic | p-value |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | SEM | Mean | SD | SEM | | |
| Gran | 4.122 | 0.845 | 0.345 | 6.885 | 1.575 | 0.485 | 3.96 | 0.001** |
| WBCs | 6.00 | 0.822 | 0.336 | 9.136 | 1.249 | 0.377 | 5.49 | 0.001** |



P-ISSN: 2664-0562
E-ISSN:2664-0554

The findings indicated a statistically significant distinction, $p \leq 0.05$, in the count of White Blood Cells (WBCs) in samples of infected individuals (9.136 (±1.249)) compared to the count of WBC in control samples (6.00 (±0.822)) and that there was a significant difference, $p \leq 0.05$, in the count of Granulocytes (Gran) in samples of infected individuals (6.885 (±1.575)) compared to the count of Gran in control samples (4.122 (±0.845)).

**Discussion**

The results agreed with what was mentioned in another study conducted on mice that proved the close link between interferon gamma and infection with the parasite, as interferon gamma reduces the process of building proteins of the parasite by binding through special receptors present in the membranes of the parasite through which it stops the process of building special proteins with the parasite, as they found that the parasite isolated from the infected tissues of the colon has a high affinity for binding to interferon gamma [16]. Some studies also indicated an increase in the value of interferon gamma in children infected with the *E. histolytica*, which confirms the important role of this cytokine during infection with this parasite [17]. In a study that also agreed with our results conducted by (Guo et al., 2008), they found a high affinity for activators to bind to interferon gamma during amoebic invasion of the colon, as this cytokine is secreted by T cells, natural killer cells, and macrophage cells located in the submucosal layer. for the colon [18].

In some studies, the role of Interferon - gamma has been demonstrated in increasing the activity of macrophages in killing amoebae, and thus reducing the incidence of dysentery [19]. This agrees with our study's findings that the value of interferon gamma differed significantly between individuals infected with the parasite and those without it.Through the results, we notice an increase in white blood cells in general in infected people, specifically with granulocyte blood cells, The results agreed with what was recorded in a study conducted on the role of granulocytes and the CXCR2 receptor in protecting against amoebic dysentery, as they found that lack of neutrophil recruitment as a result of decreased gene expression of neutrophils for the receptor CXCR2 on their surface increased the severity of colitis, as granulocytes play Specifically, neutrophils play an important role in cellular immunity [12] .

Also, some studies have demonstrated the prominent role of white blood cells, specifically macrophages, in killing the parasite through oxidative and non-oxidative pathways [20]. This explains why parasite carriers tend to have more white blood cells than healthy individuals. Some cytokines, like interferon gamma and tumour necrosis factor alpha, have also been shown to have a role in prior research., in recruiting neutrophils and macrophages. Specifically, neutrophils and macrophages are



P-ISSN: 2664-0562
E-ISSN:2664-0554

very important in resisting the parasite and protecting against amoebic dysentery, as this was proven in mice, this study agreed with our results [19]. Also, white blood cells, neutrophils, in particular, are thought to be the first immune cells to engage the parasite, which are activated by interferon gamma, and this explains the reason for the higher numbers of granulocytes in infected people with the parasite than in healthy people [11].

**Conclusion**

The present study showed the role for Interferon gamma and granulocytes protection from *Entamoeba histolytica* infection. The results showed that there were significant differences in the concentrations of interferon gamma between patients that infected with the parasite and healthy people, in addition to the presence of significant differences in the numbers of white blood cells and granulocytes between patients and healthy people, $P < 0.05$.



P-ISSN: 2664-0562
E-ISSN:2664-0554

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P-ISSN: 2664-0562

E-ISSN:2664-0554