論文の内容の要旨

論文題目　Development of clinical index in islet cell transplantation for type 1 diabetes: Association between hypoglycemic events, self-monitoring of blood glucose and graft function

(1 型糖尿病に対する膵島移植における臨床指標の開発: 移植片機能と低血糖発作、自己血糖測定の関係)

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Islet cell transplantation (ICT) is a promising treatment option for patient with brittle type 1 diabetes. Although ICT is a potentially curative treatment and a minimally-invasive procedure, one of the major difficulties is maintaining long-term graft function and insulin independence due to the loss of transplanted islets by inflammatory reactions, allogenic rejection, autoimmune recurrence and senescence. Thus, one of the greatest concerns in ICT is to clarify clinical features as well as basic backgrounds to develop islet graft dysfunction.

Self-monitoring blood glucose (SMBG) is traditionally but still widely used for glycemic control and has kept its value by providing day-to-day or intra-day glucose excursions in clinical practice of diabetes mellitus. Studies on the interpretation of SMBG assessments in ICT, which is accompanied with dramatic change of glycemic control from largely fluctuated profile to stable pattern, has been limited since one of the difficulties in analyzing SMBG is that investigators have to deal with a large amount of data from daily measurements and complex calculations to evaluate the quality of glucose control. Cluster analysis has been frequently used in the field of genomics and proteomics together with heatmap to handle and interpret large volume of data by classifying them on the basis of their similarity. Whereas, the application of cluster analysis coupling with heatmap

to SMGB data has not been employed as far as we searched.

A well-designed and easily-accessible graft index is highly desirable to manage islet recipients clinically, since physicians have to control immunosuppression. Clinical islet graft indices of the secretory unit of islet transplant objects (SUITO) index, C-peptide per glucose ratio (CP/G) and β score have been proposed; however, reports on extended study to link between these graft indices and hypoglycemic events are limited. We developed the secretory unit of islet transplant objects (SUITO) index and reported that the SUITO index is associated with daily insulin dose, HbA1c, intravenous glucose tolerance test (IVGTT) and scores from QoL questionnaires of the short form 36 health survey questionnaire (SF-36).

Therefore, we hypothesized the following two possibities; (I) Cluster analysis and heatmap display are useful for the evaluation of SMBG in ICT. (II) The SUITO index should be related to the frequency and severity of hypoglycemic episodes. The aim of this study is to investigate the association between self-monitoring of blood glucose (SMBG), hypoglycemic episodes and islet graft function.

Eleven islet recipients were included in this study. This study was performed under the acceptance of study guidance between University of Tokyo and Baylor Research Institute (Dallas, TX, USA). The patients visited the clinic monthly after ICTs and provided blood samples for fasting C-peptide which was used to calculate the clinical islet graft indices. The number of hypoglycemic events was also reported. Hypoglycemic events were defined as those with SMBG levels below 3.8 mmol/L and severe events were below 2.2 mmol/L or with hypoglycemic unawareness. The SMBG data for three days immediately before each clinic visit were evaluated with 27 assessment tools including M-value, mean amplitude of glycemic excursions (MAGE), J-index, index of glycemic control, average daily risk range (ADRR), Glycemic Risk Assessment Diabetes Equation (GRADE). The hierarchical cluster analysis was performed for both SMBG assessment tools and samples. The optimal number of clusters and their stabilities were determined with 1,000 bootstrap resampling. The multivariate logistic model was used to select statistically significant SMBG clusters for the prediction of islet graft function. The receiver operating characteristic (ROC) analysis was employed to evaluate the discrimination ability of selected SMBG clusters or clinical graft index for islet graft function or hypoglycemic events, correcting repeated observations.

Basic characteristics of patients before ICT were followings; age; 43.8 ± 3.3 (mean ± *S.E.*) years old, gender; nine females and two males, body weight; 67.0 ± 3.6 kg and body mass index (BMI): 24.3 ± 1.1 $m^2$/kg. Eight patients achieved insulin independence after ICTs. Four, five and two patients received one, two and three dose of islets, respectively.

Cluster analysis for SMBG assessments revealed five types of clusters with 100% of stabilities in 1,000 resamplings, named as euglycemia, hypoglycemia, semi-hyperglycemia, hyperglycemia and glucose fluctuation cluster on the basis of the elements in each cluster. These clusters showed similar patterns according to islet graft function on the heatmap. The euglycemia cluster ($p < 0.001$) and hypoglycemia cluster ($p = 0.001$) were observed as significant factors in the multivariate logistic model to predict islet graft function. The final prediction model demonstrated 0.927 of area under the curve (AUC) of ROC curve (95% confidence interval: 0.887 – 0.967, $p < 0.001$).

Significant increase of SUITO index and significant decrease of the numbers of hypoglycemic events were observed within 14 months when compared to pre-transplant data using ($p$ with repeated-measure ANOVA < 0.05). The SUITO index, CP/G and β score had significant discrimination with outstanding accuracy for total hypoglycemic events (AUC of ROC curve [95% confidence interval]; 0.924 [0.885 – 0.964], 0.936 [0.901 – 0971] and 0.929 [0.891 – 0.967], respectively. $p$ value for all indices < 0.001) as well as severe events (0.925 [0.884 – 0.967], 0.923 [0.881 – 0.965] and 0.910 [0.868 – 0.952], respectively. $p$ value for all indices < 0.001). No significant differences of AUC were found in these indices. The cut-off points of SUITO index 26 had 88.3% of sensitivity and 93.2% of specificity for the occurrence of total hypoglycemic events while 10 of SUITO index showed 90.0% and 82.0% for severe events. The frequencies of both total and severe hypoglycemic events were inversely correlated with the SUITO index (Spearman $r = -0.663$ [$p < 0.001$] and $r = -0.521$ [$p < 0.001$]).

This report demonstrated that the cluster analysis of SMBG could provide helpful information on glycemic profiles and discrimination of graft function in islet recipients, and the SUITO index, as a clinical graft index of islet function, has statistically significant association with hypoglycemic events after ICT.

The advantages of the SMBG clusters are advanced visualization of glucose profile, representation of 27 SMBG assessment tools previously published and linking between glucose profile and islet graft function by the prediction model. The disadvantages in current study include limited clinical availability due to complex and time-consuming calculation process, interpretation requiring special training and the lack of factors on insulin treatment and carbon intake when the prediction model for islet graft function was developed. These issues should be considered in the future study and the following extensive studies also should be directed; demonstration of the SMBG clusters with newly onset type 1 diabetic patients and data availability for continuous glucose monitoring (CGM).

Single-donor ICT does successfully make type 1 diabetic patients insulin independent.

One of the advantages of ICT is the relative ease of repeated transplantation. The SMBG cluster analysis can be used to estimate the timing of additional ICT, providing uninterrupted periods of long-term insulin independence by utilizing re-transplantation.

ROC analysis showed the accuracy of the clinical graft indices in predicting hypoglycemic episodes. AUCs of ROC curves of the SUITO index for the occurrence of total and severe hypoglycemic events indicated outstanding accuracy (AUC > 0.9) as well as those of CP/G, β score and LBGI. The similar AUCs among the SUITO index, CP/G, β score and LBGI suggest that the islet graft indices would be useful as a predictor for hypoglycemia. Values of 26 and 10 on the SUITO index provided reasonable cut-off points for the occurrence of total and severe hypoglycemic episodes respectively and it was consistent with previous results; SUITO index > 26 predicted insulin independence after ICTs and SUITO index > 10 was associated with higher scores of the following QoL questionnaires; the physical functioning, energy/fatigue and emotional well-being subscales in SF-36. The benefits of the SUITO index are simple calculation compared to β score and available linking with insulin independence, exogenous insulin amounts, glucose tolerance test, QoL and hypoglycemia from previous and current studies. However, these findings were reported using small population and no statistically significant differences were observed against other indices to date. Large cohort study should be prepared to justify which graft indices are most reasonable in clinical field.

In summary, the SMBG cluster analysis provided excellent discrimination of islet graft function and helpful information on glycemic profiles in ICT and the SUITO index could predict hypoglycemic episodes including severe events with excellent accuracy. Limitations of this study include the small number of subjects and retrospective design. Further prospective investigations with a larger group size will provide definitive conclusions.