Chapter 1

Introduction and Review of Literature

1.1. Introduction

The liver assists to battle infections and cleans blood. It also assists to absorb food and stores a outward appearance of sugar in a body make use for energy. Cirrhosis is particular condition of liver to be obtained fascinated within the sea of mark and struggle to regenerate. It manipulates the slow contraction of the size of the liver. Liver transplantation is surgical procedure to remove a contaminated or wounded liver and replace it with good physical shape one from another person, called a donor (Fig 1). There are an anticipated 300,000 hospitalizations and 30,000 deaths in the world due to persistent liver diseases each year. Orthopedic (solid or whole organ) liver transplantation (OLT) has considerably enhanced the prediction in patients with end-stage liver disease and metabolic liver diseases.

![Living-Donor Liver Transplant](image_url)

Fig 1: Demonstrates the liver transplantation procedure.

However, the number of patients requiring OLT far goes above the number of solid livers accessible for transplant and the risks and difficulty of OLT in chronically incapacitated, often underfed, patients are considerable and the financial costs are expensive during the first year following OLT. Over the past thirty years, a considerable body of scientific literature has collected representing (in animals) the ability of infused hepatocytes to en-graft in host tissue, survive, proliferate, function and contribute in the regenerative process. Transplantation of
hepatocytes into the spleen or liver has been exposed to correct inherited imperfections in metabolism in numerous animal models, to almost completely repopulate a host liver under conditions where the host liver cells have a reduced life-span (as in the FAH-deficient mouse model), to provide hepatic function during acute liver failure induced by a variety of insults and to get better liver function and extended survival in carbon tetrachloride-induced models of cirrhosis.

Data from a number of clinical reports propose that the cells did certainly engraft, survive and functions well. Many of these patients were tremendously ill, with grade four encephalopathy (coma). Within days, some patients in these studies awoke from their hepatic comas, were weaned off their respirators, demonstrated improvements in cerebral blood flow and intracranial pressures and experienced a significant drop in blood ammonia levels. In one study, the artificial capabilities of the liver showed enhancement 4 to 6 months post-transplant as supported by improved albumin levels and prothrombin time (PT).

In humans, case studies and case reports published (or otherwise documented) by independent researchers explain the organization of other preparations of human liver cells to over fifty patients. Robert A. Fisher, MD and his colleagues at College in Virginia have carried out liver cell removes in the biggest number of these patients and the outcomes of their work were vital in deciding the dosages (number of cells) that will be handled and the splenic artery route of administration that will be applied in this early Phase one trial of Incara's cryopreserved liver cells.

Incara's cryopreserved human liver cells are obtained from whole livers (procured from heart-beating donors) that are found to be unsuitable for OLT. Furthermore, it is expected that a single donated (whole) liver may provide a sufficient number of cells to treat multiple patients (based on animal data, in which a relatively small number of transplanted hepatocytes repopulated livers in certain animal models of liver disease). Therefore, the availability of cryopreserved human liver cells would greatly increase the "donor pool" and thereby provide a treatment option for patients who are denied an OLT because of lack of an available whole (solid) liver or who have co-morbidities that make the patient unsuitable for OLT. Having a bank of cryopreserved cells also offers the advantage of being able to use the cells electively, when and where they are
needed. If cells can bank from a sufficient number of donors, the potential also exists for closer matching of host and donor tissue antigens.

In liver cirrhosis problem, the duration between transplantation to recover is crucial period for patients. Patients are generally measured through follow up periods with liver functioning effects. The disease severity in patient with liver cirrhosis is measured through the model for end stage liver disease (MELD). It is continuous disease severities scale with highly predictive of the risk of dying from liver. The score is adopted by UNOS (the United Network for Organ Sharing) for use in allocating livers to patients on the liver transplantation waiting list. Set of respondent are divided into two parts as cured and non-cured. The main goal of this work is to look on the cure and non-cured rate among liver cirrhosis patients through MELD score. The patients alive at any point of times are defined as cured otherwise non-cured. The mixture distribution is useful to formulate the cure rate model. The deep consideration is important on patients’ treatment effects through long term observations. The model is ultimate choice for treatment effect comparison. It is widely applied in the field of economic, reliability and criminology. Recently, Cure rate model in medical research is elaborated and extended. It is applied for AIDs in HIV positive patients. The survival experience is defined with cure rate model. It is also used in the recidivism time among the prisoners in Western Australia. The product of log-normal survival function is appropriate for non-cured portion of patients. The simple log-normal distribution as a choice of mixture model is elaborated. It provides stratified results in drug treatment effect of lymphoma patients. The exponential distribution as a choice of mixture model is useful for non-cured patients. The MELD Score (UNOS Modification) is calculated as follows

\[
\text{MELD Score} = 9.57 \times \log e(\text{serum creatinine}) + 3.78 \times \log e(\text{bilirubin}) + 11.2 \\
\times \log e(\text{INR}) + 6.43.
\]

The MELD is useful tool to detect the liver status in patients. It is widely used method for organ allocation in liver transplantation. The score function is formulated with consideration of liver and renal functions. Several studies have concluded that the liver transplanted patients having low MELD score can be influenced for death. There is different biochemical parameters used to calculate the MELD score. The details to compute the MELD score can be cited with www.mayo.edu/int-med/gi/model/mayomodl-5 unos.htm.
In interpreting the MELD Score in hospitalized patients are described as below:

<table>
<thead>
<tr>
<th>MELD Score</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 40</td>
<td>71.3% mortality</td>
</tr>
<tr>
<td>30–39</td>
<td>52.6% mortality</td>
</tr>
<tr>
<td>20–29</td>
<td>19.6% mortality</td>
</tr>
<tr>
<td>10–19</td>
<td>6.0% mortality</td>
</tr>
<tr>
<td>&lt; 9</td>
<td>1.9% mortality</td>
</tr>
</tbody>
</table>

1.2. Review of literature

Late stage progressive hepatic fibrosis differentiated by deformation of hepatic architecture, necrosis of hepatocyte and formation of regenerative nodules leads to cirrhosis. The liver has a not likely regenerative ability but, subsequent chronic liver injure, this starts to fall short, and then fibrosis, and finally cirrhosis develops. Presently the just curing treatment for advanced liver cirrhosis is liver transplant. While liver transplant has revolve into a technique with a fairly good five-year survival, organ contribution has not kept up with demand, which has resulted in an growing number of patients on the liver transplant coming up list waiting longer for a donor organ, which leads to amplified morbidity and mortality. Although there is emerging facts that extending the donor organ criteria may impact on mortality rate, there is obviously still an urgent need to expand another strategies for treatment of advanced liver disease, and numerically cirrhosis is most significant target. Strom et.al and Mitry et.al reported that responsibility of hepatocyte transplantation in dealing of acute and chronic liver infection is less obvious due to complexity in organizing large-scale clinical studies. Indeed, the main factor restrictive the practice of hepatocyte transplantation is again accessibility of liver grafts for cell separation. Mito and Kusano were first to try hepatocyte transplantation in cirrhotic patients. Hepatocytes were isolated from the parts of the cirrhotic livers of the patients and transplanted by injection into the splenic pulp, splenic artery, splenic vein, or portal vein.
Storm et. al. reported that transplantation of minute numbers (0.1% or less of normal hepatocyte mass) of ABO-matched, freshly isolated human hepatic progenitor cells via injection into hepatic artery in two adults with ALF due to hepatitis B virus infection and phenytoin hepatotoxicity, respectively.

Bilir et.al. from the University of Colorado consequently transplanted bigger numbers of primary human hepatocytes (in order of 5% of the normal hepatocyte mass) by percutaneous injection in 5 adult patients with ALF and grade IV encephalopathy who were not candidates for OLT. Three (60%) patients survived more than 72 hours, with evidence of improved encephalopathy score, serum ammonia levels and prothrombin times. Although substantial progress has been made in hepatic stem/progenitor cell transplantation in pre-clinical trials, however, there is lack of proven safety data to treat the end stage liver cirrhosis. Despite contradictory results from animal models, they investigated serial clinical and pathological features and the clinical impact after human hepatic stem cell infusion in patients for advanced liver cirrhosis.

With these points in view, the present study is an attempt to apply advanced statistical tools in estimating the HFPLC (Human Fetal Liver Progenitor Cell) therapy effect as compared to conventional therapy on patients suffering with liver cirrhosis. Also, it is aimed to extend Bayesian approach on cure rate model on patients responded to transplantation vs. non responders and also analyze missing data in longitudinal data analysis and apply as a new methodology.

Yakovlev, et.al. suggested that parametric cure rate model through Bayesian and Frequency approach are initiated nearly two decades earlier and it is built with assumption of two groups of patients i.e. (I) cured and (II) non-cured. However, the distributional assumptions of group of patients are sometimes become problematic. Non-cured part is applicable for those samples that are subject to the event of death and rest of them are as cured. Chen et. al. suggested that those patients are survived till the end of the study is defined as cured group. The finite mixture of Weibull distributions and Generalized Gamma distribution in survival function has been elaborated in melanoma clinical trial data and found suitable. However, the models are quite complicated to apply for analysis. Lambert et.al expressed that the flexible parametric model in survival analysis is discussed and extended with relative survival function.
There are many statistical methods are developed to analyze survival data. A most frequent approach to analyze survival data is by assuming that study population is homogeneous.

Beard et.al expressed that the term frailty was introduced in order to account for unobserved heterogeneity with random effects, and association in survival models. This concept of frailty was later introduced to biostatistics by applying it on population mortality data. Clayton et.al suggested that the applications of model to multivariate survival data were applied on chronic disease incidence in families.

Diggle and Zeger et.al presented a detailed outline of models used for longitudinal data. A wide range of statistical models for analyzing outcomes with missing data is available, but their validity depends on the nature of the missing-data mechanism as well as on the assumptions used. Satty et. al and Little R.J.A suggested that selection models partition function of outcome and response data indicator as the product of function of outcome and function of outcome given response data indicator. This requires explicit modeling of the missing-data mechanism where the probability that a subject is missing may depend on observed and unobserved values.

1.3. Objectives

The objectives of this study are,

I. Survival analysis on patients with liver cirrhosis
   a. Exploratory data analysis (preliminary analysis on survival data like, K-M estimator, Log-rank test etc...)
   b. Bayesian approach on Fitting of Cure rate model on data of patient with liver cirrhosis
   c. Fitting of shared frailty model for survival data with various baseline and frailty distributions

II. Assuming that missing values will occur and to deal with such problem proper algorithm will be developed.

III. To fit statistical model with working correlation structure (the application of GEE) on liver cirrhosis patients.
To apply Bayesian Computation for the Concordance Correlation Coefficient to establish the agreement between two surgical procedures in liver cirrhosis patients

1.4. Data Methods

The secondary data used in this thesis has been obtained from a clinical trial conducted on liver transplant patients. A total of 325 patients have been screened over a long period of time. Among them a total of 172 patients have been found suitable to participate in study. A total of 172 subjects with cirrhosis are randomized in two different treatment group i.e. 86 in each group. In the treated group, subjects are given consenting for participation with new surgical procedure and in the control group; subjects are given with consenting for participation along with conventional surgical procedure. The duration of the study is 36 months with 7 visits (Baseline, 3 months, 6 months, 9 months, 12 months, 24 months & 36 months). The parameters under consideration is MELD Scores, Serum Albumin, SGOT, SGPR, AlkPhos, INR, Serum Bilirubin & S. creatinine, weight of liver and triglycerides . Each patient observation has been taken in seven different time points of each patient. The variable “Subject”, being the patient's code, the time from liver transplantation to death or censoring is stored in Duration while status is 1 when subject died and 0 for censored observations. Four covariates are available: Therapy, subject received consenting for participation with new surgical procedure or subjects are given with consenting for participation along with conventional surgical procedure, Gender, being 1 for males and 2 for females, Centre, being 1 for T1 and 2 for T2, Meld, being the meld score of each subject and triglycerides are considered into the model.

Assessments for initial determination of patient eligibility (14 to 10 days prior to the day of intended cell transplantation) were included a complete physical examination and medical history, grading of encephalopathy, clinical laboratory studies (hematology, blood chemistry, urinalysis and coagulation tests), a serum pregnancy test (for females of child-bearing potential), ABO blood typing (if not known), HLA Class I antigen typing (if not known) and a Panel Reactive Antibody test with identification of any preformed antibodies, serologic testing for Epstein-Barr virus (EBV) and cytomegalovirus (CMV) and calculation of the patient’s MELD and Child-Pugh Scores. Only cells from an ABO-compatible donor with no HLA Class I antigen to which the recipient has performed antibodies were selected for transplant. Furthermore, cells
from an EBV-positive or CMV-positive donor were only administered to EBV-positive or CMV-positive recipients.

Intravenous administration of adequate hydration was begun at 2:00 AM and last until about one hour before discharge. Assessments and procedures that was preceded the catheterization procedure include clinical laboratory studies, assessment of donor-specific HLA Class I antigens (expected to be absent prior to cell transplantation), grading of encephalopathy, a brief physical examination, calculation of the patient’s MELD and Child-Pugh Scores and assessment of trough plasma tacrolimus concentration. Prophylactic antibacterial and antifungal agents were administered before (both agents) and after (antibiotics only) the catheterization procedure. Oral Pepcid (famotidine) and a 30-minute infusion of 250 mg of Solu-Medrol (methylprednisolone) were administered 2 hours before splenic artery catheterization.

The patients were taken to the radiology suite approximately 30 to 60 minutes before splenic artery catheterization. After induction of conscious sedation, a catheter was inserted into the femoral artery and, under fluoroscopic guidance, passed into the splenic artery. The final position of the catheter was confirmed with a small volume of contrast dye. Blood pressure, heart rate, respiratory rate and O2 saturation was monitored frequently during cell infusion and until the catheter is removed. Confirmation of splenic artery and splenic vein patency with contrast media was performed just before cell infusion and again just after cell infusion.

Discharge medications were included Prograf (dosage adjusted, if necessary, based on assessment of trough tacrolimus concentration assessed that morning), prednisone (20 mg p.o. once daily for two days; then 10 mg p.o. once daily for the next five days; then 5 mg p.o. once daily until the Week 24 Visit) and Diflucan (fluconazole), 100 mg p.o. once daily for ten days. Patients were scheduled to attend follow-up visits twice in the first week after cell transplantation, then weekly until eight weeks after cell transplantation, then once every four weeks until 24 weeks after cell transplantation, then once every three months until two years after cell transplantation. Routine clinical laboratory testing (hematology, blood chemistry and urinalysis) and assessments of trough plasma tacrolimus levels were also performed once each month between visits (that were scheduled every three months) after the Week 24 Visit.
1.5. Design and patients

The secondary data used in this thesis considered from the path http://www4.stat.ncsu.edu/~boos/var.select/pbc.html; accessed on January 6, 2013. All patients gave written, informed consent to participate in the study, and institutional ethics committee approval was obtained before the starting of the study. The study was conducted according to the requirements of Good Clinical Practice (GCP) of the (ICH-E5). The two combined surgical procedure have been studied by consenting for participation HFLPC Infusion and consenting for participation alone group (Fig 1.2).

![Study flow diagram](image)

**Fig 1.2: Study flow diagram**

The major inclusion and exclusion criteria are as follows:

A). Inclusion Criteria

Patients must be at least 18 years of age. Patients must be able to understand the explanation of the study and be competent to read and voluntarily sign the patient informed consent form. Female patients of childbearing potential (i.e., who have not had a hysterectomy and/or bilateral ophorectomy OR who are not postmenopausal with at least one year having passed since the last
menses) must have a negative serum pregnancy test and must use (and agree to continue to use) an approved barrier (e.g., condom, diaphragm with spermicide), intrauterine device (IUD) or hormonal method of birth control. Patients must have a diagnosis of cirrhosis and end-stage liver disease, based on all three of the following criteria:

- A histological (biopsy) diagnosis of cirrhosis or evidence of a nodular pattern of cirrhosis obtained by sonography, CT scan or MRI.
- A history of hepatic encephalopathy or clinical evidence of portal hypertension
- A Model for End-Stage Liver Disease (MELD) Score of 12 to 24.

Patients must be considered ineligible for solid liver transplantation because of documented co-morbidities (such as advanced cardiopulmonary disease or cancer). Patients must have a patent splenic artery and splenic vein. Patients must be residing outside the hospital.

B). Exclusion Criteria

Patients will be excluded from participation in the study if he/she is an HIV +ve. He/she has preformed antibodies to class I antigens that are present on all available donor samples. (Only cells from an ABO-compatible donor with no HLA Class I antigen to which the recipient has preformed antibodies will be selected for transplant.). He/she has tested positive for Hepatitis B Surface Antigen (HBsAg), Hepatitis Be antigen (HBeAg) or HBV DNA. He/she not has a credible history of abstinence for at least six months (for patients with alcoholic cirrhosis). He/she has serum creatinine concentration more than 1.5 mg/dL. Female patients who are breast feeding. The use of any investigational drug within 30 days or five elimination half-lives of the drug, whichever is longer. He/she has used any other medical condition that, in the opinion of the investigator, makes the patient unsuitable for participation in this trial.

1.6. Organization of the study

The thesis is organized as a collection of 5 research papers which have been submitted for peer reviewed international journals. Out of these 5 papers, 2 papers have been published and the others are still in review.

Each paper has been written as a stand-alone article that can be read separately from the rest of the thesis but draws separate conclusions that link to the overall research objectives and questions.
In Chapters 2 focuses on cure rate modeling on liver cirrhosis data in conjunction with Bayesian is the central idea, while Chapters 3 focuses on shared frailty modeling approach to adjust for heterogeneity factor into the model. Chapter 4 discusses on comparison of information criteria of CIC & QIC by taking into consideration of importance of working correlation structure. The focus of chapter 5 is on fitting of selection modeling approach for analyzing the missing observation. In Chapter 6, the focus is on concordance correlation co-efficient with application of Bayesian counterpart. While most chapters are stand-alone, the study is easier to follow and understand. Chapter 7 should be read when all the other chapters have been read as it summarizes the findings. A brief outline follows:

Chapter 1: This chapter serves as an introduction and review of literature of the study.

Chapter 2: This chapter focuses on cure rate model, with particular focus on Bayesian counterpart. In cure rate modeling, the group of patients can be directly stratified into cured and non-cured strata. The mixture models are natural choice for estimation of cure and non-cure rate estimation. The estimation of cure rate is an important parameter of success of any new intervention. The cure rate model is illustrated to compare the surgery of liver cirrhosis patients with consenting for participation HFLPC (Human Fatal Liver Progenitor Cells) Infusion vs. consenting for participation alone group. The MELD (Model for End-Stage Liver Disease) score is considered as response of interest for cured and non-cured group. The primary efficacy of surgery is considered as covariates of interest. Distributional assumptions of the cure rate are solved with Markov Chain Monte Carlo (MCMC) techniques. The risk of death due to liver transplantation in liver cirrhosis patients including time dependent effect terms has also been explored. Finally, this chapter ends by summarizing the result of application.

Chapter 3: In this chapter, the effectiveness of treatment is assessed after adjustment of heterogeneity factor. The approach mention in chapter 2 could not account for heterogeneity factor into the model. Hence, this factor has been addressed with the help of shared frailty model. The frailty model consists of two components firstly baseline hazard. If Baseline hazard is known in advance then, the parametric estimation approach can be used advantageously. The second component of frailty model is frailty distribution. In this chapter, we compare the different parametric frailty models namely (Gamma, Inverse Gaussian, Positive stable &
Lognormal) with respect to AIC and BIC values on liver transplantation patients. Lastly, the chapter ends up with a discussion of the findings.

Chapter 4: In this chapter, we examine criterion of quasi-likelihood information criterion (QIC) for selecting a working correlation structure, and have compared with the performance of the correlation information criteria (CIC) of the correlation structures on liver cirrhosis patients. The performance of both information criteria are compared by fitting generalized estimating equations (GEE) model, which is popular for analyzing correlated responses. It is important to select a proper working correlation matrix because an inappropriate choice will lead to inefficient parameter estimation. The covariates like therapy and visit are used to predict Meld scores (It is continuous disease severities scale with highly predictive of the risk of dying from liver cirrhosis) in GEE model. The computation code for CIC is performed into open source software R. The study indicates that the CIC is useful for selecting appropriate correlation structures for liver cirrhosis data from phase III clinical trial. Discussion and conclusions are drawn from the comparison.

Chapter 5: This chapter focuses on technique that is based on selection modeling approach to analyze missing observations. Data setting and necessary notation in terms of the missing assumptions are introduced. The data setting and modeling framework with the emphasis on dropout mechanisms are described. A background to the selection model is provided, followed by descriptions of the selection model and detailed discussion of the linear mixed model and dropout model. An application example including a description of liver cirrhosis data set in the form of a multi-centre clinical trial data used in the analysis is presented. In the current application, the MCMC approach has been used to fit selection model. The results of the estimation of the selection model are then described. In conclusion, a discussion of the results is given.

Chapter 6: This chapter deals with assessment of agreement between two raters for continuous responses carried out by concordance correlation coefficient. This chapter carries out the Bayesian counterpart to compute concordance correlation coefficient estimator and established the performance of proposed estimator. The methodology is illustrated on Liver Cirrhosis marker data. It is found handy to compute concordance correlation coefficient through application of prior information.
Chapter 7: Finally, this chapter gives a synthesis of the study. The findings are summarized and conclusions are derived from the preceding chapters. For future work on the applications, relevant recommendations are made. A reference list is given at the end of the thesis.