Reviewer's report

Title: Erythrocyte membrane anionic charge in type 2 diabetic patients with retinopathy

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Reviewer: Tsuneharu Baba

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General
Dr. Budak et al. studied the erythorcte anionic chanrge (EAC) and urinary glycosaminoglycan in 49 Type 2 diabetic patients and 38 non-diabetic control subjects. The authors observed a decreased level of EAC in diabetic patients with retinopathy as compared to those without retinopathy and the control subjects, whereas urinary glycosaminoglycan (CAG) was not associated with retinopathy. The study rationale is sound and the study was well conducted. There are, however, a few points to be considered.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. The number of patients in each group is small, particularly the group 3 (proliferative retinopathy) has only 13 patients. This may make it difficult to reach a reliable conclusion. Some parameters might not have reached a statistical significant level because of type II statistical error due to the small sample size.
2. Urinary tract infection (UTI) is not mentioned in the subject exclusion criteria (Method, Patients, Paragraph 2). UTI could be a cause of microalbuminuria. How did the authors excluded those those with asymptomatic UTI?
3. How the authors selected the patients in each group is not clear. Were the patients each group prospectively selected according to the definition of each group, or the whole patients were later divided into 3 subgroups? The group comparison method is applicable in the first case, and correlation analyses for the latter case.
4. How many time of 24 hour urine sampling was made to calculate the urinary albumin excretion in each subject? As the day-to-day variation is not so small, this may have influenced the results of correlation analyses. In addition, all 3 groups of diabetic patients present a similar level of microalbuminuria (Table 2). What are the range of microalbuminuria in each group? Nearly 20 - 30 % of microalbuminuria seen in Type 2 diabetic patients without retinopathy are associated with no diabetic kidney demise or other cause than nephropathy.
5. It is not clear whether urinary excretion of CAG would represent the local change, such as seen in retina. Does kidney function has no influence on its urinary level?
6. The study demonstrated a decrease in EAC in diabetic patients with retinopathy, which observation was more prominent in patients with proliferative retinopathy. This nevertheless does not lead to the conclusion that “Type 2 diabetic patients with low erythorcpyte anionic charge are susceptible to the development of retinopathy” (Conclusion), as one does not know yet from this study whether the observation is the cause or the result of retinal change, or general changes in body, which progress in parallel with development of diabetic microvascular changes. The conclusion in the abstract and the discussion need to be changed accordingly.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
Discretionary Revisions (which the author can choose to ignore)

**What next?:** Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No

**Declaration of competing interests:**

None