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Original Article

Hematological profile among cutaneous leishmaniasis patients before and after treatment with sodium stibogluconate in Diyala, Iraq

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Abstract

Introduction: Cutaneous leishmaniasis (CL) is a common protozoan disease in Iraq characterized by localized ulcers, primarily on exposed skin. This study aimed to investigate the hematological parameters of infected patients using a complete blood count (CBC) in the endemic area of Diyala Governorate, northeast of Baghdad. This has been studied in newly diagnosed, untreated individuals and patients receiving sodium antimony gluconate.

Methodology: Hematological screening was performed on blood samples from 161 patients with microscopically diagnosed cutaneous leishmaniasis before and after treatment. Anti-Leishmania IgG was also assessed by ELISA in seropositive and seronegative subjects.

Results: The newly diagnosed, untreated patients showed no significant differences in blood cell counts, whereas treated patients had significant changes in white blood cell composition, including absolute neutrophil count (ANC), absolute monocytes (MID), eosinophils Granulocytes and Neutrophil-Lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), platelets count (PLT) and Mean Platelets Volume (MPV), following the administration of five consecutive sodium stibogluconate injection. In addition, the anti-Leishmania IgG seroprevalence was highest (85%) in the newly diagnosed, untreated group and gradually decreased with continued treatment. However, there was no significant difference in red blood cell components including red blood cells (RBC), hematocrit test (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW) before and after treatment.

Conclusions: The current data gave an insight into certain hematological factors regarding WBC subtypes, along with cutaneous leishmaniasis treatment. In addition, the anti-Leishmania IgG may be considered a marker for therapeutic monitoring.

Key words: Cutaneous leishmaniasis; sodium stibogluconate; CBC; anti-leishmanial IgG, Iraq.

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Introduction

Leishmaniasis is a group of tropical parasitic infections transmitted by female sandflies and caused by *Leishmania* species. The most common hosts of this zoonotic and anthroponotic disease are humans and dogs. The most common types of infection are cutaneous leishmaniasis (CL), visceral leishmaniasis (VL), and mucocutaneous leishmaniasis [1,2]. The most endemic and least fatal form of the disease is the cutaneous form, which is characterized by ulcerative skin ulcers. According to the World Health Organization (WHO), 1.5 million people are infected with cutaneous leishmaniasis each year, and millions more are at risk of infection in endemic areas of the Old and New World [3,4]. *Leishmania* eradication depends on components of the innate and adaptive immune systems that regulate host defense. Furthermore, the extent of the host immune response determines how CL manifests as a persistent granulomatous infection of the skin [5]. Phagocytic leukocytes are a key component of the anti-pathogen and tumor-healing immune response, and after a brief invasion period, neutrophil host cells begin to spread to areas where *Leishmania* infection occurs [6]. When *Leishmania* promastigotes are consumed by immune cells, a variety of microbial factors are produced that fight the infection [7]. Neutrophils also have a protective role against the majority of *Leishmania* infections. However, their effectiveness depends on the strain and neutrophiles' apoptotic or necrotic state [8]. Lymphocytes (usually T cells) play a key role in the production of cytokines that can activate or inhibit the antiparasitic effects of macrophages [9]. A complete blood count (CBC) is critical in a variety of settings, including the assessment of local or systemic inflammation and the diagnosis and treatment of disease, where hematological changes are closely related to disease prognosis [10]. However, some

studies suggest that hematological changes may be related to the severity of cutaneous leishmaniasis ulcers rather than to the diagnosis [11]. Worldwide, sodium stibogluconate (Pentostam) is the only successful clinical treatment for *Leishmania* ulcers. Intralesional injections are effective, but continuous administration (10–20 days) should be monitored, and drug side effects frequently occur during patient follow-up [12,13]. Additionally, thrombocytopenia and leukopenia have been shown to be associated with long-term Pentostam therapy [14].

On the other hand, in both cutaneous and visceral forms of leishmaniasis, the humoral immune response has an inconsequential role in immunological protection, in which the anti-leishmania IgG is found to promote the ulceration during the active stage of CL by inducing macrophage IL-10 [15,16]. Furthermore, high levels of IgG have been related to parasite persistence, despite little being known about how antibodies contribute to the healing of cutaneous leishmaniasis and protective immunity [17]. In this study, we have investigated the hematological profile and anti-leishmanial IgG of cutaneous leishmaniasis patients in newly diagnosed individuals and in those who were undergoing Pentostam treatment.

Methodology

Study area

The study was conducted from January to June 2022 at the Baqubah Teaching Hospital in the endemic area of Diyala City, northeast of Baghdad, Iraq. Baqubah is the administrative center of Diyala Province. It is located in the north of the country (-3.33 and -35.6), east longitude (-44.22 ºN and 45.56 ºE), 64 kilometers east of Baghdad and 416 kilometers from the Iranian border. Diyala has a population of approximately 467,895 inhabitants [18]. Blood samples were collected from patients visiting the central hospital from different parts of the city (Figure 1). Age, gender, lesion type, and number of all participants were documented.

Ethical considerations

Prior to engaging with participants, the collection of samples from patients received approval from the Ethical Committee of the College of Science/University of Baghdad (Approval No: CSFC/1221/0088/December 1, 2021). In accordance with the ethical guidelines outlined in the Declaration of Helsinki for medical research involving human subjects, each volunteer or legal guardian of patients under the age of 18 provided verbal consent.

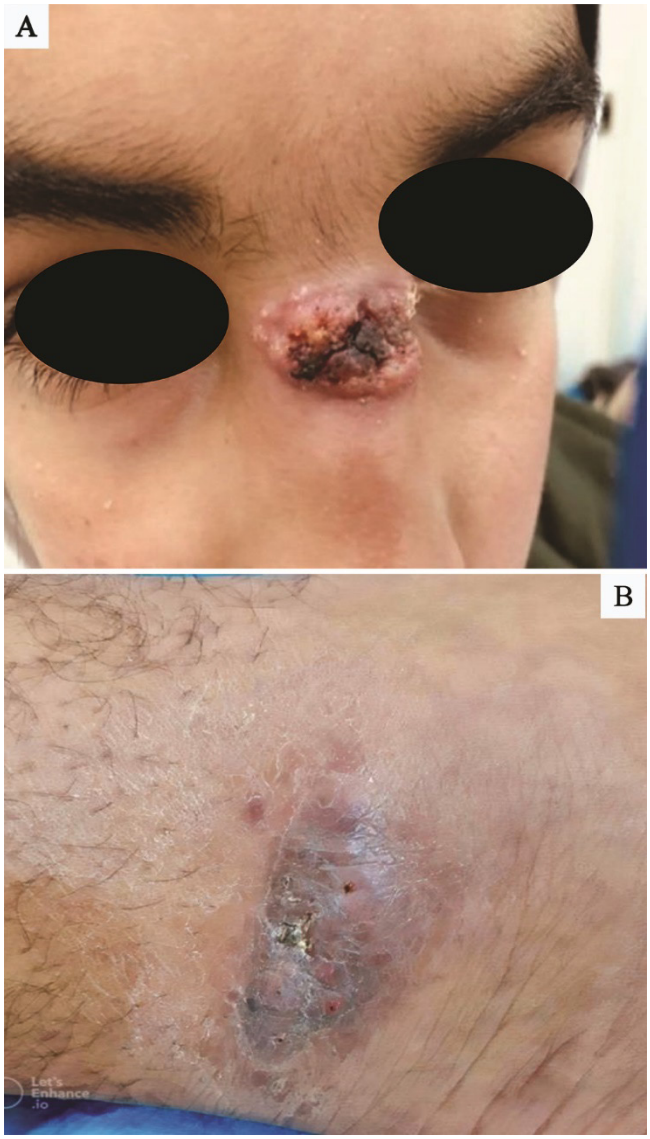
Figure 1. Map of Iraq shows the sample collection area - Diyala City - northeast of Baghdad, the capital city.

Observational study design and sample collection

Under the guidance of resident dermatologists, confirmed cases of cutaneous leishmaniasis were identified and treated with Pentostam at Baqubah Teaching Hospital's dermatology department. The diagnosis of cutaneous ulcers relied on the clinical assessment conducted by the dermatologists. To further confirm the diagnosis, scrapings from suspected lesions were subjected to Giemsa staining microscopy (Merck, Germany) [19,20]. Additionally, the characteristics of the lesions, such as dryness or wetness, were recorded. The investigation in this particular study focused on 161 individuals, consisting of 77 men and 84 women, all of whom had been diagnosed with CL. The age range of the participants spanned from 9 to 65 years old, with the exclusion of individuals who had chronic illnesses or had tested positive for coronavirus COVID-19. To establish a control group, a total of fifty blood samples were randomly obtained from the healthy population residing in the designated city. The collected samples were categorized into six primary groups. The first group consisted of individuals who had recently been diagnosed with CL and had not yet received any treatment. The remaining five groups consisted of patients who had received a series of five consecutive Pentostam injections every week. These patients suffered from various types of ulcerations, characterized by lesions with a diameter ranging from 2-5 cm and accompanied by inflammation at the ulcer

site. Inpatients in the dermatology section were treated if they had lesions causing chronic cosmetic issues or if the lesions were located on the legs and face (such as the eyelids and cheeks) and resulted in dysfunction or deformity. Additionally, CL patients with multiple lesions (more than five) were also treated as inpatients. The treatment protocol involved administering a 10 mg/kg injection of sodium stibogluconate (manufactured by Albert David Ltd. India) once a week. Blood samples were collected from the antecubital vein and placed in vacuum tubes containing EDTA (15% K3 EDTA, 0.054 ml/4.5 ml blood). These samples were screened within a few hours of collection [21].

Figure 2. A, erythematous ulcerated nodular lesion on the nose of female patient coated with black crusts; B, a cutaneous lesion on the leg of a patient with Cutaneous leishmaniasis after the fifth dosage of Pentostam treatment.



An automated complete blood counter (hematology analyzer) was used for the complete blood counts (CELL-DYN RUBY /Abbott Germany). The pre and five-post Pentostam treatment subjects were examined and compared with the healthy group for the following hematology profile: WBC, ANC, MID, eosinophils, NLR, PLR, PLT, MPV, RBC, HTC, MCV, MCH, MCHC, RDW and for serology of anti-leishmanial IgG.

Detection of anti-leishmanial IgG antibodies by ELISA

Every newly infected patient who was not receiving treatment had an IgG serology performed on the first day of their visit to the hospital. Serology results for patients receiving treatment were generated weekly. According to the manufacturer's instructions for a commercial kit (SUNLONG, China, catalogue number: SL3381Hu), anti-leishmanial IgG was found in the serum. ELISA plate reader (Promega Instrument), with optical density (OD) serving as the unit of measurement. The cut-off value for the indirect ELISA assay for anti-leishmania IgG antibodies was established by measuring absorbance at 450 nm.

Data analysis

Statistical analysis was performed using a student t-test using SPSS, where $p \leq 0.05$ was considered significant for each patient's group with the control.

Results

Patients

In this study, 161 local patients who visited the Baqubah Central Hospital and had defined cutaneous leishmaniasis ulcers were evaluated. As indicated in Table 1, there were 77/161 (47.8%) male subjects and 87/161 (52.1%) female subjects. The face, upper and lower extremities of the arms, legs and feet were the most often affected areas (Figures 2 and 3).

Diagnosis of CL

In the hospital's laboratory, all smears were viewed under a microscope for direct amastigote observation after lesion samples from every patient were collected and stained with Giemsa (Figure 4).

Hematological investigation

For white blood cells, the results showed a significant increase in the total number of white blood cells after dose 3 compared with the control group. Absolute monocyte count (MID), eosinophil count, and platelet count (PLT) increased significantly during treatment follow-up. In addition, absolute neutrophil count (ANC), neutrophil-lymphocyte ratio (NLR), and

Table 1. Distribution of clinical features of patients according to gender and age group.

Patients' information		Male		Female	
		number	%	number	%
Gender	160 patients	77	47.8%	84	52.2%
	50 controls	29	58%	21	42%
Age	>18 Years	27	35%	38	45.2%
	19-30 Years	28	36%	30	35.7%
	31-50 Years	20	25%	13	15.4%
	< 50 Years	2	2.5%	3	3.5%
	Total	77	47.8%	84	52.1%
	Control	29	58%	21	42%
Body sites affected by lesions	Upper limps				
	Face	3	6.2%	6	13.9%
	Arm	12	25%	8	18.6%
	Hand	25	52%	16	37.2%
	Chest	4	8.3%	5	11.6%
	Total	44	91.6%	35	81.3%
	Lower limps				
	Femur	1	2%	2	4.6%
	Leg	10	20.8%	12	27.9%
	Foot	10	20.8%	15	34.8%
	Toes	4	8.3%	0	0%
	Total	25	52%	29	67.4%
Lesion Type/patient	Dry	13	27%	15	34.8%
	Semi-dry	10	20.8%	13	30.2%
	Wet	9	18.7%	4	9.3%
	Semi- wet	15	31.2%	11	25.5%
Lesion Number/patient	One	16	33.3%	19	44.1%
	Two	9	18.7%	12	27.9%
	Three	8	16.6%	2	4.6%
	> Three	16	33.3%	10	23.2%
Employment activity	Farming	37	77%	38	88.3%
	Employee	15	31.2%	16	37.2%
	Studying	25	52%	30	69.7%

mean platelet volume (MPV) decreased during follow-up. However, no significant changes were detected in absolute lymphocyte count (ALC) and basophil count, as shown in Table 2. Regarding red blood cell composition, no significant changes were found in RBC, HBG, MCV, MCH, MCHC, and RDW (Table 3).

Anti-leishmanial IgG antibodies serology

Cut-off values for the indirect ELISA were calculated using results from 161 CL patient samples and 50 local healthy individuals (without a history of CL). Sixty-nine of 161 patients (42.8%) had positive ELISA results, whereas 92 patients (57.2%) had negative serum results. Interestingly, the greatest percentage of seropositive anti-Leishmania IgG was observed in the newly diagnosed, untreated group with 40 cases (85%), with only 7 cases (15%) being seronegative. Furthermore, there was a progressive decrease in seropositivity in the treatment groups at doses 1, 2, and 3. Additionally, all subjects showed seronegative results after doses 4 and 5 (Table 4).

Figure 3. Percentages of lesions in relation to various bodily parts.

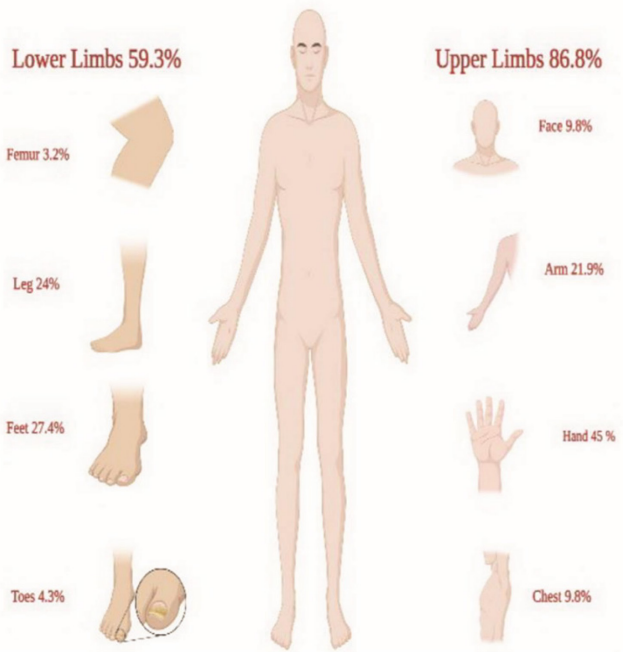


Table 2. Hematological parameters / WBC groups in CL patients and control subjects (∗↑ = significant increase, ∗↓= significant decrease).

Parameter	NEW	Ctrl	Pen-d1	Ctrl	Pen-d2	Ctrl	Pen-d3	Ctrl	Pen-d4	Ctrl	Pen-d5	Ctrl	*p*
WBC	7.23	6.84	6.8	6.84	6.92	6.84	7.38 ∗↑	6.68∗	7.01	6.84	6.94	6.84	≤ 0.05
ANC	3.83	3.64	3.57	3.64	3.6	3.64	3.93	3.93	3.62	3.64	2.75*↓	3.83*	≤ 0.05
ALC	2.39	2.44	2.2	2.44	2.4	2.44	2.37	2.37	2.47	2.44	2.62	2.44	≤ 0.05
MID	0.62	0.51	0.65	0.51	0.57	0.51	0.61*↑	0.47∗	0.59*↑	0.48∗	0.56	0.51	≤ 0.05
EOS	0.2	0.16	0.2	0.16	0.18	0.16	0.27*↑	0.13*	0.24*↑	0.16*	0.27 *↑	0.13*	≤ 0.05
BAS	0.19	0.15	0.19	0.15	0.17	0.15	0.14	0.15	0.14	0.15	0.17	0.15	≤ 0.05
NLR	1.73	1.55	1.74	1.55	1.57	1.55	1.72	1.55	1.63	1.55	1.12*↓	1.53*	≤ 0.05
PLR	128.54	90.12	105.56	90.12	97.8	90.12	107.05	90.12	103.48*↑	82.82*	107.83	90.12	≤ 0.05
PLT	267.58	209.5	222.95	209.5	223.07	209.5	247.39*↑	197.81*	255.44 *↑	195.56*	247.71*↑	209.5*	≤ 0.05
MPV	2.8	3.68	2.67	3.68	2.51*↓	3.68∗	2.93*↓	4.4∗	2.09*↓	3.68∗	1.79*↓	3.68∗	≤ 0.05

NEW, newly diagnosed; Ctrl, Control; Pen-d 1, 2, 3,4 or 5, Pentostam-dose- 1, 2, 3, 4 or 5; EOS, eosinophils; BAS, basophils.

Table 3. Hematological parameters / HBG groups in CL patients and control subjects.

Parameter	NEW	Ctrl	Pen-d1	Ctrl	Pen-d2	Ctrl	Pen-d3	Ctrl	Pen-d4	Ctrl	Pen-d5	Ctrl	*p*
RBC	5.44	5.48	5.31	5.66	5.3	5.48	5.45	5.48	5.37	5.48	5.49	5.48	≤ 0.05
HBG	13.59	13.81	13.14	13.81	12.81	14.06	13.62	13.81	13.38	13.81	13.33	13.81	≤ 0.05
HCT	40.51	40.8	38.99	40.8	38.45	40.8	39.88	40.8	39.56	40.8	38.9	40.8	≤ 0.05
MCV	72.89	74.43	73.71	74.44	73.29	74.43	73.49	74.44	73.84	74.44	70.44	74.44	≤ 0.05
MCH	25.13	25.18	24.82	25.18	24.78	25.18	25.11	25.18	24.91	25.18	24.16	25.18	≤ 0.05
MCHC	34.45	33.91	33.67	33.91	33.85	33.91	34.2	33.91	33.09	33.91	34.27	33.91	≤ 0.05
RDW	11.913	11.39	11.36	11.4	11.4	11.39	11.32	11.39	11.41	11.39	11.76	11.4	≤ 0.05

NEW, newly diagnosed Ctrl, Control; Pen-d 1, 2, 3,4 or 5, Pentostam-dose- 1, 2, 3, 4 or 5.

Table 4. Anti-leishmania lgG antibodies ELISA result.

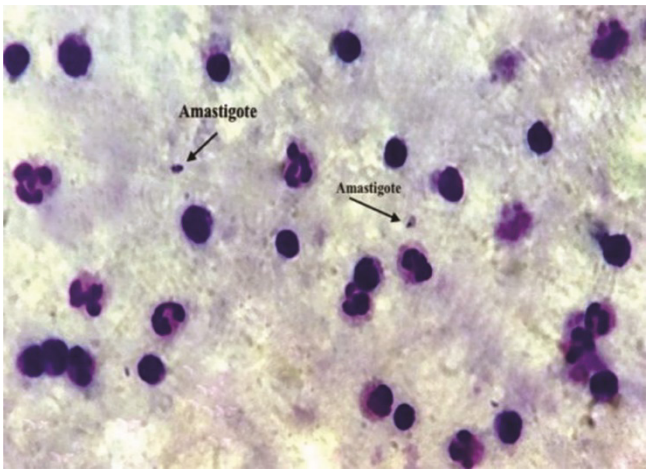
Groups	Seropositive	Seronegative	Total
NEW (no treatment)	40 (85%)	7 (15%)	47
Pen-d1	12 (31%)	26 (69%)	38
Pen-d2	8 (30%)	19 (70%)	27
Pen-d3	9 (35%)	17 (65%)	26
Pen-d4	0 (0%)	16 (100%)	16
Pen-d5	0 (0%)	7 (100%)	7
Total	69 (42.8%)	92 (57.2%)	161

NEW, newly diagnosed; Pen-d 1, 2, 3,4 or 5, Pentostam-dose- 1, 2, 3, 4 or 5.

Discussion

In this study, hematological characteristics and anti-leishmanial IgG were evaluated before and after treatment in patients with cutaneous leishmaniasis, in both genders regardless of age. According to research results in CL endemic areas, women under 18 years of age have the highest infection rate (45.2%), while men are more susceptible to infection due to outdoor activities [1]. There were 123 patients in young men and women under 30 years of age, accounting for 34% and 42% of all subjects, respectively, as this age group is more susceptible to sandfly bites than older adults in rural areas [22]. Furthermore, although older adults may have less lymphadenopathy and larger, disseminated skin lesions, the number of subjects older than 30 years is smaller [23]. In the studied Diyala province, the highest incidence of CL is endemic [24], but data on gender prevalence are sparse [25].

Figure 4. Amastigotes in blood smear under light microscope (1000 ×).



Although some studies mentioned that men are more susceptible to CL infection than women [26,27], other studies concluded that men's reported infection and exposure to hazardous occupations contribute to this epidemiological finding [28]. Hematological features have attracted attention, particularly in visceral leishmaniasis, either specifically or nonspecifically, and have been studied extensively [29]. Although the cutaneous form results in local non-systemic infection, hematological and nutritional characteristics are involved in the formation and clearance of erythematous nodules [30]. Most researchers focused on patients in the primary stages of skin infection and found that granulocytes such as

neutrophils were present in normal numbers early in lesion development [31]. Patients in the current study had normal blood cell counts early in the infection, even during the first two doses of Pentostam.

However, detection of high eosinophil counts late in treatment may trigger cellular mechanisms to eradicate amastigotes [32]. Number of granulocytes, monocytes, or lymphocytes play an important role in protection against *Leishmania* [32]. Higher monocyte numbers during treatment may indicate active anti-inflammatory effects but lower phagocytic function in the visceral form or may be related to lesion size in the cutaneous form [33,34]. Furthermore, a significant decrease in platelet counts and the development of thrombocytopenia was found in a murine model of visceral leishmaniasis [35]. The opposite result was found in CL patients where thrombocytosis was observed [36]. Interestingly, few studies have considered immune-inflammatory markers of leishmaniasis. Patients in this study showed significant increases in NLR and PLR during the later stages of treatment. This supports previous studies showing high rates during malaria infection [37]. Additionally, research conducted on visceral experimentation revealed that the administration of a single dose of Amphotericin B medication effectively restored the low blood platelet count to its normal levels [38].

The present study also assessed the levels of anti-leishmania IgG antibodies before and during therapy. It has been established that the presence of anti-leishmania IgG antibodies serves as a significant indicator for the development of CL infection [39,40]. Within our research population, the highest number of individuals testing positive for these antibodies was observed in the newly identified group. However, as treatment progressed, a clear reduction in seropositivity was observed, eventually leading to complete seronegativity. Previous research has also demonstrated a correlation between the severity of CL and high levels of anti-leishmania IgG and IgG subclasses [41,42]. Additionally, successful treatment has been shown to decrease the titers of Leishmania-specific antibodies in CL patients [43,44]. The humoral immune response in CL patients has been found to vary based on clinical factors such as lesion size, type and number [42]. Likewise, in Iraq, the main endemic species of *Leishmania* is *L. tropica*, making it possible to use anti-leishmania IgG as a diagnostic marker specifically for this species [45,46].

Conclusions

In the current study, the blood profile of patients receiving treatment for CL was observed to exhibit variability, particularly in relation to WBC components. This finding is significant in terms of monitoring treatment progress, alongside the examination of anti-leishmanial IgG levels for seropositive analysis.

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