# Study of phagocytosis in diabetic patients

Raheem T. Obai*s Abdul Adeem .Y. A. AL-Barrack**
Shakir .H. M. Al-Alwany*** Hala Abd Al-Hadi Chabok****
(Central public health Laboratory of Babylon/ Department of immunology)*
(College of medicine, Almustansarya university)**
(College of science, Babylon University) ***
(College of medicine, Babylon University)****

## Abstract

This study deals with the host resistance to infection in diabetes and the influence of an acute infection on the endocrinologic- metabolic status of the diabetic patient, while it is well known that acute infection lead to defecting in controlling blood sugar levels and that infection is the most frequently documented cause of ketoacidosis. The patient with diabetes mellitus are more susceptible to infection than age and sex matched non diabetic subject "the data were being studied as phagocytic activity or (leucocytes function) to ingest bacteria (This study consist of 72 diabetic patients and 19 apparently healthy individuals. These samples in this study have been grouped according to the severity of diabetes, duration of disease , insulin non insulin dependant, controlled and noncontrolled for all patient and control sample. Phagocytic activity is done as well as bacterial cells per neutrophil and rate of ingestion of phagocytic cells to the bacteria among time of incubation).

The result among 72 diabetic patients showed a highly significant decreased($P<0.01$) in phagocytic activity of neutrophil among this patients and direct correlation between the phagocytic activity of polymorphonucleur neutrophil (PMN) ($P< 0.05$) and the mean glucose level after comparsim with the control samples. The effect of duration of disease give a highly significant differences ($P< 0.01$) with the phagocytic activity after comparism with control group as well as less than 5 years and more than 5 years. There is a significant diminution in phagocytosis of ***staphylococcus aureus*** in subject poorly controlled diabetes in comparism with the control group. The result from these study suggest that good control of blood sugar in diabetic patient is a desirable goal in the prevention of continuous infection (Bacterial, Viral, Fungal….etc) and to ensure maintenance of normal host defense mechanism that determine resistant and response to such infections.

***Key words:- Phagocytosis , staph aureus , diabetes mellitus .***

## الخلاصة

تتضمن هذه الدراسة اليه المقاومة الذاتية للجسم ضد الاخماج البكتيرية في مجموعه من المرضى اللذين يعانون من داء السكري حيث تم جمع ٧٢ عينة وتم دراسة عملية البلعمة باستخدام بكتريا العنقوديات الذهبية ***staphylococcus aureus*** وبتراكيز معينة وقد تبين من النتائج أن هناك فرق معنوي كبير يقدر إحصائيا ($P>0.01$) باتجاه خفض عمليه البلعمة لدى( مرضى السكري مقارنه بالأشخاص الأصحاء أي أن هنالك انخفاض ملحوظ في عمليه البلعمة للعنقودية الذهبية

***staphylococcus aureus*** في مرضى داء السكري غير المسيطر عليهم مقارنه بغيرهم ضمن مستويات الكلوكوز الطبيعي أو المسيطر عليه ، اضافه إلى أن هنالك علاقة مباشره بمستوي معنوي ($P<0.05$ ) بين عمليه البلعمة ومستوى السكر في الدم مقارنة مع نماذج السيطرة. وقد تبين من خلال هذه الدراسة أن هنالك علاقة مهمة بين مده حدوث (فتره استمرار المرض) وعمليه البلعمة بمستوى عالي المعنوية( $P>0.01$) باتجاه خفض عمليه البلعمة . يمكن الاستنتاج من خلال هذه الدراسة بأن السيطرة على مرض السكري أو مستوى الكلوكوز في الدم لدى المريض سيؤدي إلى الوقاية من بعض الأمراض أو الاخماج الانتهازية أو غيرها (الجرثومه والحميه و الفطرية ) وكذلك لغرض المحافظة على مستوى طبيعي لاليه الدفاع التي تحدد الاستجابة أو المقاومة لمثل هذه الاخماج.

## Introduction:-

In diabetic patient there increase susceptibility to pyogenic infection, the latter may be due to altered metabolism, elevated glucose level, influx phagocytic cells and depression of phagocytosis (1). Abnormalities in host defense mechanism to account for this have no been convincingly shown various steps involved in host response to invading microorganism including neutrophuil chemotaxis, adherence to vascular endothelial, phagocytosis, intracellular killing have been depressed in some diabetic patient (2). The surface protein in cell wall component of ***Staphylococcus aureus*** . (Protein –A)may interfere with phagocytosis and pathogenic type not kill white blood cells and may phagocytosis as affectively as non pathogenic variants and capable of very active intracellular multiplication while non pathogenic organism tend to die inside the cells. Diabetic patients are susceptible to infective by pyogenic bacteria such as ***Staphylococcus aureus*** more than other type of microorganisms. (1). Polymorphonuclear leucocytes or neutrophils are critical for human innate immunity and kill invading bacteria, however, pathogens such as *Staphylococcus aureus* avoid destruction by PMNs to survive, thereby causing human infections. The molecular mechanisms used by pathogens to circumvent killing by immune system remain largely undefined. (3). Hyperglycemia accompanied by the classical symptoms of diabetes occur only when 90% of insulin secreting cells are already destroyed, the immune system retains the capacity to recognized and destroy transplanted insulin secreting cells indefinitely, HLA linked genetic predispotion and associated with other autoimmune disorders, inheritance of human D.M (I.D.D.M) is polygenic it has been estimated that HLA class II (4). Various steps involved in the host response to invading microorganisms including neutrophil, chemotaxis, adherence to vascular endothelium phagocytosis, intracellular killing, serum opsonins and cell mediated immunity, have been found to be depressed in some diabetic patient .(2). Diabetic state characteristic by many bacterial abnormalities may interfere with phagocytic function of PMNL in 1964 reported studies on invitro phagocytosis in diabetic patient employing ***Staphylococcus epidermides*** and ***Staphylococcus aureus***, they showed that only the avdotic diabetic patient manifested decreased phagocytic capacity and that the defect disappeared upon correlation of the acidosis (5). Baghdadi and associates (1974) studied phagocytosis and microbicidal rate of PMN in nonketotic patient with poorly controlled diabetes before and after diabetic therapy was instituted, the test organism in this assay was ***Strep. Pneumonia*** type (25), prior to treatment, both phagocytosis and bactericidal rates were significantly

reduced, these function improved following diabetic therapy but were still less than in control subject. (2; 6). Granulocytes from diabetic patient treated with either insulin or oral diabetic treatment reveal a significant diminution of intercellular bactericidal activity in diabetic subject for 2 organism ***Staphylococcus aureus*** and ***E. coli*** (7). Neither age and sex of patient non chronic complication caused affected the phagocytic activity of leucocytes in diabetic patient there was no significant difference in phagocytic activity of leucocytes between patient with (IDDM)and these with NIDDM (8)..There are many cases in which that phagocytosis and intracellular killing of microorganism are inhibition such as autoimmune disease (e.g. IDDM) and some of endocrinological and metabolic disorder as IDDM. (4) .Bacterial or viral infection might cause some form of IDDM this done either by distraction of the pancreatic beta cells by direct glycolysis result from infection or dependent on the genetic background of the host cells (9). Activated macrophages produce some harmful factors such as the cytokine (IL–1) or other chemical (10). The damage results from interaction between activated macrophages and cells, removal of necrotic cells by phagocytosis is identical to the removal of apoptotic cells, Hence for slow level of inflammation in the pancreas, the positive feedback leading to self destruction of B-cells is negligible, a very rapid and complete destruction of the B-cell mass could occur even before the adaptive immune system get involved .( 11). Immunologic research has however, demonstrated several defects in host immune defense mechanisms in diabetics subjects. Phagocytic capabilities of polymorphonuclear leucocytes (PMNLs) are adversely affected by hyperglycemia. Several PMNLs defects occur in diabetic subjects including impaired migration, phagocytosis, intracellular killing and chemotaxis (12), which may be due to decreased PMNLs membrane fluidity. (13).Diabetic patient are susceptible to infection by pyogenic bacteria such as ***Staph. aureus*** more than other type of microorganism.(1). Acidotic patient manifest decreased in phagocytic capacity of ***Staph. aureus***. and that the defect disappeared upon correlation of the acidosis (14). Diabetic and neutropenic patients are susceptible to infection by ***Staph. aureus, E. coli, Pseudomonas*** and ***Aspargellus***, chemotaxic defect may occur in diabetic patient, some of autoimmune disorder (SLE, Rheumatoid arthritis and IDDM) these defect predispose patients to infection by gram positive and negative bacteria .(11).

## ***Materials and Methods: -***

### *A) Sample collection:-*

From a fasting diabetic patient take about 5ml of blood in test tube, 2 ml to obtained serum for glucose level measurement, and 2 ml mix with (20 i.u/1ml)of heparin solution to prevent clotting the samples. The sample should be used within (2-3) hours from collection because the granulocytes (neutrophil) have a short life span (15)

### *B) Bacterial isolates:-*

***Staph. aureus*** are cultured over night in neuterint broth to make certain that they well be in a logarithmic growth phase, they are then washing by normal saline and diluted to give suspension of about ($10^7$ cells/ml) counting by neubeur chamber (16 ).

***C) White blood cells count:-***

Total W.B.C.s count and neutrophils number should be estimate, the volume of bacterial suspension used in this experiment depend on the number of neutrophil in the sample in ratio about (1 neutrophil for each 10 cells of bacteria ).(15;17 ).

***D) Phagocytosis Test :-***

The test occurred according to (17)

***Calculation:-***

1. Percentage of positive neutrophil (%)= (No. of Phagocytic cell with bacteria/ Total cells count) x100
2. Average number of bacteria/ 100 cells (Neutrophils).
3. Rate of ingestion = (Bacteria /Neutrophil / Time of incubation).

## ***Results and Discussion:-***

Seventy two (72) patient samples have been used in this study and 19 samples used as control (apparently healthy with no diabetes) and the results as the following:-

***A; - Effect of diabetic on phagocytic activity of neutrophil as in the following: -***

Measurement of positive phagocytic cells ((neutrophil with bacteria = N %) Table (1), showed that highly significant value in N% ($p<0.01$) between test and control, while no significant difference in No. of bacteria / Neutrophil and rate of ingestion compared with control, it mean that the diabetic is the mean factor caused impaired of phagocytic activity of neutrophils. (18) in his study demonstrated a defective function of leucocytes in diabetic patients. (19) studied impairment of leucocytic function in diabetic patients and all steps of PMN functioning which are altered in these patients. (20) pointed out that a high Glucose correlate through the activation of calcium channels (augment in vitro calcium entering into cells dysfunction, among there cells are PMN and phagocytosis is reduced. (21) pointed out that hyperglycemia with out acidosis inhibit phagocytosis in addition to many factors which may lead to impairment in immune response. Phagocytic capabilities of polymorphonuclear leucocytes (PMNLs) are adversely affected by hyperglycemia. Several PMNLs defects occur in diabetic subjects including impaired migration, phagocytosis, intracellular killing and chemotaxis, which may be due to decreased PMNLs membrane fluidity.(13 ).

***Table (1)*** Effect of diabetic on phagocytic activity

| Groups | Numbers | N%<br>Mean ± stander Error |
|---|---|---|
| Test | **72** | 30± 1.4 |
| Control | **19** | 44± 205 |
| | | **B/N**<br>**Mean± stander Error** |
| Test | **72** | 3± 0.36 |
| Control | **19** | 305± 0.31 |
| | | **Rate of ingestion**<br>**Mean± stander Error** |
| Test | **72** | 0.08± 0.006 |
| **Control** | 19 | 0.06± 0.005 |

***B;- Effect of glucose level on phagocytic activity n diabetic patients:-***

There is a significant difference (P<0.05) between results obtained in patient with different concentration of glucose level on reducing the phagocytic activity as well as immune response (table (2)). There is no significant difference in (B/N) and rate of ingestion among diabetic and control samples. These results are similar to the results obtained from the studies of some workers which showed a great relationship between glucose level and phagocytic activity. (20); (18); (19). There is direct correlation between $Ca^{+}$ of PMNs and blood glucose level and an inverse relationship between phagocytosis and blood glucose level, this done on experimental animals (rats) by inducing diabetes and treatment uses and the results show that $Ca^{+}$ of PMNL increases and phagocytosis decrease rapidly after the induction of diabetes, high $Ca^{+}$ is responsible for the impaired phagocytosis in association with hyperglycemia (20)

Table (2) Effect of Glucose level on Phagocytosis .

| Groups | Numbers | N%<br>Mean ± stander Error |
|---|---|---|
| Test (a)<br>Equal or < 200 mg/dl | **39** | 34± 1.07 |
| Test (b)<br>>200 mg/dl | **33** | 36±1.15 |
| Control | **19** | **39**±2.5 |
| | | **B/N**<br>**Mean± stander Error** |
| Test (a)<br>Equal or < 200 mg/dl | **39** | 3 ± 0.32 |
| Test (b)<br>>200 mg/dl | **33** | 3.1 ± 0.1 |
| Control | **19** | **3.6** ± 0.31 |
| | | **Rate of ingestion**<br>**Mean± stander Error** |
| Test (a)<br>Equal or < 200 mg/dl | **39** | **0.05**± 0.004 |
| Test (b)<br>>200 mg/dl | **33** | **0.05** ± 0.004 |
| **Control** | 19 | **0.06**± 0.006 |

This tables showed that glucose level has a significant effect (P<0.05)on phagocytosis, it mean causes decrease in phagocytic activity compared with control and no variation between low level and high level of glucose, as well as no significant difference.(p>0.05) in B/N and Rate of ingestion.

***C;- Effect of duration of diabetic on phagocytic activity :-***

Table (3) showed that there is a highly significant difference in result obtained from this study on diabetic patient with different duration as well as under and out of control after comparism with the control group. (20). pointed out that the phagocytic activity could be reduced just one day after diabetes . The reduction of phagocytic activity could be due to the deterioration in immune response and phagocytic activity with chronic of diabetic disease under control and out of control as stated by other workers.( 8).

**Table (3**) The Effect of duration of disease on phagocytosis.

| Groups | | numbers | N% Mean ± stander Error |
|---|---|---|---|
| Test(1) equal or < 5 years | **Under control (a)** | **20** | 35 ± 1 |
| | **Out of control (b)** | **21** | 31 ± 1.04 |
| Test(2) >5years | **Under control (a)** | **17** | 27 ± 1.96 |
| | **Out of control (b)** | **14** | 38 ± 3.1 |
| Control | | **19** | 44 ± 2.5 |
| | | | **B/N Mean± stander Error** |
| Test(1) equal or < 5 years | **Under control (a)** | **20** | 3.4± 0.29 |
| | **Out of control (b)** | **21** | 2.6 ± 0.41 |
| Test(2) >5years | **Under control (a)** | **17** | 2.8 ± 0.46 |
| | **Out of control (b)** | **14** | 2.3± 0.37 |
| Control | | **19** | 3.4± 0.31 |
| | | | **Rate of ingestion Mean± stander Error** |
| Test(1) equal or < 5 years | **Under control (a)** | **20** | 0.06 ± 0.006 |
| | **Out of control (b)** | **21** | 0.05±0.007 |
| Test(2) >5years | **Under control (a)** | **17** | 0.04±0.00 |
| | **Out of control (b)** | **14** | 0.03±0.005 |
| **Control** | | 19 | 0.06±0.006 |

This tables show the following results:-

< 5 years compared to control give H.S result (P<0.01). It mean that the duration of disease caused decrease phagocytosis of diabetic patient compared with control.

>5 years (under controlled) diabetes give H.S (P <0.01) in direction of decrease phagocytosis compared with control. While there is a significant (P<0.05) decrease in phagocytosis in uncontrolled diabetic patients compared with control

<5 years controlled compared with < 5 years uncontrolled; give H.S (P<0.01) in controlled lees then uncontrolled.

There is a same result in B/N for each group controlled and uncontrolled .While no significant (P>0.05) in rate of ingestion of bacteria.

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