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BRIEF REPORT

Impaired Phagocytosis of Capsular Serotypes K1 or K2 *Klebsiella pneumoniae* in Type 2 Diabetes Mellitus Patients with Poor Glycemic Control

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Context: Diabetes mellitus (DM) and capsular serotypes K1 and K2 *Klebsiella pneumoniae* have been identified as risk factors for liver abscess and complicated endophthalmitis.

Objective: The objective of this study was to determine whether poor glycemic control contributes to the development of capsular serotype K1 or K2 *K. pneumoniae* liver abscess.

Design and Setting: Neutrophil phagocytosis in patients with type 2 DM and nondiabetic controls was compared with isolates from liver abscess. Phagocytic rates of 18 K1/K2 and nine non-K1/K2 *K. pneumoniae* strains were evaluated by flow cytometry and electron microscopy.

Patients or Study Participants: Forty patients with type 2 diabetes, 14 with good glycemic control, 26 with poor glycemic control, and 13 age-matched healthy normal subjects, were studied.

Main Outcome Measures: Phagocytic rate of *K. pneumoniae* was measured.

Results: Phagocytosis of serotype K1/K2 isolates by neutrophils from diabetics was significantly less than normal controls ($P < 0.01$). Further analysis revealed that, in type 2 DM patients with poor glycemic control, phagocytosis of K1/K2 was remarkably impaired at 10 min (25.2 ± 1.7 *vs.* $42.4 \pm 1.8\%$) and persisted until 60 min (51 ± 1.2 *vs.* $59.4 \pm 1.4\%$; $P < 0.01$), but in type 2 DM patients with good glycemic control were similar at 10 min ($38.2 \pm 1.7\%$ *vs.* $42.4 \pm 1.8\%$) and at 60 min ($57 \pm 0.3\%$ *vs.* $59.4 \pm 1.4\%$; $P = 0.2$). No significant difference in the phagocytosis of non-K1/K2 *K. pneumoniae* among all subjects was observed.

Conclusions: Poor glycemic control plays a role in impairing neutrophil phagocytosis of K1/K2 *K. pneumoniae*, but does not significantly affect the phagocytosis of non-K1/K2 *K. pneumoniae*. This study identifies poor glycemic control as a risk factor for susceptibility to serotype K1/K2 *K. pneumoniae* liver abscess and complicated endophthalmitis. **(*J Clin Endocrinol Metab* 91: 3084–3087, 2006)**

*K*LEBSIELLA *PNEUMONIAE* HAS been identified as the predominant bacteria responsible for pyogenic liver abscess worldwide (1–5). The capsular serotypes K1 and K2 are most prevalent in liver abscess and metastatic infections, including endophthalmitis (2). Although *magA* gene has recently been found as the cause behind *K. pneumoniae* liver abscess and septic metastatic complications (6), its association appears to be restricted for serotype K1 *K. pneumoniae* (7). It is generally accepted that *magA* is part of the gene cluster for serotype K1 capsular formation. Apart from the bacterial factor, the incidence of *K. pneumoniae* liver abscess has been documented to be frequently associated with type 2 diabetes mellitus (DM) (2, 3, 8, 9). Seventy-five percent of patients with *K. pneumoniae* liver abscess and 93% of those with complications of septic endophthalmitis have underlying type 2 DM (2, 4). To our knowledge, the relationship between type 2 DM and liver abscess has yet to be delineated.

Neutrophils are involved in the innate immunity against bacterial infection. Impaired neutrophil activity in type 2 DM is partly responsible for the increased susceptibility to infection (10). Previous studies showed that patients with liver abscess and endophthalmitis had unrecognized DM (9, 11). However, whether susceptibility to serotypes K1/K2 *K. pneumoniae* infection is due to impaired neutrophil phagocytosis in patients with poor glycemic control remains unclear. This study compared neutrophil phagocytosis of serotypes K1/K2 and non-K1/K2 *K. pneumoniae* among type 2 DM patients with either good or poor glycemic control with healthy subjects.

Subjects and Methods

Subjects

Forty patients with type 2 DM and 13 age-matched healthy subjects were enrolled in this study. Type 2 DM diabetics with fasting blood glucose level

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Abbreviation: DM, Diabetes mellitus.

above 130 mg/dl, 2-h postprandial blood glucose above 180 mg/dl, or hemoglobin A1c above 7% under control with oral hypoglycemic agents as definition of American Diabetes Association (12) were classified as those with poor glycemic control. Diabetics that met any one of the criteria of poor glycemic control were selected for this study. In contrast, diabetics with physiological indicators below the respective criterion stated above were classified as those with good glycemic control. The good glycemic control group consisted of seven men and seven women. Their mean age was 61 ± 14 yr. The mean fasting blood glucose was 117 ± 10 mg/dl, and hemoglobin A1c was 6.6 ± 0.3%. The poor glycemic control group consisted of 16 men and 10 women. Their mean age was 63 ± 15 yr. The mean fasting blood glucose was 229 ± 31 mg/dl, and hemoglobin A1c was 10.2 ± 2.3%. Thirteen normal healthy subjects with a mean age of 62.7 ± 4.7 yr constituted the normal control group. The body mass indexes of subjects with good glycemic control, poor glycemic control, and the healthy nondiabetics were 21.3 ± 2.2, 23.4 ± 2.5, and 22.7 ± 2.4 kg/m^2, respectively. All the participants had no evidence of infection 4 wk before this study. This study protocol was approved by the Ethics Committee in our institutes, and informed consent was obtained from all the participants.

Isolation of human neutrophil and preparation of pooled serum

Neutrophils from normal healthy subjects and type 2 DM patients were separated as previously described (13). Viability was over 95% as determined by trypan blue exclusion.

Pooled normal human sera from 13 healthy volunteers and pooled type 2 DM sera from 14 good and 26 poor glycemic control patients were prepared, respectively. Pooled serum was obtained and stored in aliquots at −70 C until required

Collection of K. pneumoniae with different serotypes and sites of infection

K. pneumoniae strains including 18 K1/K2 and nine non-K1/K2 isolates were obtained from liver abscess patients with and without the complication of endophthalmitis and from nonliver abscess of nondiabetic subjects (2, 14, 15). One isolate of K3, K6, K16, K28, K36, K38, and K54 was obtained, except K55, where two isolates were obtained. The diabetics contributed nine strains of K1, three strains of K2, and seven strains of non-K1/K2, whereas the nondiabetics contributed four strains of K1, two strains of K2, and two strains of non-K1/K2. Five strains of *K. pneumoniae* were isolated from patients with endophthalmitis.

Phagocytosis measurement

Fluorescence (fluorescein isothiocyanate) labeling of 2×10^8 cells per milliliter in PBS was performed as previously described (13) and stored at −70 C before use.

Phagocytosis was measured using a standard assay (13). A FACScan (Becton Dickinson Immunocytometry Systems, San Jose, CA) was used to measure phagocytic rate. Percentages of phagocytosis were counted at 2, 5, 10, 30, and 60 min. A non-fluorescein isothiocyanate-labeled tube served as 0-min control. The experimental procedures and FACS settings were followed as previously (16).

Electron microscopic evaluation of phagocytosis

Purified neutrophils were mixed with live bacteria for 60 min under the conditions described above for flow cytometry. Details of experimental procedures were described previously (16). Ultra-thin sections were stained and examined by transmission electron microscopy (JEM 1230; JEOL, Peabody, MA) using standard operation conditions (17).

Statistical analysis

One-way ANOVA with repeated measures was performed to compare the differences of phagocytosis rates from time 0–60 min between groups. Student's *t* test was used to assess the differences in the phagocytosis rates at time 60 min between the groups. Data were expressed as mean ± SEM. Pearson correlation test was used to evaluate the correlation between blood glucose level and phagocytic uptake rate. *P* values less than 0.05 were considered to be statistically significant.

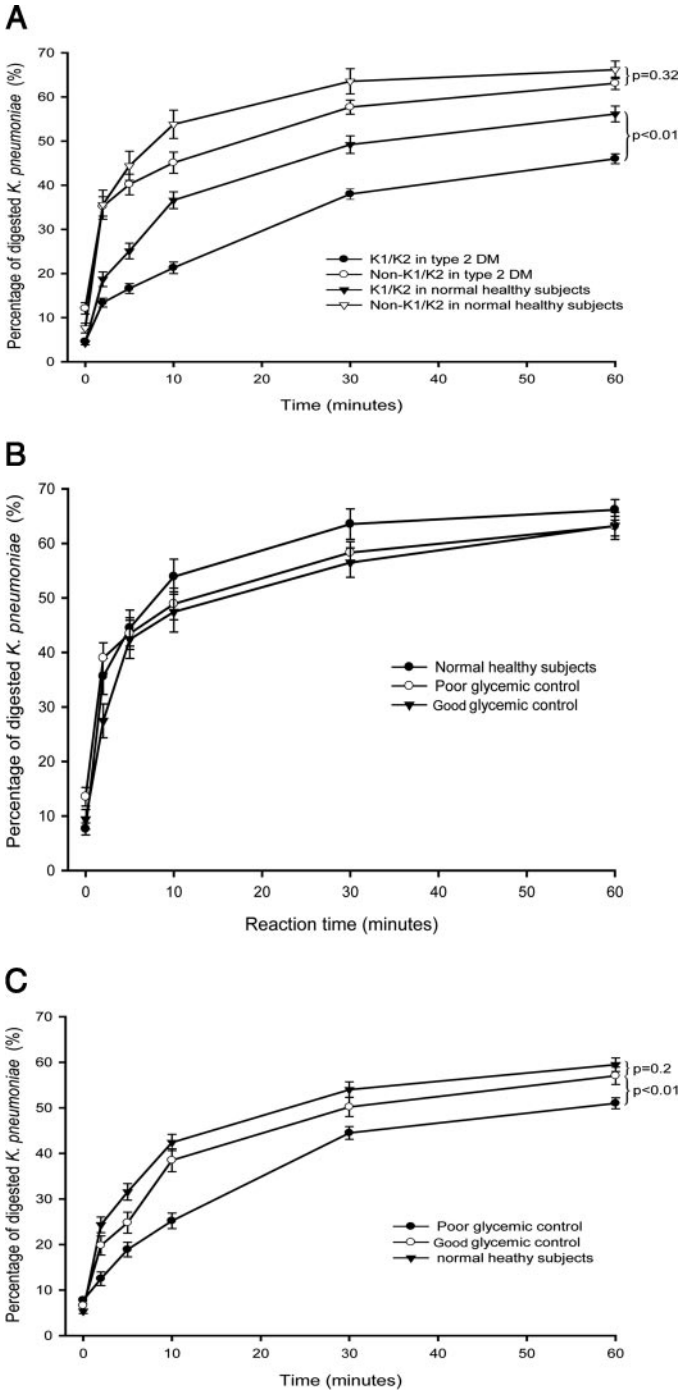

FIG. 1. Comparisons of neutrophil phagocytosis of serotypes K1/K2 and non-K1/K2 isolates between patients with type 2 DM and normal healthy controls (A). Neutrophil phagocytosis among type 2 DM patients with poor glycemic control and good glycemic control and normal healthy controls against serotypes non-K1/K2 *K. pneumoniae* (B) and serotypes K1/K2 *K. pneumoniae* (C).

Results

Effect of type 2 DM on neutrophil phagocytosis of K. pneumoniae serotypes K1/K2 and non-K1/K2

To differentiate between diabetic and capsular effects, *K. pneumoniae* were grouped into serotypes K1/K2 and non-K1/

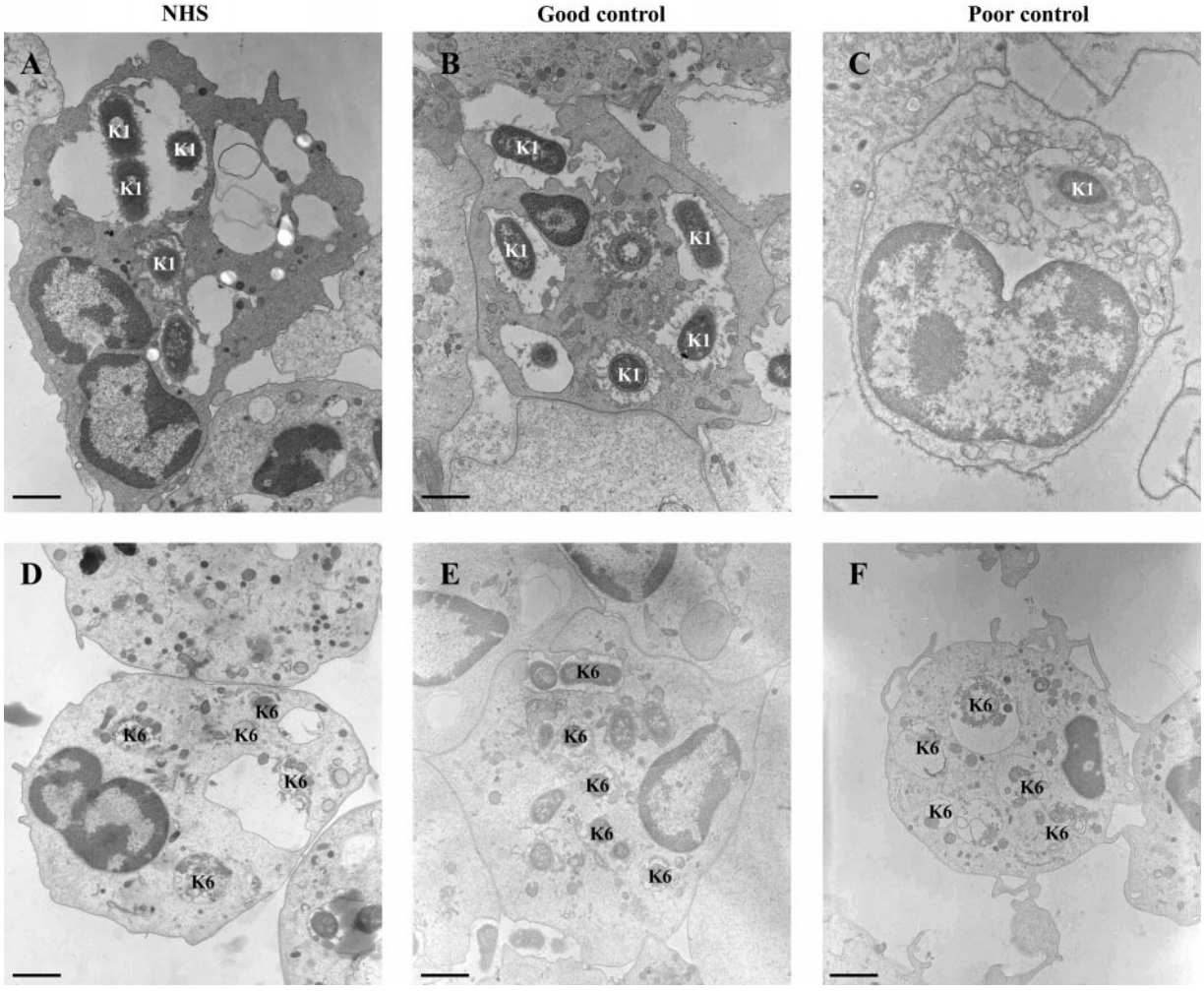

FIG. 2. Evaluation of serotype K1 and non-K1 (K6) that were phagocytosed for 60 min with neutrophil from normal healthy controls and good and poor glycemic control type 2 DM patients by transmission electron microscope. A–C show serotype K1 phagocytosed by neutrophil of normal healthy controls and good and poor glycemic control type 2 DM patients (*bar*, 1 μm). D–F show serotype K6 phagocytosed by neutrophil of normal healthy controls and good and poor glycemic control type 2 DM patients. Intracellular lysis in vacuoles was shown in K6. NHS, Normal healthy subjects.

K2, to compare phagocytic rate between healthy subjects and type 2 DM patients (Fig. 1A). For non-K1/K2 isolates, no significant difference of phagocytic rate at 60 min (66.15 ± 1.9 and 63.1 ± 1.4%; $P = 0.32$) was observed between healthy subjects and type 2 DM patients. In contrast, a significant decreased phagocytic rate at 60 min (46 ± 1.1 and 56.1 ± 1.8%; $P < 0.01$) for serotype K1/K2 isolates was observed, respectively, from type 2 DM patients and healthy subjects (Fig. 1A).

Effect of glycemic control of type 2 DM on neutrophil phagocytosis in serotypes K1/K2 and non-K1/K2 isolates

To ascertain the type 2 DM effect on impaired phagocytosis, patients with type 2 DM were further divided into good glycemic control and poor glycemic control groups. Non-K1/K2 isolates were all highly susceptible to neutrophil phagocytosis in healthy, good, and poor glycemic control groups at 60 min with phagocytic uptake rate of 66.1 ± 1.9, 63.1 ± 1.8, and 63.2 ± 2.5%, respectively. No significant difference in phagocytic rate of non-K1/K2 was observed in all samples (Fig 1B). In contrast, there was a significant impairment of phagocytosis for poor glycemic control groups against serotypes K1/K2 strains at 10 min (25.2 ± 1.7 *vs.* 38.2 ± 1.7 and 42.4 ± 1.8%) and persisted until 60 min (51 ± 1.2 *vs.* 57 ± 0.3 and 59.4 ± 1.4%, $P < 0.01$) in comparing to good glycemic control and healthy control group, respectively (Fig. 1C). The neutrophil phagocytic rate was inversely correlated to the fasting blood glucose level of serotype K1/K2 ($R = -0.32228$, $P < 0.01$), whereas there was no correlation between phagocytic rate and glycemic control ($R = -0.05178$, $P = 0.6359$) of non-K1/K2 serotypes. A similar correlation was also observed when hemoglobin A1c instead of fasting blood glucose level was compared with neutrophil phagocytic rate (data not shown). Although the phagocytic activity appears to be less efficient in the good glycemic control group compared with the healthy individuals, the data were not statistically significant.

Electron microscopic results

Viable *K. pneumoniae* serotype K1 and K6 isolates were further selected to assess neutrophil phagocytosis. For serotype K1, the phagocytosed bacteria in the neutrophils from type 2 DM patients with poor glycemic control were less than

those of good glycemic control and normal healthy controls. In poor glycemic control neutrophils, phagocytosed serotype K1 still maintained an intact cell structure and capsule in a vacuole without evidence of intracellular lysis 60 min after uptake (Fig. 2C). In good glycemic control neutrophils, phagocytosis and partial intracellular lysis of serotype K1 was comparable to the normal healthy controls (Fig. 2, A and B). For serotype K6, cell wall rupture and complete lysis in larger vacuoles within the neutrophil was observed. The phagocytosed numbers and intracellular lysis of serotype K6 were not significantly different among the three groups of subjects (Fig. 2, D–F).

Discussion

Previous studies observed that DM was the only underlying factor highly correlated to the *K. pneumoniae* liver abscess, especially with the complication of septic endophthalmitis (2–4). In this study, neutrophil phagocytosis of K1/K2 *K. pneumoniae* was lower in patients with type 2 DM than normal healthy controls. However, no difference in phagocytosis of non-K1/K2 isolates was observed, indicating that a bacterial factor instead of DM was the major factor affecting the phagocytic rate. DM was an additional factor influencing phagocytosis when tested against virulent K1/K2 isolates. This observation may explain why only K1/K2 *K. pneumoniae* were found in complicated endophthalmitis, with over 93% of the cases involving DM (2).

Cases with complicated endophthalmitis or meningitis in unrecognized DM have been documented frequently (2, 3, 8, 9). Poor glycemic control of these patients was of concern in the development of the complication. Further analysis showed that type 2 DM with good glycemic control did not show a significant reduction of phagocytosis against K1/K2 *K. pneumoniae*. In contrast, poor glycemic control markedly reduced phagocytosis of virulent K1/K2 *K. pneumoniae*. For non-K1/K2 *K. pneumoniae*, there was no significant difference in neutrophil phagocytosis among good and poor glycemic control type 2 DM patients and normal healthy controls. As the age of the patients increased, phagocytosis of K1/K2 *K. pneumoniae* decreased further in poor glycemic control subjects but not in good glycemic control type 2 DM patients (data not shown). The results further support the notion that poor glycemic control plays a role in K1/K2 liver abscess. Thus, strict metabolic control may improve the phagocytic function of neutrophils to virulent strains of *K. pneumoniae*. Improving neutrophil function by way of blood glucose control will reduce the incidence of infection and improve response to antibiotic therapy (18). Impaired neutrophil bactericidal function was strongly associated with poor glycemic control and improved positively with good glycemic control (19, 20). Our study disclosed that good glycemic control improved neutrophil phagocytosis of serotypes K1/K2 *K. pneumoniae* implicating this factor as a prevention of complication.

In summary, type 2 DM with poor glycemic control was a factor in the phagocytic-resistance of K1/K2 *K. pneumoniae*. Strict metabolic control may improve the neutrophil phagocytosis of K1/K2 *K. pneumoniae* in patients with type 2 DM and may also prevent the development of serious metastatic complication.

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