WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 3, 103-119. Research Article ISSN 2277– 7105

IMMUNOLOGICAL STUDY ON ASTHMATIC PATIENTS WORKING IN OIL AND GAS REFINERIES IN KIRKUK, IRAQ

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Article Received on
19 Dec 2014,

Revised on 13 Jan 2015,
Accepted on 07 Feb 2015

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ABSTRACT

Background: Asthma is a global health problem affecting around 300 million individuals of all ages, ethnic groups and countries. It is estimated that around 250,000 people die prematurely each year as a result of asthma.[1,2,3] work-related exacerbation of asthma particularly in oil and gas refineries ranging from single transient exacerbations after an unusual exposure to daily work-related worsening that can mimic occupational asthma.[24] This disease might occur in up to 25% of working persons with asthma. **Methods**: This study was carried out at Kirkuk city in the North Oil Company (N.O.C.), which is a state company within the Ministry of Oil of Iraq. 100 subjects were included in this study from both genders. Their ages ranged from 25 to 65 years. 50 of them were attending allergy/ immunology center in Kirkuk city. The other 50 adult asthmatic patients were working in North Oil Company. **Methods**: All subjects included in this study were investigated for total and differential white blood cell count by using Quintus automatic haematological analyzer, serum interleukin-6 (IL-6), serum interleukin-8 (IL-8), serum tumor necrosis factor-α (TNF-α) and serum immunoglobulin E (IgE) using ELISA technique, serum immunoglobulin A (IgA) and serum complement component 4 (C4) radial immunodiffusion plate. **Results**: Regarding differential white blood cells count, the mean of different lymphocytes were significantly different among the three groups of patients examined in North Oil and Gas Company patients, other patients and control group ($P < 0.01$).The values of Interleukins IL-6,IL-8 and TNF-α were also different with type of patient tested. The evaluation of immunoglobulins IgE was statistically different but IgA did not show a significant difference ($P \geq 0.05$). Complement C4 revealed a significant difference between patient groups tested and the mean level of serum C4 was

(29.1), (35.9) and (18.7) in North Oil and Gas Company patients, Other patients and the control group respectively. **Conclusions**: There was a significant association between asthma and serum IL- 8 level between study groups. The highest level of serum IgE appeared in the other asthmatic patients and there was a significant relationship with oil and gas refineries asthma and control group while IgE level in oil & gas refinery patients was non-significant. The present study showed a highly significant difference in serum C4 level among groups studied.

KEYWORDS: Quintus automatic haematological analyzer, immunoglobulins IgE.

1. INTRODUCTION

Asthma is one of the most common chronic diseases worldwide. Among several noninfectious respiratory disorders affecting human being, Bronchial asthma is the most common chronic disease that can impede breathing**.** Asthma is a global health problem affecting around 300 million individuals of all ages, ethnic groups and countries. It is estimated that around 250,000 people die prematurely each year as a result of asthma.[1,2,3] A continuing increase in the number of people with asthma worldwide over the two next decades has been predicted such that it is estimated that there may be an additional 100 million people with asthma by the 2025.[1] The concepts underlying asthma pathogenesis have evolved dramatically in the past 25 years and are still undergoing evaluation as various phenotypes of this disease are defined and greater insight links clinical features of asthma.[4] Unfortunately, despite the availability of effective therapies, with genetic patterns suboptimal asthma control exists in many patients on a world- basis.[4,5] The increasing global outbreak of asthma, with its large burden over patients and the high health care costs have led to extensive research about its mechanisms and treatment.[6]

Work-related or occupational asthma is defined as a chronic inflammatory disorder of the airways with recurrent episodes of respiratory symptoms such as coughing, wheezing, chest tightness, dyspnea, shortness of breath at rest, and reversible airflow limitations caused by a particular occupational environment.[7] The prevalence of occupational diseases shows the quality of working conditions and health of working environment. Respiratory diseases are common entities in occupational industries, because the lungs are the route of entry for noxious particles and gases. These agents can be inhaled in the form of fibers or dusts. The development of occupational respiratory disease is dependent on several factors including the chemical nature and physical state of the inhaled substance, the size and concentration of the

dust particles, the duration of exposure, and individual susceptibility.[8] work-related exacerbation of asthma — ranging from single transient exacerbations after an unusual exposure to daily work-related worsening that can mimic occupational asthma.[9]

2. MATERIALS AND METHODS

Description of the refinery

This study was carried out at Kirkuk city in the North Oil Company (N.O.C.), which is a state company within the Ministry of Oil of Iraq. The company contains more than fifty installations comprising pump stations, process units, oil tank fields, degassing stations, gas compressor stations, water treatments plants, electric generation stations , and a large number of oil wells which are connected with a network of flow lines and pipelines dispersed throughout the company‘s area of operation, This company occupies first position among extracting oil companies in the Middle East and the world.

The number of workers at this company is about 12000 workers distributed in six main sections and these sections are divided into major sub-sections. There are roughly three types of gases emitting from oil companies: suffocating gases (H2, CH4, CO2) where they take a space in the air of the working environment which leads to lowering the percentage of oxygen, irritating gases (Cl2, F) in which they cause irritation and inflammation in both skin and respiratory tissues and the erosion of these tissues leads to the death of the cells, and toxic gases (CO, H2S) poisoning with CO causes stress, mental illusion, losing the ability to concentrate and unconscious while H2s affects the respiratory center in the brain. There are heavy metals and other substances in which the continuous exposure to them causes chronic poisoning among the workers as a result of their exposure to them either in the form of dust or smoke or steam vapours in the working environment and injury is caused either by inhalation or precipitation the major heavy metals are Pb, Cu, Ni, Cr, Cd, S, P and CCl4.

Study design

This cross-sectional study was conducted in kaiwan General hospital in the North Oil Company and Allergy /Immunology Center in Kirkuk city during the period from January 2014 to August 2014. Data were collected by personal interview and using questionnaire. Participants were informed about the study, and those who agreed to participate were included.

Patients selection

100 subjects were included in this study from both genders. Their ages ranging from 25 to 65 years. 50 of them were attending allergy/immunology center in Kirkuk city. The other 50 adult asthmatic patients were working in North Oil Company. Asthmatics were defined as having work-related asthma if they answered positively the questions: ―Have you ever had respiratory symptoms in relation to your work? Did the symptoms improve on absence from work?

A number of studies carried out on asthmatic populations.[10,11] and community samples.[12,13] have tried to assess the proportion of asthma of occupational origin. The term occupational asthma is often used in these studies. This term implies a causal relationship between the exposure and the disease.[14] However, in none of the studies cited is it possible to tell whether the asthma had started prior to the exposure or if it had started after.

Exclusion criteria

1. patients with chronic obstructive pulmonary disease (COPD).
2. pregnant women.
3. patients with cardiovascular disease.
4. Children and adolescents.
5. Uncooperative patients.

Control group

Fifty individuals were considered as a control group in this study. They were apparently healthy, after taking details of history of asthma and clinical examination the same methods and instruments were used in all stages for the control group as for the asthmatic group.

Diagnosis

Clinical evaluation

Patients previously diagnosed with asthma by physician were chosen. The inclusion criteria for all cases were bronchial asthma, where the diagnosis was established through demonstrating reversible airway obstruction.

Blood sampling

Alcohol 70% is used to sterilize the area of blood aspiration from basalic vein in the anticubital fossa. Five ml blood sample was collected from all subjects (patients and control)

using disposable syringes. Each blood sample was divided in 2 divisions as follow:
(a) 2 ml of blood sample was obtained in tube containing (EDTA) as anticoagulant for the estimation of total WBC count and complete blood picture.
(b) 3 ml of blood sample collected in plane tube for the estimation of serum IgA, IgE, IL-6, IL-8, TNF-α and C4.

The blood sample in tube (a) was shacked gently for mixing the blood with anticoagulant to prevent clot formation. While the blood in tube (b) was allowed to clot and then centrifuged at 3000 rpm for 15 minutes and the serum was then removed to another plain tube and stored at - C for further studies. ny sample showed haemolysis was discarded.[15]

Estimation of complete blood count

Complete blood picture was done using automatic haematological analyzer Quintus (Boule medical AB, Sweden).

Estimation of serum interleukin 6(IL-6), Interleukin 8 (IL-8) and tumor necrosis factor-alpha (TNF-α)

The concentration of IL-6, IL-8 and TNF-α in the sera of patients and control group was quantitatively measured utilizing sandwich enzyme linked immunosorbant format following the manufacturer instructions.

Estimation of serum immunoglobuline E (IgE) (IU/ml)

Serum IgE was estimated by a solid phase enzyme-linked immunosorbent assay. The IgE quantitative enzyme immunoassay provides a rapid, sensitive, and reliable assay for total serum IgE. The minimal sensitivity of this assay is about 5.0 IU/ml.

Determination of the serum complement 4 (C4) (mg/dl) protein and serum immunoglobuline A (IgA) (mg/dl)

Serum C4 protein and serum IgA was determined by radial immunodiffusion plate.

Statistical analysis

Computerized statistically analysis was performed using Minitab version 11 statistic programme. Comparison was carried out using Chi-square (X2), ANOVA and probability (P value).

Ethical consideration

Eathical consideration for study was obtained from North Oil company, Kaiwan hospital and Allergy/Immunology Center at Kirkuk city. A verbal consent was taken for all participants in this study.

3. RESULTS

Count of white blood cells WBCs (10 3/ML) of asthmatic patients and control group

Table 1 shows that the mean level of white blood cell count was 8.17, 8.32 and 7.96 in North Oil and Gas Company working patients, other patients and the control group respectively. There was a statistically non-significant difference between these groups using Anova test ($P > 0.05$).

Table 1. Count of white blood cells WBCs (10 3/ML) of asthmatic patients and control group:

Group	Oil & Gas company patients	Other patients	Control
Number	50	50	50
Mean	8.17	8.32	7.96
St. Deviation	2.19	2.37	2.36
Std. Error of Mean	0.31	0.33	0.33

Mean neutrophil (%) of asthmatic patients and control group

Table 2 shows that the mean neutrophil count was 50.4%, 44.9% and 56.4% in North Oil and Gas Company working patients, other patients and control group respectively. There was a statistically highly significant difference between these groups using Anova test ($P < 0.01$).

Table 2. Neutrophil count (%) of asthmatic patients and control group:

Group	Oil & Gas company patients	Other patients	Control
Number	50	50	50
Mean	50.4	44.9	56.4
St. Deviation	12.8	14.7	8.9
Std. Error of Mean	1.8	2.1	1.2

Mean lymphocyte (%) of asthmatic patients and control group

Table 3 shows that the mean Lymphocyte count was (24.3), (14.2) and (34.2) in North Oil and Gas Company worker patients, Other patients and the control group respectively. There was a statistically highly significant difference between these groups using Anova test ($P < 0.01$).

Table 3. Lymphocyte count (%) of asthmatic patients and control group:

Group	Oil & Gas company patients	Other patients	Control
Number	50	50	50
Mean	24.3	14.2	34.2
St. Deviation	10.5	4.1	7.7
Std. Error of Mean	1.5	0.58	1.1

Mean monocyte (%) of asthmatic patients and control group

Table 4 shows that the mean monocyte count was 19.7, 30.68 and 7.5 in North Oil and Gas Company working patients, other patients and control group respectively. There was a statistically highly significant difference between these groups using Anova test ($P < 0.01$).

Table 4. Monocyte count (%) of asthmatic patients and control group:

Group	Oil & Gas company patients	Other patients	Control
Number	50	50	50
Mean	19.7	30.68	7.5
St. Deviation	12.9	8.4	7.2
Std. Error of Mean	1.8	1.2	1

Mean eosinophil (%) of asthmatic patients and control group

Table 5 shows that the mean eosinophil count was 0.92, 1.06 and 0.21 in North Oil and Gas Company working patients, other patients and the control group respectively. These results were a statistically highly significant using Anova test ($P < 0.01$).

Table 5. Eosinophil count (%) of asthmatic patients

Group	Oil & Gas company patients	Other patients	Control
Number	50	50	50
Mean	0.92	1.06	0.21
St. Deviation	1.5	1.3	0.27
Std. Error of Mean	0.2	0.1	0.03

Mean basophil (%) of asthmatic patients and control group

Table 6 shows that the mean level of basophil count was 3.87, 4.66 and 1.09 in North Oil and Gas Company working patients, Other patients and control group respectively. These results were highly significant using Anova test ($P < 0.01$).

Table 6. Basophil count (%) of asthmatic patients and control group

Group	Oil & Gas company patients	Other patients	Control
Number	50	50	50
Mean	3.87	4.66	1.09
St. Deviation	3.38	3.8	2.44
Std. Error of Mean	0.4	0.5	0.3

Mean serum interleukin-6 (IL-6) (pg/ml) level among asthmatic patients and control group

Table 7 shows that the mean serum level of IL-6 was 23.8, 23.5 and 21.4 in North Oil and Gas Company working patients, other patients and the control group respectively. The result was not significant using Anova test ($P > 0.05$).

Table 7. Estimation of serum interleukin-6 (IL-6) (pg/ml) level of asthmatic Patients and control group

Group	Oil & Gas company patients	Other patients	Control
Number	50	50	50
Mean	23.8	23.5	21.4
St. Deviation	18.18	28.4	19.44
Std. Error of Mean	2.5	4	2.7

Mean serum interleukin-8 (IL-8) (pg/ml) level among asthmatic patients and control group

Table 8 shows that the mean serum level IL-8 was 34.06, 60.6 and 26.3 in North Oil and Gas Company working patients, other patients and the control group. These results were significant using Anova test ($P < 0.05$).

Table 8. Estimation serum inerleukin-8 (IL-8) (pg/ml) level of asthmatic patients and control group

Group	Oil & Gas company patients	Other patients	Control
Number	50	50	50
Mean	34	60.6	26.3
St. Deviation	65.2	127.3	26.7
Std. Error of Mean	9.3	18.1	3.7

Mean serum tumor necrosis factor (TNF-α) (pg/ml) level of asthmatic patients and control group

Table 9 shows that the mean serum level of TNF-α was 59, 36.9 and 19. in North Oil and Gas Company working patients, other patients and the control group respectively. The result was non-significant using Anova test (P > 0.05).

Table 9. Estimation serum tumor necrosis factor (TNF-α) (pg/ml) level of asthmatic patients and control group

Group	Oil & Gas company patients	Other patients	Control
Number	50	50	50
Mean	59	36.9	19.2
St. Deviation	242	94.7	50
Std. Error of Mean	34.5	13.5	7.1

Mean serum immunoglobulin E (IgE) (IU/mL) level of asthmatic patients and control group

Table 10 shows that the mean serum level of IgE was 130.9, 309.9 and 88.7 in North Oil and Gas Company working patients, other patients and the control group respectively. These results were highly significant using Anova test (P < 0.01).

Table 10. Estimation of serum immunoglobulin E (IgE) (IU/mL) level of asthmatic patients and control group

Group	Oil & Gas company patients	Other patients	Control
Number	50	50	50
Mean	130.9	309.9	88.7
St. Deviation	177.7	312.8	110.6
Std. Error of Mean	25.3	44.6	15.8

Mean serum immunoglobulin A (IgA) (mg/dl) level of asthmatic patients and control group

Table 11 shows that the mean level of serum IgA was 189, 201 and 188 in North Oil and Gas Company working patients, other patients and the control group respectively. The result was non-significant using Anova test (P > 0.05).

Table 11. Estimation of serum immunoglobulin A (IgA) (mg/dl) level of asthmatic patients and control group

Group	Oil & Gas company patients	Other patients	Control
Number	50	50	50
Mean	189	201	188
St. Deviation	91.5	108	116.8
Std. Error of Mean	13	15.4	16.6

Mean serum complement 4 (C4) (mg/dl) level of asthmatic patients and control group

Table 12 shows that the mean level of serum complement C4 was 29.1, 35.9 and 18.7 in North Oil and Gas Company working patients, other patients and the control group respectively. These results were highly significant using Anova test ($P < 0.01$).

Table 12. Estimation of serum complement 4 (C4) (mg/dl) level of asthmatic patients and control group

Group	Oil & Gas company patients	Other patients	Control
Number	50	50	50
Mean	29.1	35.9	18.7
St. Deviation	15.6	25.3	11.3
Std. Error of Mean	2.2	3.6	1.6

Table 13. Comparison of changing patterns of different immunological parameters Tested

Parameter Mean±SD	Oil & Gas company patients	Other patients	Control
W.B.C (10^3/ML)	8.17±2.19a	8.32±2.37a	7.96±2.36A
Neutrophil count (%)	50.4±12.8b	44.9±14.7c	56.4±8.9A
Lymphocyte Coun (%)	24.3±10.5b	14.2±4.1c	34.2±7.7A
Monocyte Count (%)	19.7±12.95b	30.68±8.4a	7.5±7.2C
Eosinophil count (%)	0.92±1.5a	1.06±1.3a	0.21±0.27B
Basophil count (%)	3.87±3.38a	4.66±3.8a	1.09±2.44B
Serum IL-6 (pg/ml)	23.8±18.17a	23.5±28.4a	21.4±19.43A
Serum IL-8 (pg/ml)	34.06±65.2b	60.6±127.3a	26.3±26.7C
Serum TNF-α (pg/ml)	59±241.9a	36.9±94.7a	19.2±50A
Serum IgE (IU/mL)	130.9±177.6b	309.8±312.8a	88.7±110.6B
Serum IgA (mg/dl)	189±91.5a	201.±108a	188±116.8A
Serum C4 (mg/dl)	29.1±15.6b	35.9±25.3a	18.7±11.3C

*The same letter in one column means that there was no significant difference between these Values

4. DISCUSSION

The relationship between lymphocyte count (%) and asthma among study groups

Mean Lymphocyte count was 24.3, 14.2 and 34.2 in oil refinery patients, other patients and the control group respectively. There was statistically highly significant difference between these groups using Anova test ($P < 0.01$). This was contraindicated by Sarah[16] who found that lymphocyte counts in peripheral blood were not related to any respiratory symptom or diagnosis. Also contraindicated by Salh[17] who found non-significant association between serum lymphocyte count in cement and oil refinery workers and control groups. This was in agreement with Wood et al[18] who found that the level of lymphocyte was lower than asthmatic patients and this difference was significant. The mean monocyte count was 19.7, 30.68 and 7.5 in oil refinery patients, other patients and control group respectively. There was a statistically highly significant difference between these groups using Anova test ($P < 0.01$). The present result was contraindicated by FRANCO[19] who found that the level of bronchoalveolar lavage monocytes was lower in both occupational and non occupational asthma than normal control. The mean Eosinophil count was 0.92, 1.06 and 0.21 in oil refinery patients, other patients and the control group respectively. These results were highly significant using Anova test ($P < 0.01$).

Eosinophils represent the main effector cell population of the inflammatory response, which underlies formation of allergic diseases such as bronchial asthma as mentioned by Fahy[20] who suggested depending on recent studies of subjects with well characterized asthma.

Eosinophilic asthma has been defined as a distinct phenotype of asthma that is associated with good pharmacological responsiveness to corticosteroids. In contrast, patients with noneosinophilic asthma, who represent a sizeable subgroup that includes patients with severe disease, appear to be relatively resistant to corticosteroid therapy.[20] The eosinophils play an important role in the defense mechanism of the body and their count increases during allergic conditions and parasitic reaction. These are responsible for disintegration, detoxification and removal of foreign particles.[21]

Increased numbers of eosinophils in peripheral blood and in airway secretions are a characteristic feature of asthma. Bousquet and coworkers[22] have shown that the number of eosinophils in peripheral blood and in bronchial lavage from subjects with asthma is associated with more severe disease. This association between eosinophilia and outcomes of

asthma severity has been confirmed and extended in several other studies. For example, Louis and colleagues[23] showed that eosinophil numbers in induced sputum are highest in patients with more severe asthma. These results were almost similar to those of Mashhadani[24] who found a significant increase in eosinophil number in asthmatic patints. The mean level of basophil count was 3.87, 4.66 and 1.09 in oil refinery patients, other patients and control group respectively. These results are highly significant using Anova test ($P < 0.01$). Basophils possess high levels of the FcεRI receptor and are capable of an immediate response to allergen. Although basophils are not present in healthy airways but they are present in the airways of asthmatic persons under a variety of circumstances. Basophils have been reported in the sputum of patients with symptomatic asthma.[25] Recent studies have demonstrated a basophil infiltration of airways in cases of fatal asthma.[26,27] Increasing basophil counts were associated with increased wheezing, nocturnal dyspnea, and with diagnosed asthma.[16] Although basophils have been viewed as having functions similar to mast cells, recent work has highlighted the unique functions of basophils and their role in allergic responses and immune regulation.[28,29]

The relationship between mean serum interleukins levels and asthma among study groups

The results of the current study revealed that the mean serum level of interleukin-6 was 23.8 in oil refinery patients, 23.5 in other patients and 21.4 in the control group. These results were non-significant using Anova test ($P > 0.05$). The serum concentration of the cytokine IL_6 is almost similar in this study and this may be attributed to the same level of environmental pollution which the study groups are exposed to. The levels of IL-6 in serum have been found to be elevated in a number of inflammatory disease.[30] As a result, IL-6 has long been considered a general marker of inflammation together with TNFα and IL-1β, two other classical inflammatory cytokines(Table 13). However, it is becoming evident that IL-6 is not simply a proinflammatory marker, but an active factor that contributes to the pathogenesis of certain inflammatory diseases such as rheumatoid arthritis, and a successful target for some of these diseases.[30] IL-6 is a pleiotropic cytokine whose role in asthma remains unclear, but It has growth regulatory effects on many cells and is involved in T cell activation, growth, and differentiation. It is a terminal differential factor for B cells and induces immunoglobulin like IgG, IgA and IgM secretion.[31] IL-6 is an important cofactor in IL-4 dependent IgE synthesis.[32] It upregulates the production of, and its response to, IL-2. IL-6 may also have anti-inflammatory effects. It inhibits the expression and release of IL-1 and TNF from

macrophages in vitro and endotoxin induced TNF production and neutrophil influx in the airways in vivo.[33–34] In IL-6 transgenic mice there is a lymphocytic infiltration around airways associated with reduced airways responsiveness.[35]

There is increased release of IL-6 from alveolar macrophages from asthmatic patients after allergen challenge.[36] Yokoyama et al showed that circulating IL-6 levels were higher in asthma patients compared with control patients, and that IL-6 levels were increased after asthmatic attacks[37] Canöz found that IL-6 level was higher in asthmatic patients than control group.[38] The mean serum level IL-8 is 34.06, 60.6 and 26.3 in oil refinery patients, other patients and the control group respectively. These results were significant using Anova test ($P < 0.05$). The results of the current study revealed that the mean level of serum interleukin-8 was significantly higher in those asthmatic patients in the city in comparison with asthmatic patients who were working in the oil refinery and control group.

Enhanced co-expression of IL-8 and GM-CSF in bronchial epithelial cells of patients with asthma has been reported.[39] Free IL-8 has been detected in the serum and bronchial tissue of subjects with severe atopic asthma but not in normal subjects or those with mild atopic asthma, suggesting that IL-8 may be a marker of severe asthma. IL-8 was also found to be complexed with IgA, levels of which were raised in bronchial tissue in asthma.[40] IL-8 might participate in development of airway inflammation and airway hyper responsiveness in asthma.[41] The mean serum level of TNF-α is 59, 36.9 and 19. in oil refinery patients, other patients and the control group respectively. The result was non-significant using Anova test ($P > 0.05$). This may be because the present samples were collected in resting state and not during asthma exacerbation or perhaps due to the fact that TNF increases along with IL-1 and IL-6 during the acute phase response to many infections and the samples were collected.

TNF- α has a number of other actions which may be relevant to asthma: it is chemoattractant for neutrophils and eosinophils.[42] it increases the cytotoxic effect of eosinophils on endothelial cells.[43] TNF- α may have an important amplifying effect on asthmatic inflammation.[44,45] There is an evidence of increased expression in asthmatic airways.[46] TNF- α is also released from alveolar macrophages of asthmatic patients after allergen challenge.[47] These results were almost similar to Rothman et al[48] who found similar peripheral levels in benzene exposed subjects and controls.

5. CONCLUSIONS

The higher number of asthmatic patients in oil and gas refineries workers were technicians. The total white blood cell count was almost similar in oil refinery patients, other patients and control group. There was a significant association between asthma and serum IL-8 level between study groups. There was a highly significant difference in serum C4 level among different groups of asthmatic patients and this would a factor needs further studies for elaboration of gas and oil refineries products effect on immune system.

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