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## Dichotomous grouped-time survival analysis with shared frailty model: A new statistical approach to test gateway hypothesis and analyze repeated measures with dichotomous outcome

Rana Mohammed Jaber

Robert Stempel College of Public Health and Social Work, USA

Dichotomous grouped-time survival analysis is a combination of the grouped cox model, the discrete time hazard model and the dichotomous approach. This method was used to test the gateway hypothesis that waterpipe smoking may predict cigarette smoking initiation among adolescents by comparing the hazards of initiating cigarette smoking between waterpipe and never smokers. In this statistical approach, survival time is represented as a set of indicators of whether or not the participant failed in each time point (until the individual experiences the eventor is censored). This approach considers the timing as well as the occurrence of the event. It also handles censoring and allows for a discrete specification of time when the data are interval-censored. Items measured at each time point were used for time-varying predictors by linking predictors pertaining to a certain time point to the risk of cigarette smoking initiation at the subsequent time points. 'ProcPhreg' commands were used in SAS with shared frailty model considering the school as a random variable to account for the unobserved heterogeneity due to clustering. This analysis allowed for maximum data use, inclusion of time-dependent covariates and relaxing of the proportional hazards assumption as well as minimizing the standard error of the estimate.

rjabe001@fiu.edu

## Closer look at the neuronal proteome

Caroline May Ruhr-University Bochum, Germany

The brain has a highly complex structure and can be sub-divided into different regions, fields and cellular layers. Moreover, different neuronal and glial cells types can be distinguished which differ in their morphology, functionality, cell numbers and their also their proteomes. In the past most of the proteomic studies were done on whole brain lysate within these studies dominant cell types such as brain glial cells can mask neuron-specific information. The separation of brain-derived cells is a challenging task especially when neurons are the focus of study. Isolating intact neurons is not feasible with traditional methods such as tissue homogenization techniques. The advent of laser microdissection techniques promises to overcome previous limitations. Here, we provide a detailed protocol for isolating and analyzing neurons from postmortem human brain tissue samples. The analysis of distinct cell types is necessary for understanding normal brain function and how certain cell types are altered in brain diseases as example in Parkinson's, Alzheimer's or Huntington's disease. Therefore, the isolation of disease-related cell types could explain the typical abnormalities and furthermore, it could clarify why some neuronal populations are widely affected earlier than others and which mechanisms could have an impact in protecting individual cells from neurodegeneration.

caroline.may@rub.de

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