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EFFECT OF AEROMONAS HYDROPHILA AND ITS BACTERIN ON THE BLOOD AND CERTAIN SERUM CONSTITUENTS OF *OREOCHROMIS NILOTICUS* FISH

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Abstract

Three groups of *O.niloticus* fish, the first was injected with *Aermonas hydrophila*, the second injected with formalized whole cell bacterin and the last with phosphate buffer saline (PBS). Red and white cell counts, serum parameters denoting liver and kidney functions were measured over a 4-weeks period. As a results of infection, most of the clinical signs and lesions indicating Aermonas septicaemia were developed, during which red cell count, haemoglobin and haematocrite values were decreased, however, white cell counts showed an increased level during the first 9 days post-infection followed by a gradual decrease up to the end of the experimental period. Gradual insignificant increase in lymphocytes, monocytes and oesinophils was noticed in infected fish group during the first 9 days post-intection, followed by a significant progressive decrease up to the end of experimental period. Significant increase in serum bilirubin, blood urea nitrogen was recorded in infected fish group, however, a significant decrease in serum proteins, cholesterol and serum glucose was also found. On the other hand, the measured parameters in bacterin infected group were mostly close to those of control group except for the recorded increase in white cell count, especially lymphocytes and the insignificant increase in serum total proteins.

INTRODUCTION

The expansive development of fish aquaculture has been paralled by the increasing range of pathogens encountered and consequently disease problems. Much losses are caused by bacterial infections among which *Aeromonas hydrophila* is considered as one of the most predominant bacteria existing in fish aquaculture and causing disease conditions usually referred to as haemorrhagic septicaemia (Post, 1987).

Infection caused by *A.hydrophila* in freshwater fish are usually characterized

externally by skin and fin haemrrhages, ulcerations and ascites and internally by congestion of visceral organs (Roberts, 1989).

Bacause of the massive destruction of various tissues during *A.hydrophila* infections, it was postulated that significant changes should occur in physicochemical parameters associated with haematopoietic, liver and kidney functions (Eissa and Abd-Alla, 1991). On the other hand, active immunization against *A.hydrophila* is one of several methods used for controlling its infection in freshwater fish.

The present study was thus planned to evaluate blood parameters and serum chemistry denoting liver and kidney functions in *O.niloticus* during *A.hydrophila* infection and immunization. These also may explain the mechanisms by which fish are reacting under such circumstances, and also, considered an early detection of deviated health status of cultured fish.

MATERIALS AND METHODS

Fish

Apparently healthy *Oreochromis niloticus* fish of an average body weight 90±5 g were collected from Nawa fish farm at Kalubia governorate. Fish were stocked on glass aquaria with aerated chlorine free tap water, thermostatically adjusted at 25±1 ^{o}C. Fish were fed commercial ration twice a day to satiation and left 2 weeks for acclimatization.

Bacteria and formalized whole-cell culture (bacterin)

A well identified pure culture of *A.hydrophila* was kindly supplied by Department of Fish Diseases, Animal Health Research Institute, Dokki, Giza, Egypt. The given strain was recorded to induce Beta type of haemolysis on 10% sheep blood agar and its LC_{50} was 6 X 10^{6} Colony forming units per ml^{-1} (Reed and Muench, 1938). A standard broth culture of *A.hydrophila* and formalized whole cell were prepared as described by Mulvey *et al.* (1995). The final cell concentration was 3 X 10^{7} colony forminy units (CFU) $^{ml-}$1. Sterility and safety of bacterin was tested according to Ward (1982).

Experimental design

Two experimental fish groups of 40 fish each and a third control group of 20 fish were used in this study. The first group was used for experimental pathogenicity where

each fish received an interaperitoneal dose of 0.2 ml of 24h *A.hydrophila* broth culture. Fish of the second group were intraperitoneally injected a dose of 0.2 ml per fish with formmalized 24h *A.hydrophila* broth culture. The third group was left as a control where each fish received a dose of 0.2 ml phosphate buffered saline (PBS pH 7.2) intraperitoneally.

Fish groups were continuously examined for gross clinical signs, lesions and mortalities where dead fish were subjected to post-mortem and bacteriological examinations. Ten blood samples were randomly collected from each fish group (Lucky, 1977) at the following time points: day 0 pre-treatment, then on day 2nd, 5th, 9th, 14th, 21th and 28th day post-reatment. Collected blood and corresponding sera samples were biochemically analysed for estimating total erythrocytic count, total and differential leukocytic counts, packed cell volume (haematocrit) and haemoglobin contents according to the methods described by Lucky (1977).

Serum samples were analysed for the assessment of renal and hepatic functions through the estimation of the following parameters: urea (Fawcett and Scott, 1960), creatinine (Husdan and Rapoport, 1968), total protein (Wotton and Freeman, 1982), total cholesterol (Richmond, 1973), total bilirubin (Walters and Gerade, 1970), SGOT and SGPT (Reitman and Frankel, 1957), alkaline phosphatase (Kilchling and Freiburg, 1951) and glucose (Trinder, 1969).

The obtained data were statistically analysed according to Hill (1972).

RESULTS AND DISCUSSION

The experimental infection of *O.niloticus* with *A.hydrophila* resulted in a sequence of progressive signs, lesions and physiochemical changes in comparison to those in immunized and control fish groups.

Morbidity among infected fish group reached 90% 48 hours posti-nfection, while, mortality percent reached 23% throughout the experimental period. Moribund fish exhibited a loss of balance, exophthalmia, darkened skin colour, skin and fins haemorrhages, inflamed vent and abdominal dropsy (Fig., 1). Post-mortem findings revealed the presence of congested internal organs and variable amounts of reddish yellow ascitic fluid. At late stages of infection, visceral adhesion and pale anaemic areas on the surface of liver were commonly seen. These signs and lesions are usually encounted during the course of haemorrhagic septicaemia caused by *A.hydrophila* as

recorded by Post (1987) and Roberts (1989). On the other hand, mortality percent did not exceed 3% and 2% in bacterin infected and control fish groups, respectively, which were proved to be non-specific after bacteriological examination.

The manifestation of the previously mentioned signs and lesions suggested that significant alterations should occur in the physiochemical parameters associated with haematopoietic, liver and kidney functions.

Data shown Table 1 clarified a significant progressive decrease in the mean red cell counts, haemoglobin percent and mean haematocrit values in infected fish group in comparison to the control and bacterin injected fish groups throughout the experimental period. These results are directly related to the induced haemolysis of RBCs mediated by the haemolytic activity of *A.hydrophila* which is clinically manifested by the observed haemorrhages, the most characteristic clinical signs of *A.hydrophila* infections (Thune *et al.*,1986).

As a consequent result of red cell loss, haemoglobin percent and mean haematocrit values were also progressively declined. These findings concised with of Amlacher (1961) who recorded sharp decrease in erythrocyte counts, haemoglobin percent and haematocrit values of carp affected by haemorrhagic septicaemia.

Regarding total leucocytic count, data in Table 1 cleared the insignificant increase of WBCs during the the first 9 days post-infection followed by gradual significant decrease up to the end of the experimental period which may be a result of early immune response to the injected bacteria followed by the destruction of haemopoietic tissues resulted from the action of bacterial toxins with subsequent loss of WBCs, as also recorded by Roberts (1989). On the other hand, WBCs count in bacterin infected group showed values close to those of control group during the first 14 days post-injection with subsequent insignificant increase up to the end of experimental periods which could be a result of immune system stimulation.

Regarding differential leucocytic count, data in Table 2 revealed the presence of gradual insignificant increase in lymphocytes, monocytes and oesinophils in infected fish group during the first 9 days post-infection followed by a significant progressive decrease up to the end of experimental period. These results may be attributed to the stimulation of haemopoietic tissues end immune system, as well as, the important role played by these cells during such bacterial infection (Roberts, 1989). However, the drastic and destructive effects of injected bacteria on haemopoietic tissues in addition to blood loss during infection may account for the subsequent recorded significant decrease of these cells (Amlacher, 1961and Roberts, 1989). On the other hand, the

recorded significant increase in lymphocytes in bacterin injected fish group on 21 and 28 day time points may reflect the critical role played by lymphocytes in mediating specific immune response (Roberts, 1989).

The marked disturbances in the hepatic functions of infected fish group was expressed by the recorded significant progressive decline in serum total protein, serum glucose and total cholesterol, as well as, the progressive significant increase in serum total bilirubin, SGOT, SGPT and alkaline phosphatase as shown in Tables 3 and 4.

The recorded decline in serum proteins and glucose may be attributed to hepatic dysfunction mediated by the action of bacterial toxins on liver cells (Benjamin, 1970). Also, the progressive loss of serum proteins and glucose may additionally be attributed to the increased permeability of ranal glomerular capillaries induced by the action of bacterial toxins (Bruno, 1984). The decreased total proteins may consequently account for the recorded decreased levels of cholesterol. Significant decrease in serum proteins, glucose and total cholesterol were also recorded by Amlacher (1961) in Carp affected by haemorrhagic septicaemia. On the other hand, insignificant increase in serum total protein was found in bacterin injected fish group which may be related to the increased gamma globulin fraction resulting from immune system response to the injected bacterin.

The recorded significant increase in serum total bilirubin, SGOT, SGPT, and alkaline phosphatase may reflect liver dysfunction resulting from the action of injected bacteria on liver cells (Benjamin, 1970). Moreove, the increased haemolysis of RBCs may additionally account for the recorded increased levels of total bilirubin (Amlacher, 1961 and Bruno, 1986).

Disturbances in the kidney functions of infected fish group was expressed as shown in Table 4 by the recorded ureamia and insignificant increase in serum creatinine levels probably resulting form the reduction in the glomerular filtration rate, cellular disruption and inflammation which occurred within the collecting duct, in addition to the possible retention caused by the swollen vent region induced by the action of bacterial toxins. These results coincide with those of Amlacher (1961) regarding carp affected by haemorrhagic septicaemia.

Such results may clarify the difference in the mechanisms of hostpathogen interrelationship through which a given pathogen exerts its effective tissue tropism within a given host (fish), as well as, the extent of tissue damage, and consequently tissue function.

In conclusion , the present study proved the drastic effect of *A.hydrophila* on haematological parameters, liver and kidney functions of *Oreochromis niloticus*, as well as, the reflection of physicochemical alterations on clinical signs and post-mortem lesions of infected fish, which may suggest the usage of studied parameters for evaluating the health status of fish and also for early detection of deviated health conditions before the onset of characteristic clinical signs of such bacterial infection.

Table 1. Effects of *A.hydrophila* and its baterin on the haematological picture of *O.niloticus* fish.

Parameter / D.P.T.	RBCs x 10^6/mm^3			Hb gm/dl			PCV%			WBCs x 10^3/mm^3		
	Control	Infected	Bacterin	Control	Infected	Bacterin	Control	Infected	Bacterin	Control	Infected	Bacterin
0	1.9±	1.9±	1.92±	7.9±	8.16±	8.20±	26.2±	26.4±	26.0±	5.16±	5.20±	5.18±
	0.8	0.64	0.74	0.46	0.70	0.46	0.92	1.20	1.06	0.18	0.32	0.16
2	1.92±	1.86±	1.91±	7.6±	7.4±	8.0±	24.9±	24.5±	25.5±	5.26±	5.36±	5.12±
	0.70	0.82	0.82	0.48	0.52	0.22	1.30	1.60	1.70	0.29	0.46	0.22
5	1.88±	1.80±	1.90±	7.7±	7.0±	7.9±	24.4±	23.5±	24.8±	5.32±	5.42±	5.80±
	0.36	0.45	0.60	0.37	0.22	0.32	1.2	1.70	1.30	0.17	0.16	0.36
9	1.90±	1.68±	1.86±	7.65±	6.90±	7.8±	24.7±	22.7±	24.6±	5.46±	5.60±	5.20±
	0.62	0.52	0.54	0.43	0.62	0.36	1.4	1.8	1.20	0.22	0.22	0.42
14	1.86±	1.10±	1.88±	7.80±	6.6*±	7.6±	24.5±	19.2*±	24.4±	5.37±	4.9±	5.0±
	0.56	0.44	0.63	0.24	0.46	0.18	1.6	1.0	108	0.29	0.38	0.30
21	1.88±	0.80*±	1.86±	7.72±	6.2*±	7.42±	24.02±	18.6**±	24.3±	5.27±	4.7*±	5.38±
	0.44	0.18	0.64	0.48	0.30	0.26	0.95	1.2	1.22	0.22	0.19	0.16
28	1.88±	0.68**±	1.84±	7.68±	5.4**±	7.18±	24.4±	16.8***±	24.0±	5.48±	4.12**	5.84±
	0.36	0.22	0.52	0.42	0.18	0.48	1.2	0.95	1.7	0.35	±0.18	0.18

* Significant at P<0.05 ** Significant at P<0.01 *** Significant at P<0.0001

Data represented as mean ± S.E. n=10

D.P.T. = days post-treatment Bacterin = bacterin injected group

Table 2. Effects of *A.hydrophila* and its bacterin on the differential leucocytic count of *O.niloticus* fish.

Parameter / D.P.T.	Lymphocyte%			Monocyte %			Neurophil %			Eosinophil %			Basophil%		
	Control	Infected	Bacterin	Control	Infected	Bacterin	Control	Infected	Bacterin	Control	Infected	Bacterin	Control	Infected	Bacterin
0	80.6± 2.5	81.4± 3.6	82.0± 4.12	1.0± 0.03	1.1± 0.02	0.99± 0.04	12.7± 0.94	13.0± 0.85	12.5± 0.65	3.9± 0.16	4.0± 0.12	4.1± 0.18	0.5± 0.09	0.51± 0.09	0.52± 0.010
2	81.5± 2.44	82.78± 4.18	82.2± 4.10	0.98± 0.06	1.1± 0.07	0.98± 0.03	13.0± 0.73	12.2± 0.82	12.7± 0.82	4.0± 0.29	4.1± 0.18	4.1± 0.20	0.50± 0.012	0.52± 0.007	0.51± 0.014
5	81.73± 3.01	85.4± 4.50	82.66± 2.70	0.98± 0.07	1.2*± 0.09	0.95± 0.05	12.9± 0.64	10.2± 1.1	12.4± 0.82	4.1± 022	4.2± 0.13	4.1± 0.25	0.50± 0.02	0.53± 0.010	0.52± 0.010
9	83.12± 2.55	91.9± 2.43	80.25 ±3.2	0.99± 0.06	1.3***± 0.03	0.94± 0.08	11.6± 1.7	8.4± 0.88	7.96± 0.97	3.9± 0.09	4.2*± 0.01	4.1± 0.18	0.46± 0.07	0.37± 0.06	0.38± 0.04
14	82.2± 3.83	86.2± 3.8	86.6± 7.2	1.0± 0.03	1.1± 0.07	0.92± 0.06	11.8± 1.9	8.2± 0.82	9.6± 1.10	4.1± 0.10	3.9± 0.16	4.0± 0.47	0.48± 0.03	0.37± 0.05	0.45± 0.05
21	82.66± 3.02	80.9± 2.30	91.75*± 4.18	0.98± 0.03	0.97± 0.04	0.94± 0.04	11.6± 1.4	8.9± 0.85	11.3± 0.87	4.0± 0.14	4.9± 0.18	4.1± 0.30	0.47± 0.02	0.41± 0.04	0.48± 0.01
28	81.5± 3.43	72.4± 3.6	95.12**± 2.76	1.0± 0.03	0.84*** ±0.02	0.96± 0.06	12.1± 0.73	11.8± 1.20	11.7± 0.90	4.2± 0.07	3.8**± 0.10	4.30± 0.18	0.48± 0.05	0.46± 0.03	0.51± 0.01

* Significant at P<0.05 ** Significant at P<0.01 *** Significant at P<0.0001

Data represented as mean ± S.E. n=10 D.P.T. = days post-treatment Bacterin = bacterin injected group

Table 3. Effects of *A.hydrophila* and its bacterin on the level of T.protein, glucose, T.cholesterol and T.bilurbin in serum of *O.niloticus* fish.

Parameter / D.P.T.	Total protein %			Glucose mg %			Total cholesterol mg %			Total bilirubin %		
	Control	Infected	Bacterin	Control	Infected	Bacterin	Control	Infected	Bacterin	Control	Infected	Bacterin
0	4.40± 0.16	4.52± 0.17	4.60± 0.13	66.20± 3.20	66.40± 3.60	86.4± 3.80	142.0± 9.8	140.0± 4.2	142.0± 7.2	0.35± 0.010	0.35± 0.02	0.36± 0.02
2	4.42± 0.13	4.40± 0.16	4.65± 0.15	65.40± 3.60	63.2± 3.20	68.0± 4.20	142.0± 8.60	163.2± 7.40	144.2± 5.60	0.36± 0.018	0.37± 0.014	0.37± 0.016
5	4.68± 0.17	4.22± 0.20	4.70± 0.19	64.70± 2.80	60.6± 3.60	66.30± 4.60	141.2± 9.20	130.0± 5.20	142± 5.60	0.37± 0.02	0.38± 0.016	0.37± 0.012
9	4.60± 0.12	4.10*± 0.16	4.72± 0.18	62.80± 3.10	58.4± 3.60	65.40± 3.40	142.6± 7.3	124.8*± 4.6	142.4± 6.40	0.36± 0.012	0.40± 0.02	0.36± 0.02
14	4.56± 0.19	4.00*± 0.14	4.80±. 0.16	63.6± 2.40	55.0*± 3.00	64.6± 3.80	141.8± 7.3	122.4* ±3.6	144.0± 7.6	0.35± 0.02	0.40*± 0.012	0.35± 0.018
21	4.56± 0.20	3.80**± 0.15	5.00± 0.17	63.50± 4.20	53.20*± 2.50	64.20± 3.20	142.0± 4.60	120.0**± 3.40	141.0± 6.2	0.36± 0.016	0.46**± 0.02	0.35± 0.02
28	4.70± 0.18	3.5***± 0.12	5.20± 0.16	62.80± 3.20	51.2**± 2.40	63.40± 2.80	141.4± 5.40	112.0± 5.20	140.0± 7.40	0.35± 0.1	0.46***± 0.012	0.34± 0.016

* Significant at P<0.05 ** Significant at P<0.01 *** Significant at P<0.0001

Data represented as mean ± S.E. n=10 D.P.T. = days post-treatment

Bacterin = bacterin injected group

Table 4. Effects of *A.hydrophila* and its bacterin on the level of urea, creatinine, glutamic oxaloacetic, glutamic pyruvic transaminases and alkaline phosphatase in serum of *O.niloticus* fish.

Parameter / weeks	Urea (mg%)			Creatine (mg5)			SGOT (U/L)			SGPT (U/L)			Alkaline phosphatase (U/L)		
	Control	Infected	Bacterin	Control	Infected	Bacterin	Control	Infected	Bacterin	Control	Infected	Bacterin	Control	Infected	Bacterin
0	2.8± 0.16	2.85± 0.18	2.85± 0.18	0.54± 0.007	0.52± 0.01	0.50± 0.007	92.5± 2.80	92.4± 1.85	63.6± 2.4	25.4± 0.25	25.6± 0.90	24.8± 0.80	19.4± 0.95	19.8± 0.70	19.6 ± 0.85
2	2.85± 0.17	2.88± 0.16	2.88± 0.22	0.50± 0.018	0.52± 0.009	0.52± 0.016	90.9± 1.90	94.6± 2.40	92.4± 1.16	25.6± 1.10	26.2± 0.80	25.0± 1.10	19.6± 1.02	20.8± 0.82	19.8± 0.78
5	2.78± 0.14	2.90± 0.22	2.84± 0.18	0.52± 0.08	0.54± 0.007	0.52± 0.01	92.4± 1.60	96.7± 3.65	94.2± 2.70	25.8± 0.92	28.2± 1.18	25.3± 0.95	19.7± 0.90	21.8± 1.06	20.4± 0.86
9	2.84± 0.16	2.95± 0.18	2.85± 0.20	0.50± 0.017	0.45± 0.01	0.54± 0.014	93.8± 1.10	100.6* ±2.40	95.4± 3.60	25.4± 0.78	30.6**± 1.10	26.8± 1.20	19.0± 0.85	22.4*± 1.00	20.8± 1.07
14	2.80± 0.14	3.20*± 0.10	2.86± 0.18	0.54± 0.019	0.56± 0.008	0.55± 0.009	94.6± 1.85	102.4* ±3.20	96.4± 1.18	25.8± 0.70	32.4*** ±1.16	27.2± 1.18	19.4± 0.92	23.2*± 1.20	20.8± 0.85
21	2.78± 0.17	3.4** 0.12	2.82± 0.16	0.52± 0.019	0.56± 0.018	0.55± 0.006	94.2± 1.20	110.6** *±0.2.6	96.8± 2.60	26.2± 1.10	34.6*** ±0.95	27.8± 0.90	20.0± 1.00	24.6**± 0.80	21.6± 0.90
28	2.80± 0.10	3.6*** ±0.41	2.86± 0.16	0.50± 0.006	0.55± 0.007	0.56± 0.012	92.8± 2.90	115.4** *±3.40	97.6± 2.80	26.4± 0.92	35.2*** ±1.19	28.0± 1.20	20.6± 0.94	26.2*** ±0.86	22.4± 0.85

* Significant at P<0.05 ** Significant at P<0.01 *** Significant at P<0.0001

Data represented as mean ± S.E. n=10 D.P.T. = days post-treatment Bacterin = bacterin injected group

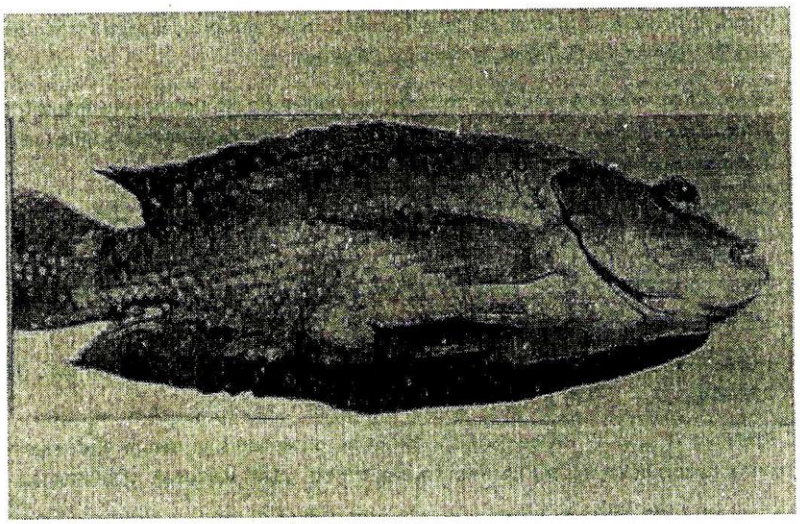

Fig. 1. *Orechromis nilocitus* showing exophthalmia, skin fin and eye haemorrhages, inflamed vent and abdominal dropsy.

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تأثير الايروموناس هيدروفيلا والبكتيرين المحضر على كفاءة الأداء الفسيولوجى لأسماك البلطى

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معهد بحوث صحة الحيوان، مركز البحوث الزراعية - وزارة الزراعة - الدقى - الجيزة .

تم اجراء هده البحث علي ثلاث مجموعات من اسماك البلطى الحية حيث حقنت اول مجموعة بميكروب الايروموناس هيدروفيلا والثانية بالبكتيرين المحضر بالفورمالين أما الثالثة تم حقنها بفوسفات بفر سالين (محلول ملح فسيولوجى).

تم عد كرات الدم البيضاء والحمراء وقياس مكونات المصل الدالة علي وظائف الكبد والكلى فى المجموعات الثلاثة على مدار أربعة أسابيع .

ظهرت اعراض التسمم الدموى على الاسماك المحقونة نتيجة العدوى البكتيرية مما ادى الى هبوط في نسبة الهيموجلوبين وسرعة الترسيب وايضا انخفاض في عدد كرات الدم الحمراء اما الكرات البيضاء فقد زادت خلال الاسبوع الاول من العدوى ثم انخفضت تدريجيا علي مدار التجربة.

كما لوحظ زيادة ملحوظة في نسبة البيلوروبين واليوريا في المجموعات المصابة مع انخفاض في نسبة البروتين والكوليستيرول والجلوكوز. اما في المجموعة المحقونة بالبكتيرين فلم يشاهد أية فروق معنوية بينها وبين المجموعة المحقونة بالفوسفات بفر سالين (محلول ملح فسيولوجى) عدا زيادة في زيادة في عدد كرات الدم البيضاء وبخاصة الليمفوسايت مع زيادة طفيفة في بروتين الدم.