การศึกษาปัจจัยเสี่ยงที่เกี่ยวข้องต่อโรคไวรัสตับอักเสบซี ในหญิงวัยเจริญพันธ์ กรณีศึกษาจากกลุ่มควบคุมในเมืองโกวยตา ประเทศปากีสถาน

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรสาธารณสุขศาสตรมหาบัณฑิต
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RISK FACTORS ASSOCIATION OF HEPATITIS C AMONG WOMEN OF REPRODUCTIVE AGE: A CASE CONTROL STUDY AT QUETTA, PAKISTAN

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A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Public Health Program in Health Systems Development

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อับดุล โกฟา: การศึกษาปัจจัยเสี่ยงที่เกี่ยวข้องต่อโรคไวรัสตับอักเสบซี ในหญิงวัยเจริญพันธุ์กรณีศึกษาจากกลุ่มควบคุมในเมืองโกวยตา ประเทศปากีสถาน (RISK FACTORS ASSOCIATION OF HEPATITIS C AMONG WOMEN OF REPRODUCTIVE AGE: A CASE CONTROL STUDY AT QUETTA, PAKISTAN) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: โรเบิร์ต เอส. แชปแมน M.D., M.P.H., 52 หน้า

การศึกษาย้อนหลังระหว่างกลุ่มศึกษากับกลุ่มควบคุม (Case-Control Studies)ในครั้งนี้มีวัตถุประสงค์เพื่อศึกษาปัจจัยเสี่ยงที่มีความสัมพันธ์กับโรคไวรัสตับซี ในหญิงวัยเจริญพันธุ์ ในโรงพยาบาล Bolan และโรงพยาบาล Sandeman เมืองโกวยตา ประเทศปากีสถาน ในช่วงเวลาระหว่าง 1 ธันวาคม 2551 ถึง 28 กุมภาพันธ์ 2552 ประชากรที่ใช้ในการศึกษาเป็นหญิงวัยเจริญพันธุ์ จำนวน 316 คน มีอายุอยู่ในระหว่าง 18 ถึง 40 ปี โดยแยกเป็นผู้ติดเชื้อไวรัสตับอักเสบซี (HCV) จำนวน 158 คน และผู้ไม่ติดเชื้อไวรัสตับอักเสบซี (non-HCV) จำนวน 158 คน ปัจจัยเสี่ยงที่ศึกษาประกอบด้วย ปัจจัยทางลักษณะประชากรและสังคม ปัจจัยเสี่ยงที่มีความเกี่ยวข้องในการได้รับการรักษาความเจ็บป่วยในอดีต และปัจจัยเสี่ยงที่มีความเกี่ยวข้องกับประวัติการคลอด โดยใช้แบบสอบถามมาตรฐานชนิดใช้ผู้สัมภาษณ์แบบตัวต่อตัว สถิติที่ใช้ในการศึกษาคือ จำนวนและร้อยละ และการวิเคราะห์การถดถอยอย่างง่าย (Logistics Regressions) ที่ระดับความเชื่อมั่น ร้อยละ 95

การวิเคราะห์ความสัมพันธ์สองตัวแปรเป็นการหาความสัมพันธ์ของปัจจัยที่เกี่ยวข้องต่อโรคไวรัสตับอักเสบซี และการวิเคราะห์หาความสัมพันธ์แบบหลายตัวแปร การวิเคราะห์ความสัมพันธ์สองตัวแปร พบว่า ประวัติการฉีดยา สถานที่ที่ได้รับการฉีดยา การอาศัยอยู่ในหลังคาเรือนเดียวกับผู้ป่วยโรคตับอ่าน และผู้ที่เคยป่วยเป็นโรคตับอ่าน มีความสัมพันธ์อย่างมีนัยสำคัญทางสถิติ กับการเสี่ยงต่อการติดเชื้อไวรัสตับอักเสบซี แต่ไม่พบว่ามีความสัมพันธ์กับรายได้ นอกจากนี้ยังพบว่ามีจำนวนตัวแปรที่ใช้ในการวิเคราะห์ความสัมพันธ์สองตัวแปร จำนวน 13 ตัวที่มีค่า p-value น้อยกว่า 0.2 ที่นำไปวิเคราะห์หาการถดถอยพหุคูณ (Multivariable Logistic Model)

ในการวิเคราะห์หาการถดถอยพหุคูณ พบว่า การรับการฉีดยาจากการรักษาพยาบาลในรอบปี และในรอบ 5 ปี ที่ผ่านมา การได้รับการฉีดยาจากการออกหน่วยให้บริการ การรับการฉีดยาจากบุคลากรที่ไม่ใช่แพทย์ และการอยู่ร่วมกันกับผู้ป่วยโรคตับอ่าน มีความสัมพันธ์ในทิศทางบวกกับการเสี่ยงต่อการติดเชื้อไวรัสตับอักเสบซี อย่างมีนัยสำคัญทางสถิติ แต่พบว่ารายได้ของครัวเรือนและประวัติการผ่าตัดมีความสัมพันธ์ในทิศทางลบกับการเสี่ยงต่อการติดเชื้อไวรัสตับอักเสบซี

เมื่อแยกวิเคราะห์ในแต่ละรูปแบบ พบว่า การเปรียบเทียบตัวแปรที่เป็นอิสระ 13 ตัวแปร ของสองโรงพยาบาล พบว่า มีตัวแปรเพียง 5 ตัวแปร ที่แตกต่างกัน อย่างมีความสำคัญทางสถิติ และพบว่าอาชีพ และรายได้ของครัวเรือนต่อเดือนมีความสัมพันธ์ในทิศทางบวก ส่วนการพักอาศัย ประวัติการฉีดยาในรอบ 1 เดือน และรอบ 1 ปีที่ผ่านมา พบว่ามีความสัมพันธ์ในทิศทางลบเมื่อเปรียบเทียบระหว่างสองโรงพยาบาล

การศึกษาในครั้งนี้ทำการศึกษาเฉพาะในกลุ่มตัวอย่างพิเศษในสองโรงพยาบาลเท่านั้น ดังนั้นจึงมีความแตกต่างกันในบางตัวแปร หากพิจารณาตามความเป็นจริงจะพบว่า ปัจจัยเสี่ยงที่พบไม่สามารถใช้เป็นตัวแทนของปัจจัยเสี่ยงทั้งหมดที่เป็นสาเหตุของการติดเชื้อไวรัสตับอักเสบซีได้ในกลุ่มประชากรทั่วไป และของโรงพยาบาลในประเทศปากีสถานทั้งหมดได้ ยังพบว่าประวัติการได้รับการผ่าตัดมีความสัมพันธ์ในทิศทางลบกับการติดเชื้อไวรัสอักเสบซีเป็นสิ่งที่อยู่นอกเหนือความคาดหมาย ซึ่งในกรณีนี้เกิดขึ้นจากตัวแปรที่เป็นตัวก่อกวน(confounder) คือสถานะภาพทางสังคมของผู้หญิง เช่น ผู้หญิงที่มีสถานภาพทางสังคมสูง มักจะมีประสบการณ์ในการผ่าตัดมากกว่าผู้หญิงที่มีสถานภาพทางสังคมต่ำกว่า และนั่นก็เป็นผลกระทบตัวแปรที่เป็นตัวก่อกวนในการผ่าตัดครั้งต่อ ๆ มาดังเช่นการเพิ่มขึ้นของจำนวนครั้งในการฉีดยาและทำแผลโดยบุคลากรที่ไม่ได้มาตรฐานทั้งในสถานบริการและที่บ้าน อย่างไรก็ตามในการศึกษาครั้งต่อไปมีความจำเป็นที่จะต้องแยกแยะปัจจัยเสี่ยงโดยใช้ชุมชนเป็นฐานในการศึกษาในหลาย ๆ พื้นที่ นอกจากนี้ยังมีความจำเป็นที่จะอธิบายถึงปรากฏการณ์ของความสัมพันธ์ในทิศทางลบของประวัติการผ่าตัดและการเสี่ยงต่อการติดเชื้อไวรัสตับอักเสบซี

สาขาวิชา : การพัฒนาระบบสาธารณสุข ลายมือชื่อนิสิต

ปีการศึกษา : 2551 ลายมืออาจารย์ที่ปรึกษาวิทยานิพนธ์หลัก Robert S. Chapman

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ABDUL GHAFFAR: RISK FACTORS ASSOCIATION OF HEPATITIS C AMONG WOMEN OF REPRODUCTIVE AGE: A CASE CONTROL STUDY AT QUETTA, PAKISTAN. ADVISOR: ROBERT SEDGWICK CHAPMAN, M.D., M.P.H., 52 pp

This case-control study was conducted to asses the risk factors for hepatitis C virus (HCV) infection among women of reproductive age at Bolan medical complex hospital (BMCH) and Sandeman provincial hospital (SPH), both in Quetta, Pakistan, during 1 December 2008 to 28 February 2009. The study subjects were 316 females of reproductive age (18 to 40 years), with cases HCV positive (158) and unmatched controls HCV negative (158) by Enzyme-Linked ImmunoSorbent (ELISA) laboratory reports. The potential risk factors considered were socio-demographic characteristics, past medical history, and obstetrical history. The data were collected by standardized, interviewer-administered questionnaires. Data were described with frequencies and percentages, and analyzed with logistic regression analysis, which gave odds ratios, 95% confidence intervals, and p-values.

A bivariate analysis was conducted to explore associations of independent variables with HCV risk, and to select variables for subsequent multivariable analysis. In bivariate analysis, history of injections (in last month, last one year and last five years), place of injection (by dispenser and by unregistered personnel), lived with jaundice patient in household and personal jaundice ever were significantly positively associated with HCV risk. Family income was significantly associated negatively. Thirteen variables with p-value less than 0.2 in bivariate analysis were included in the multivariable logistic model.

In multivariable analysis, health care injections in the last year, health care injections in the last five years, hospitalization for deliveries, injections by dispenser and by unregistered persons, and household contact with jaundice were associated positively and significantly with HCV risk. Family income and history of previous surgeries were associated negatively and significantly with HCV risk.

In separate logistic models, the 13 independent variables were compared between the two study hospitals. This comparison showed significant or marginally significant ($0.05<p<0.10$) for 5 of 13 variables. Occupation and monthly family income showed positive significant positive association and living place and history of injections for last one month and for last one year showed significant negative association when BMCH was compared to SPH.

The study was conducted in a specific population at two hospitals and some of the risk factors showed significant differences between these hospitals in the same city. Considering the above facts only these risk factors may not represent all the risk factors which lead to HCV infection in general population and all the hospitals of Pakistan. The observed negative association of HCV risk with surgery history was unexpected. This might partially reflect confounding with socioeconomic status; women with higher socioeconomic status (SES) were more likely to have had previous surgery than those with lower SES. It might also reflect confounding with post surgical consequences like increased numbers of injections and wound dressing by unregistered health practitioners and/or at home. However future research is needed to characterize the risk factors through community based studies at multiple locations. Further studies are also needed to explain the observed negative association of surgery history with HCV risk.

Field of study: Health System Development Student's Signature _____
Academic Year: 2008 Advisor's Signature Robert S. Chapman

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LIST OF ABBREVIATIONS

HCV	Hepatitis C Virus
BMCH	Bolan Medical Complex Hospital
SPH	Sandeman Provincial Hospital
ELISA	Enzyme-Linked ImmunoSorbent Assay
OR	Odds Ratio
CI	Confidence Interval
PKR	Pakistani Rupees
US	United States
CDC	Center for Disease Control
NANBH	Non A Non B Hepatitis
cDNA	complement Deoxyribonucleic acid
ALT	Alanine Aminotransferase
D&C	Dilatation and curettage

CHAPTER I

INTRODUCTION

1.1 Background and significance of the problem

Hepatitis C virus (HCV) infection is major public health problem worldwide. HCV is one of the major etiological agents of parenterally acquired hepatitis. The worldwide literature on HCV prevalence has increased considerably over the past decade (Kumar, 2007).

It is estimated that approximately 130-170 million people worldwide are infected with hepatitis C virus (HCV). According to data from WHO community and blood donor surveys, the African and Eastern Mediterranean countries report the highest prevalence rates (> 10%). The rates of infection in the general population and the incidence of newly-acquired cases indicate an appreciable change in the epidemiology of the infection in recent years (Baldo, 2008).

Intermediate rates 2.15% of HCV have been reported from Asia. From 1995-2000, 0.49% anti-HCV antibodies were detected among 3,485,648 blood donors in Japan. In China, prevalence rates were generally low with rates around 1% among donors in Beijing and Wuhan. However, rates may be higher in certain areas such as Hubei province (30.13%) and Inner Mongolia Autonomous Region (31.86%). Low rates have been found in Malaysia (around 1.6%) and Singapore (0.54%). Higher rates of HCV have been found in Thailand (3.2-5.6%) (Sy, 2006).

Regarding community prevalence in Pakistan, few population based studies are available, the most comprehensive sample size from a population of 150,000 in Hafizabad and found an overall sero-prevalence of 6%. This increased to 30% with increasing age. The same group also found a 16% sero-prevalence rate in household

members of HCV infected cases. Other smaller studies have reported a population prevalence of 16% from Lahore and 23.8% from Gujranwala. Based on an average prevalence rate of 6%, it could be estimated that approximately 10 million people are infected with HCV in Pakistan. The sero-prevalence of HCV in children appears to be low in Pakistan, with 0.2% and 0.4% children infected under the age of 12 and between 12-19 years respectively (Hamid, 2004). Women are expected to be the major victims of infection HCV because of greater exposure to syringes, blood and blood products, especially during pregnancy, delivery, ear and nose piercing and especially in the province of Balochistan where females have low literacy rate and less awareness as compare to other provinces of Pakistan. Investigation of risk factors for HCV among women in a high prevalence country will help in promoting their health by identifying and hopefully preventing these risks.

1.2 Research question of the study

What are the risk factors associated for hepatitis C virus infection in females during active reproductive age in Quetta, Pakistan?

1.3 Objectives

- To identify risk factors for hepatitis C infection among females visiting Bolan medical Complex Hospital (BMCH) and Sandman provincial hospital (SPH), Quetta. (please see conceptual frame work)
- Compare risk factors at BMCH to those at SPH.

1.4 Purposes of the study

In the Balochistan province, Pakistan, no study in my knowledge has attempted to evaluate the risk factors in females in reproductive age. The purpose of the study is to investigate risk factors for HCV among women in reproductive age, and to compare findings to those of hepatitis C studies in other locations.

1.5 Benefits of the study

Investigation of risk factors in the females in a high prevalence country will help in promoting their health by identifying and hopefully preventing these risks.

1.6 The study area

After the approval of proposal from Chulalongkorn University, The proposed study was conducted in the following two tertiary hospitals care hospitals in Quetta district, Balochistan. Quetta city is the capital and biggest city with population of about 1.2 million.

- Bolan Medical complex Hospital, an undergraduate as well postgraduate teaching hospital in the province.
- Sandeman Provincial Hospital, post graduate hospital located in the center of the city.

These hospitals are the only Government hospitals which provide facilities for low cost HCV screening by ELISA method. As in other parts Balochistan such facilities are not available patients are referred to these hospitals from other cities of the province. Hepatitis control programs are also run by these hospitals. These hospitals are located at a distance of 8 km from each other.

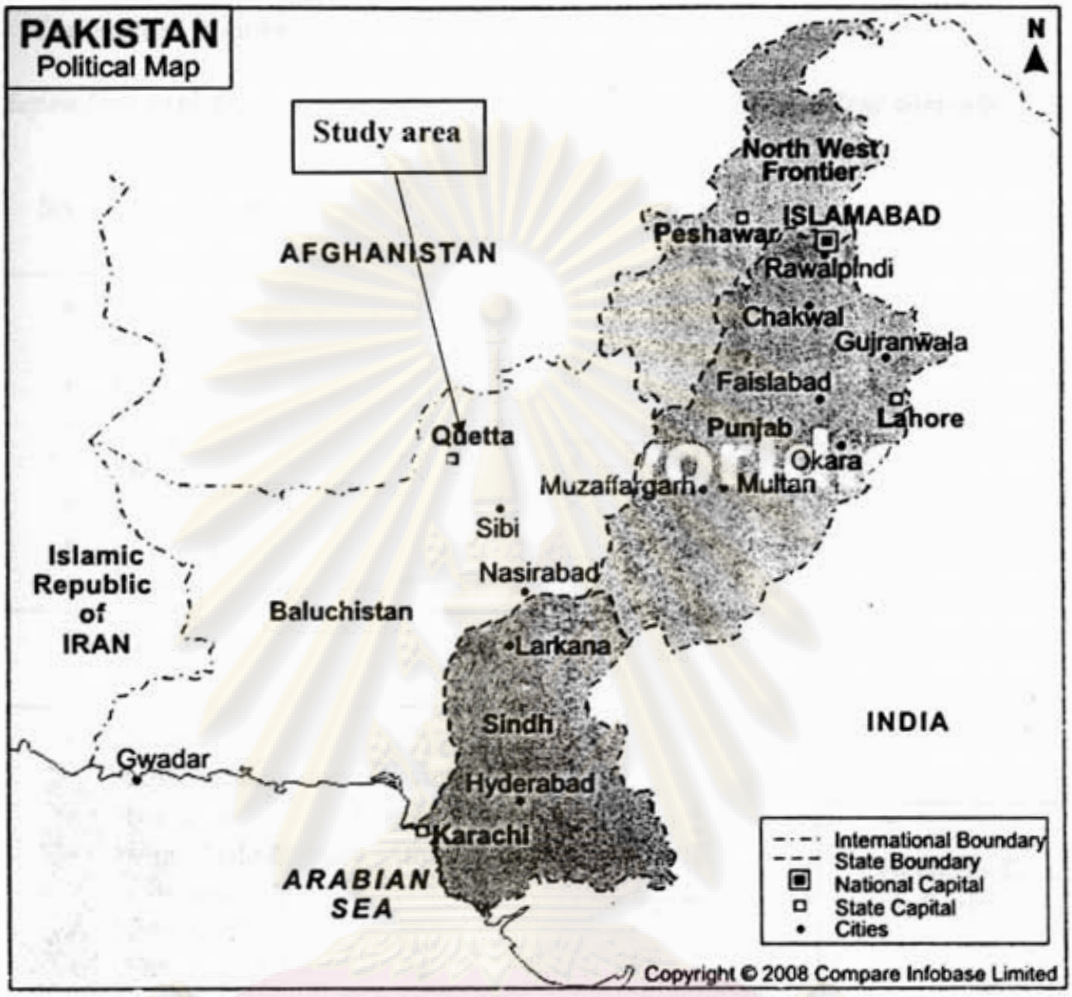

Figure 1 Map of Pakistan, showing the study area

Conceptual framework

Independent variable ***Dependent variable***

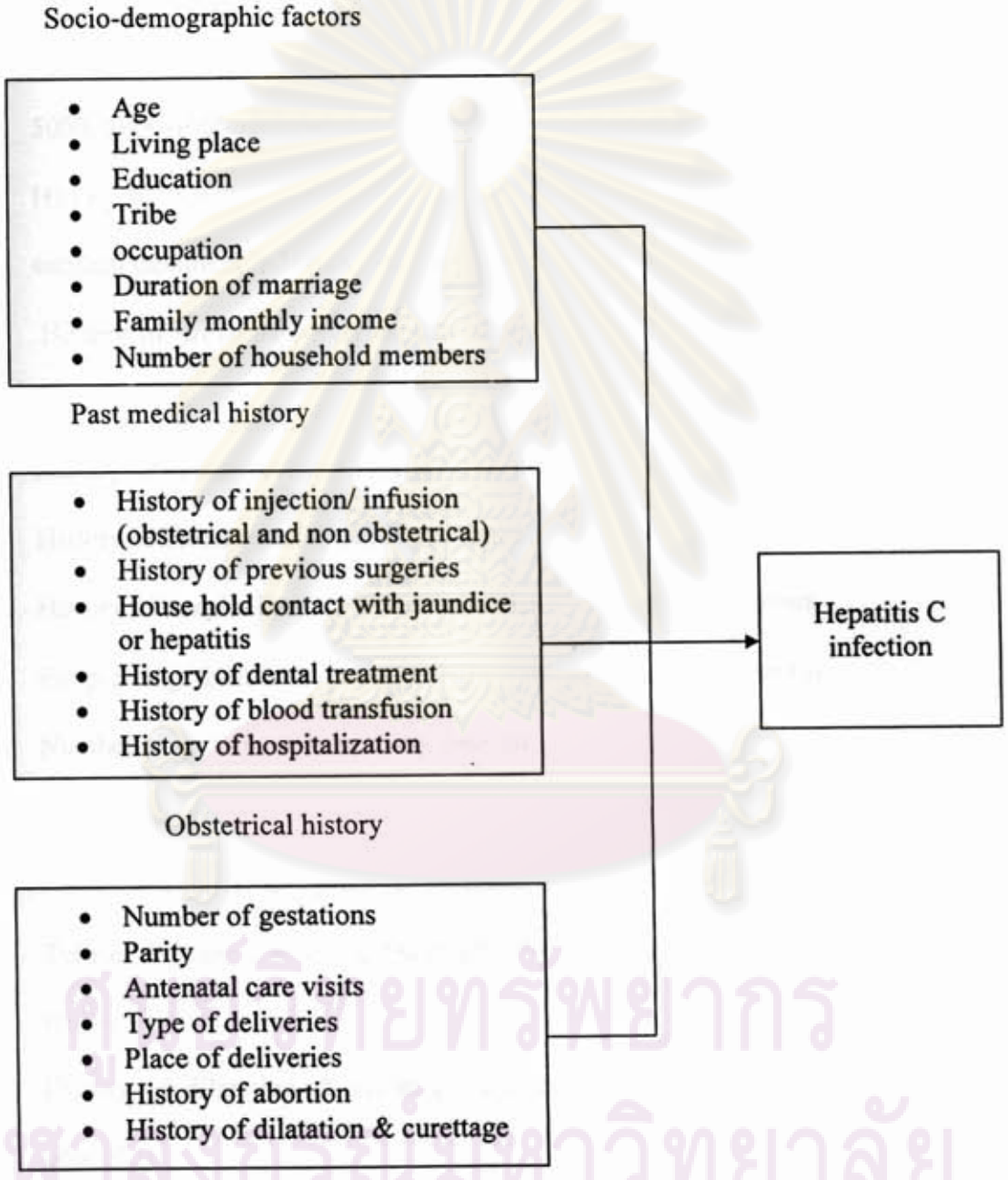

Figure 2 Conceptual framework

1.7 Operational definitions

- Living place: the respondents live in Quetta city or suburbs
- Duration of marriage: how long the female is marriage in years.
- Family monthly income: how much money the family is earning per month , PKR 5000, 5000-10000, more then 10000
- History of injection/ infusion: refers to the number of injections for medical or surgical treatment in last month, last one year and five years.
- History of previous surgeries: any surgical procedure except for gynecological and obstetrical procedure
- History of dental treatment: refers to tooth extraction or dental scaling
- History of blood transfusion: blood transfusion one or more units
- History of hospitalization: any stay in hospital more than 48 hours
- Ear piercing: traditional piercing that covers entire pinna of the ear
- Number of gestations: how many time the female got pregnant
- Parity: how many full term births, including Primipara, Parity 1-3, Parity > 3
- Antenatal care visits: visited for antenatal clinic for her obstetrical problems
- Type of deliveries: how she delivered baby by normal vaginal delivery or cesarean section
- History of dilatation & curettage: did she went to doctor or a nurse for the procedure
- Number of injections during obstetrical history: number of injections only she got during per obstetrical procedure, less than 5, 5-10, or more than 10

CHAPTER II

LITERATURE REVIEW

On April 21, 1989, Michael Houghton and his colleagues at the Chiron Corporation in Emeryville, CA, in collaboration with Daniel Bradley of the Centers for Disease Control in Atlanta, GA, announced in a landmark publication in Science their discovery of hepatitis C virus. Their words were Thus; our data indicate that clones 5-1-1 and 81 are derived from the genome of a blood-borne non-A non-B Hepatitis (NANBH) virus that we now term the hepatitis C virus (HCV). The cDNA clones reported here were obtained in the absence of prior knowledge concerning the virus, the viral genome, and the presence of circulating antibodies. As such, this represents cloning without characterization of the infectious agent. This approach should be relevant to studies of other diseases in which an unknown infectious agent (viral or otherwise) might be involved (Choo, 1989).

Hepatitis C virus is a RNA virus belonging to *Flaviviridae* virus family. The HCV genome is consists about 9400 nucleotides with one large open-reading frame encoding for a polypeptide consisting for structural and non structural domain (Hwang, 2001).

HCV is divided among six genotypes with numerous subtypes. These genotypes can differ up to 30% from each other in nucleotide sequence. Depending on the HCV genotype, length of treatment can differ. Genotype 1b is less responsive to alpha-interferon therapy compared to genotypes 2 and 3. It is therefore important to track the different genotypes of the HCV virus (Sy, 2006). Epidemiological studies from the largest province included a total of 6817 participants. Of these, 5678 (83.3%)

were male and 1139 (16.7%) were female. The ages of the participants ranged from 6 years to 70 years. Anti-HCV antibodies were detected in 998 (15.09%) samples. Positive anti-HCV antibodies were found in 857 (14.59%) of the samples collected from male participants, and in 141 (12.37%) of the samples collected from female participants. The presence of anti-HCV antibodies was significantly higher in the male than female participants ($P < 0.009$). The prevalence of anti-HCV antibodies is different in male and female age groups, with the lowest prevalence being between 6 and 15 years and the highest in the 56–65-year age groups. There were also significant differences in sero-prevalence among the age groups tested ($P < 0.05$). The prevalence of anti- HCV antibodies was found to be significantly higher in the male than female participants among the various age groups, such as those aged between 26–35, 36–45 and 46–55(Idrees, 2008).

The results of the above study showed that injection drug use, a higher age, the male sex, residence in rural areas, major or minor surgeries, reusing syringes and blood transfusions were independently associated with HCV infection (Idrees, 2008).

Women use more health care than men due to antenatal care and child birth, which may result in surgical procedures, hospitalization, blood transfusion or unsafe medical injections. Hence these events may lead to more exposure to women HCV risk factors.

Study conducted in Karachi, the largest city of the Pakistan among pregnant women revealed that five or more gestations (OR = 1.99; 95% CI = 1.08–3.33), ‡1 injection (OR = 2.33; 95% CI = 1.38–3.91) per month, hospitalization (OR = 1.78; 95% CI = 1.01–2.99) and household contact with jaundice / hepatitis (OR = 3.32; 95% CI = 1.89–5.83) were independently associated with HCV(Khan, 2008).

Natural history of the disease

Average incubation period of HCV is 6-7 week and ranges from 2- 26 weeks. Most patients with newly-acquired HCV infection do not present with an acute hepatitic illness – most estimates suggest that only 10-15% of cases are acutely jaundiced (Blackard, 2008).

In the remainder, the infection is either asymptomatic, or may present with mild constitutional symptoms (nausea, loss of appetite, fatigue, vague abdominal pain), with an alanine aminotransferase (ALT) which peaks below 1,000 Ul/ml (Irving, 2008).

A minority of newly infected patients (15%–50%) will clear the infection, but in most (50%–85%) the infection will become chronic. The risk of chronicity after acute infection is less (50%–70%) with community-acquired infections and higher (70%–85%) in post-transfusion cases. Of those chronically infected with HCV, approximately 5% of individuals aged <40 years and 34% to 58% of individuals aged ≥40 years develop cirrhosis of the liver 20 years after exposure and may ultimately die from cirrhosis (end-stage liver disease) or hepatocellular carcinoma. Younger age, female sex, non-African-American race, and certain histocompatibility genes are all associated with improved spontaneous clearance of the virus and a lower likelihood of chronic infection (Hwang, 2008).

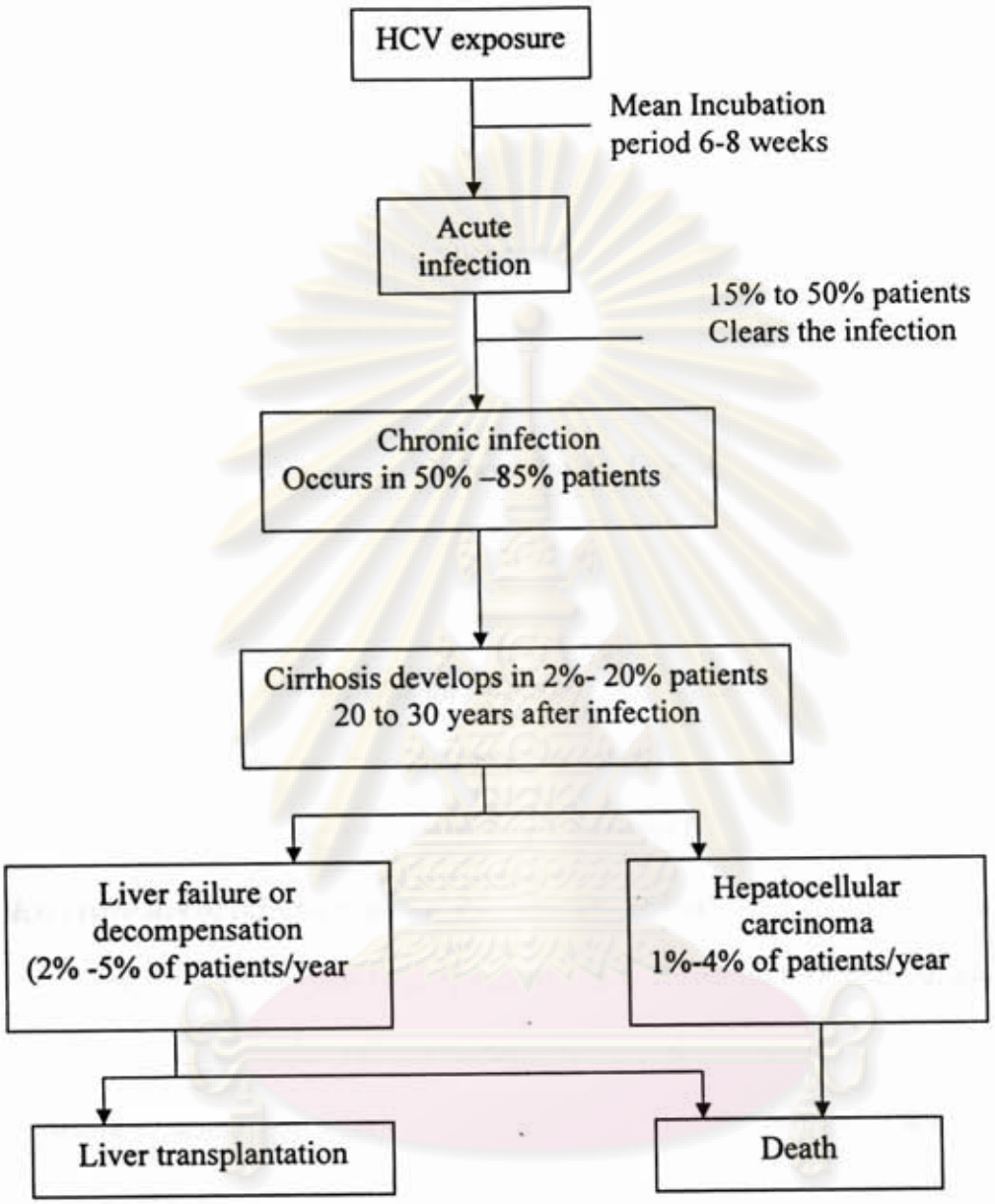

Figure 3 Natural history of HCV infection

2.1 Modes of transmission

Initially, it was believed that blood transfusion and intravenous drug abuse were the most common routes of transmission of HCV. Further epidemiological studies revealed the existence of other possible modes of transmission of the disease (Morton, 1998). The other modes of transmission of HCV include unsafe injections reuse of glass syringes or needles by medically unqualified personnel, vertical transmission, non-sexual contact in households, face or armpit shaving at community barber shops, ear piercing, tattooing and inadequately sterilized surgical or dental instruments (Khokhar, 2004).

Modes of transmission in Pakistan

Unfortunately, HCV infection is not a notifiable disease in Pakistan and there is no national data collection system for evaluation of routine risk factors.

Excessive use of injections and use of unsafe needles

Health care workers usually practice unsafe injections or reuse of inadequately sterilized syringes and needles. The majority of health care workers are not medically qualified or scientifically trained and are unaware of standard sterilization procedures or the importance of safe injection practice. Apart from this, the general population of Pakistan typically prefers to be treated by injection rather than oral medication. Thus, patient demand and financial incentives favor the use of injectable treatment in patient care (Luby, 2005).

Blood transfusion

In Pakistan, blood transfusion is still a major source of HCV transmission. Possible reasons for this include lack of resources, weak infrastructure, ill-equipped resources, poorly trained staff, inadequate policy implementation, frequent power

breakdown and ineffective screening of blood donors for anti-HCV antibody (Aslam, 2005).

Community barber shops

Another significant risk factor of HCV transmission which has previously been reported from different regions of Pakistan is daily face and armpit shaving at community barber shops. The delicate skin of the face and armpit are susceptible to microtrauma, leading to possible exposure to HCV through a contaminated traditional long-handled razor (Bari, 2001).

Intravenous drug abuse and sexual preference

Homosexuality and intravenous drug abuse are on the rise in Pakistan, although at present these make only a nominal contribution to HCV prevalence infection in this country. However, data are lacking regarding these possible routes of entry, and intravenous drug abuse is the leading risk factor for HCV transmission worldwide (Aslam, 2001).

Perinatal transmission

The average rate of infection in infants born to infected mothers is 5% to 6%. The chance of infection has been shown to be greater with higher serum levels of HCV-RNA (17%) and in mothers coinfected with HCV and HIV (14%). HCV-RNA can be detected within 2 to 3 months of birth (Hwang, 2001).

Household transmission

Nonsexual transmission of HCV between family members may infrequently occur, presumably via percutaneous and permucosal exposure to blood. HCV seropositivity concordance rates average 4% in nonsexual household contacts, including siblings and others (Ackerman Z, 2000).

Sexual transmission

The relevance of sexual transmission to the epidemiology of HCV remains controversial. Epidemiologic surveys suggest that 20% of patients with recently acquired hepatitis C have no self-reported risk factor other than multiple sexual partners or an infected sexual partner (Alter, 1997).

Nosocomial transmission

The average incidence of seroconversion after needle stick or sharp exposure from an HCV-seropositive source has been estimated to be 1.8% (range 0% to 7%).One study showed a 1.2% incidence of seroconversion after injury from a hollow-bore needle but no instances of seroconversion from injuries with sharp objects or from contamination of mucosa or nonintact skin (Puro, 1995).

Epidemiologic surveys show that the prevalence of seropositivity in patients in hemodialysis centers averages 10% and, in some centers, can be greater than 60%.during the last 15 years, there has been an increase of iatrogenic HCV transmission not related to dialysis (Alter, 1997).

Diagnosis

Screening assays The commonly available screening test for anti-HCV is an enzyme immunoassay (EIA, also called enzyme-linked immunosorbent assay or ELISA) that detects HCV antibodies. The hepatitis C virus is a small 40 to 60 nm virus with a lipid envelope and a single-stranded RNA viral genome comprising approximately 9500 nucleotides

Confirmatory assays These confirmatory assays have been supplanted by tests which directly detect HCV RNA. However, for individuals who are anti-HCV positive by screening tests (EIA) and negative for HCV RNA by sensitive molecular

tests, the supplemental antibody tests, called RIBA-2 and RIBA-3 (meaning recombinant immunoblot assay, second version and third versions), can be used to distinguish a false positive antibody result from a case of past (but resolved) HCV infection. In the former, the RIBA test will be negative and in the latter the RIBA test will be positive

Molecular testing — HCV RNA detection and quantification are essential tools in the management of individuals with chronic HCV infection. As discussed above, HCV RNA tests are used to confirm the presence or absence of infection and to quantify the amount of specific time points during therapy to guide decisions regarding duration of treatment.

Examples of qualitative HCV RNA assays are Amplicor PCR assay (Roche Diagnostics) and Versant TMA assay (Norah, 2008).

2.2 Treatment

Treatment of HCV is mainly composed of antiviral therapy and depends on the clinical status of the patient. The standard therapy for chronic HCV infection is a course of weekly subcutaneous injections of pegylated interferon alfa (PEG-IFN) combined with once-daily oral ribavirin. Duration of treatment is 48, 24, or 12 weeks, depending on HCV genotype and other treatment prognostic factors.

The principal goal of treatment is a sustained virologic response (SVR), defined as elimination of detectable virus during treatment and continued absence of virus 6 months after the end of treatment (Strader, 2004).

Treatment groups for patients with hepatitis C virus (Strader, 2004).

Individuals recommended for treatment

(i) Patients with persistently elevated alanine aminotransferase (ALT) levels

(ii) Patients with detectable HCV ribonucleic acid

(iii) Patients with a liver biopsy indicating either portal or bridging fibrosis or at least moderate degrees of inflammation and necrosis

Individuals for whom treatment is unclear

(i) Patients with compensated cirrhosis (without jaundice, ascites, variceal hemorrhage, or encephalopathy)

(ii) Patients with persistent ALT elevations but with less severe histologic changes (i.e., no fibrosis and minimal necroinflammatory changes)

(In these patients, progression to cirrhosis is likely to be slow, if at all; therefore, observation and serial measurements of ALT and liver biopsy every 3–5 years is an acceptable alternative to treatment with interferon)

(iii) Patients <18 years of age or >60 years of age (note that interferon is not approved for patients younger than 18 years)

Individuals for whom treatment is not recommended

(i) Patients with persistently normal ALT values

(ii) Patients with advanced cirrhosis who might be at risk for decompensation with therapy

(iii) Patients who are drinking excessive amounts of alcohol or who are injecting illegal drugs (treatment should be delayed until these behaviors have been discontinued for ≥6 months)

(iv) Persons with major depressive illness, cytopenias, hyperthyroidism, renal transplantation, evidence of autoimmune disease, or who are pregnant.

CHAPTER III

RESEARCH METHODOLOGY

3.1 Research design

This was a case-control study to assess the risk factors for Hepatitis C virus (HCV) infection among women of reproductive age in Quetta, Pakistan.

3.2 Study population

The study population for both the case and controls were females in active reproductive age (18 to 40 years), who were screened for HCV at Bolan Medical Hospital complex and Sandeman provincial Hospital in Quetta.

CASES

Were Enzyme-Linked ImmunoSorbent Assay (ELISA) antibody test positive for HCV from Bolan Medical Complex Hospital and Sandeman provincial Hospital Quetta

CONTROLS

Were ELISA-negative after screening for HCV visiting any department of the above said hospitals

3.3 Exclusion criteria

The subjects who were severely ill due to HCV infection or any other disease were not included in the study. Patients from provinces other than Balochistan and countries other than Pakistan were excluded from the study. Cases and controls having HBV infection and dual infection with HCV and HBV were also excluded because risk factors are overlapping for hepatitis B and C.

3.4 Sample size calculation

Considering the fact that general population of Pakistan typically prefers to be treated by injection rather than oral medication, and health care workers usually practice unsafe injections or reuse of inadequately sterilized syringes and needles, injection history were of special interest in my study.

Table 1 Review of literature also revealed that odds ratio and CI values were significant.

% age of exposure in controls	% age of Exposure in cases	Odds ratio	Sample size needed(1:1)
18.9	32.0	2.0	187+187 = 374
18.9*	36.0*	2.4	117+117 = 234
18.9	39.5	2.8	85+85 = 170
18.9	41.5	3.0	72+72 = 144
18.9	48.0	4.0	47 + 47 = 94
18.9	54.0	5.0	34 +34 = 68

* observed in case-control study in Karachi, Pakistan.

An odds ratio between 2 and 2.4

Confidence level or 1- α 95%

Power 1- β 80%

Assume equal members of cases and controls

A sample size of 300 subjects is calculated: Cases = 150 and controls = 150, and add 10% for safety a total of 165 for cases and 165 for controls was the size of sample (total 330 patients).

These figures were calculated using the Statcalc procedure, Epi Info software. Source: Fleiss, "Statistical Methods for Rates and Proportions" 2nd ed., Wiley, 1981, pp.38-45.

3.5 Sampling method

Patients visiting BMCH and SPH to different specialties like gynae and obstetrics, general surgery and allied departments and patients with jaundice in gastroenterology departments are referred to pathology laboratory for the screening of different infectious diseases like hepatitis and HIV/AIDS before surgical interventions. Both cases and controls were selected from these laboratories on same day after conformation of diagnosis. Cases were positive for HCV antibodies on ELISA and controls were negative for HCV antibodies.

Controls were randomly selected from the pathology departments at both the hospitals. At each hospital, one control was selected for each case (1:1 ratio of cases to controls).

3.6 Research Instruments and measurements

A standardized interviewer–administrated questionnaire on the demography, possible risk factors related to past medical and obstetrical history were given to both anti-HCV positive subjects and controls.

Pretest: 20 women 18-40 years old at some other hospitals in Quetta. The questionnaire was revised in accord with findings of the pretest.

All the interviews will be carried out by the researcher with a female assistant.

Demographic details include age, residence, education, and mother tongues, and tribe, address living in rural or urban area, household members and monthly income.

Independent risk factors included were,

Past medical history

Self reported history of injection/ infusion for last month one year, and five year, History of previous surgeries, Household contact with jaundice or hepatitis, History of dental treatment, number of history of blood transfusion, History of hospitalization and ear piercing were asked.

Past obstetrical history

Number of gestations, Parity, Antenatal care visits, Type of deliveries, Place of deliveries, History of abortion, History of dilatation & curettage, No of injections during obstetrical history were asked by the respondent.

3.7 Data collection

After the grant of permission by the Chulalongkorn University, and permission from the medical superintendents of the concerned hospitals the data was collected.

Subjects were approached during January, February, and March 2009 in the outpatient department with a female assistant for the interview.

Before interview the subjects were briefed on purpose of the research and signature informed consent was obtained. In case if the subject is illiterate she was briefed with accompanying person and her thumb impression or signature of the blood was taken.

3.8 Data analysis

3.8.1. Data entry and editing

Data was coded and entered twice by using SPSS.

3.8.2. Statistical technique

SPSS software was used for data analysis. Mean ± SD were calculated for age, duration of marriage and number of house hold members.

Descriptive statistics were computed for categorical variables for cases and controls by computing their frequencies for the two groups. To asses bivariate associations between HCV seropositivity and potential risk factors like socio-demographic characters, past medical history and factors related to obstetrical history , odds ratio (OR) and their 95% confidence intervals (CIs) were computed by logistic regression. All the risk factors with $p < 0.20$ on bivariate analysis were considered for inclusion in the multivariable logistic model. For each hospital risk factors with $p< 0.20$ were considered for multivariable logistic model separately.

All risk factors with $P < 0.05$ were considered significant.

3.9 Ethical Considerations

According to the declaration of the World Medical Association in Geneva, the right of the respondent must always be respected. Therefore, every precaution was taken to respect the privacy of the subjects and to minimize the impact of the subject's physical, mental integrity and on the personality of the subject. The respondent was adequately informed for the objectives, methods and benefits of the study prior to the questionnaires distribution and informed consent was taken. They were informed that they are free to abstain from participation in the study and free to withdraw any time. The names of the respondent were included in the electronic data files. After collecting the data, the confidentiality of the data was ensured.

CHAPTER IV

RESULTS

This study was a case-control study conducted to elucidate the risk factors association of HCV infection in females of reproductive age at Bolan Medical Complex Hospital and Sandeman Provincial Hospital Quetta, Pakistan, related to socio-demographics and past medical and obstetric histories.

Data has been collected through personnel interview and laboratory based evidence for positive anti-HCV antibody ELISA tests cases and negative anti-HCV antibody ELISA tests for controls. 158 cases and equal number of controls were included and data was collected during 1 December 2008 to 28 February 2009. Cases and controls who were positive for HBV and dual infection with HBV and HCV were not included. There was no refusal among cases and controls.

The data were computerized and analyzed by SPSS for Windows software. The result of this study will be presented in 4 tables as follows:

- Socio demographics of Cases (HCV Group) and Controls
- Past medical history
- Past obstetrical history
- bivariate analysis was applied to thirty seven variable (risk factors)
- The multivariable logistic regression model risk factors associated with Hepatitis virus C infection

4.1 Socio-demographics characteristics of Cases (HCV Group) and Controls:

The demographic characteristics of all study participants are given in table2. Majority of the study subjects 60.0% were from BMCH and about 40.0% of the subjects were from SPH. Mean (±SD) age for case was 32.37 (±6.68) and for controls was 31.71 (±6.89) years (OR 1.01, 95% CI 0.98 to 1.05).

About 45.6% cases were from rural areas and 37.9% of the controls were from rural areas (OR =1.37, CI = 0.87 to 2.14). Age not differ significantly among cases and controls. The mean (±SD) duration of marriage for the cases was 11.17 (±7.2) and for the cases was 10.62 (±7.6) years. A total of 51.5% of cases and 48.5% of controls never went to school. About 52% of cases were house wives and 48.1% were house wives among controls (OR =0.64, CI = 0.35 to 1.15). The mean number of households was 9.66 (±5.1) for cases and 10.09 (±4.9) for controls.

Regarding social division 35.4% were belonging from Baloch tribe both in cases and controls (OR 1.19, CI 0.68 to 2.07), and 35.6% were Pashtoons in cases and controls (OR 1.02, CI 0.58 to 1.76), while remaining 29% were from other clans.

More than half of the study subjects had a monthly income of between PKR 5000 to 10000 (63 to 126 US$).

Table 2 Socio-demographics characteristics of Cases (HCV Group) and Controls

Characteristics	Number (percentage)		Odds ratio	95% CI	*P value*
	Case	Control			
Age (year)			1.01	0.98 to 1.05	0.384
Range	18-40	18 - 40			
Mean ± SD	32.37±6.68	31.71± 6.89			
Hospital			1.00	0.64 to 1.57	1.000
Bolan medical complex	96 (50.0)	96 (50.0)			
Sandmen provincial	62 (50.0)	62 (50.0)			
Marital status					
Married*	147 (50.9)	142 (49.1)	1	---	---
Unmarried	11 (40.7)	16 (59.3)	0.66	0.30 to 1.48	0.317
Living place					
Urban *	86(54.4)	98(62.1)	1	---	---
Rural	72(45.6)	60(37.9)	1.37	0.87 to 2.14	0.172
Duration of marriage			1.01	0.98-1.04	0.507
Range	0-25	0-32			
Mean ± SD	11.17±7.2	10.62±7.6			
Tribe					
Baloch	59(52.7)	53(47.3)	1.19	0.68 to 2.07	0.540
Pashtoon	55(48.7)	58(51.3)	1.02	0.58 to 1.76	0.964
Others*	44(48.4)	47(51.6)	1	---	---
Education			0.86	0.62 to 1.19	0.358
No schooling	118 (51.5)	111 (48.5)			
Primary to secondary	25 (47.2)	28 (52.8)			
High school and above	15 (44.1)	19 (55.9)			
Occupation					
Housewife*	136 (51.9)	126 (48.1)	1	---	---
Other	22 (40.7)	32 (59.3)	0.64	0.35 to 1.15	0.137
Family Income			0.69	0.50 to 0.94	0.019
<5000	56 (52.8)	50 (47.2)			
5000-10000	83 (56.1)	65 (43.9)			
>10000	19 (30.6)	43 (69.4)			
No. of Household members			0.98	0.94 to 1.03	0.438
Range	2-40	2-32			
Mean ± SD	9.66±5.1	10.09±4.9			

* Reference group, are for categorical data not for continuous data

4.2 Past medical history

Past medical histories of cases and controls are summarized and compared in Table 3. A total of 41.1% of the case and 38.8% of the controls ever had hospitalization.

History of injection was asked for last month, last year and last 5 years. For last month 25.9% among cases and 23.4% among controls had up to three injections, and 12% of the case and only 5.7% of controls had more than 3 injections. In the last year 40.5% of the cases and 36.7% of the controls had 2 to 5 injections, 33.5% of the cases and 8.9% of the controls had more then 6 injections. In last five years 30.4% cases had 5-10 injections and 19.0% had more than 20 injections, while 51.9%controls had less than five injections only 5.7% of the controls had more than 20 injections.

Most of the cases (69.6%) and controls (74.1%) got injections at government hospitals, 24.7% cases and 25.9% of controls used private hospitals to be injected for the medication, the dispensers were also draining a significant number of cases 51.3% and 32.9% of the controls. Unregistered health practitioners provided injection facilities to total of 88 patients out of which 42.4% of the subjects were controls.

History of previous surgery was dominating the control group 31.0% and 24.1 of the cases had surgeries, 41.2% of the cases and 58.8% of the controls had major surgeries and 48.1% of the cases and 51.9% controls had minor surgeries.

Sixty three patients had history of dental treatment 18.4% were cases and 21.5% were among controls. About 30.4% of the cases and 25.3% controls had history of blood transfusion.

Three hundred and nine subjects had ear piercing, and 270 of the respondents had nose piercing.

Regarding living with jaundice patient in household 46.8% of the cases and 20.09% of the controls were living or ever lived. Personal jaundice was reported by 36.1% of the cases and 25.9% controls.

Table 3 Past medical history

Characteristics	Number (percentage)		Odds ratio	95% CI	*P value*
	Case	Control			
History of hospitalization					
Never*	93 (58.9)	98 (62.0)	1	---	---
Ever	65 (41.1)	60 (38.0)	1.14	.73 to 1.79	0.565
History of injection					
In last month			1.44	1.02 to 2.04	0.039
Nil	98 (62.0)	112 (70.9)			
1-3	41 (25.9)	37 (23.4)			
> 3	19 (12.0)	9 (5.7)			
In last year			2.71	1.95 to 3.76	<0.001
Nil	41 (25.9)	86 (54.4)			
2-5	64 (40.5)	58 (36.7)			
≥ 6	53 (33.5)	14 (8.9)			
In last 5 years			1.92	1.51 to 2.44	<0.001
< 5	41 (25.9)	82 (51.9)			
5-10	48 (30.4)	47 (29.7)			
11-20	39 (24.7)	20 (12.7)			
> 20	30 (19.0)	9 (5.7)			
Place of injection					
Government hospital					
No*	48 (30.4)	41 (25.9)	1	---	---
Yes	110 (69.6)	117 (74.1)	0.80	0.49 to 1.31	0.382
Private hospital					
No*	119 (75.3)	117 (74.1)	1	---	---
Yes	39 (24.7)	41 (25.9)	0.93	0.57 to 1.55	0.796
Dispenser					
No*	77 (48.7)	106 (67.1)	1	---	---
Yes	81 (51.3)	52 (32.9)	2.14	1.36 to 3.38	0.001

Unregistered					
No*	91 (57.6)	137 (86.7)	1	---	---
Yes	67 (42.4)	21 (13.3)	4.80	2.75 to 8.39	<0.001
History of previous surgeries					
No*	120 (75.9)	109 (69.0)	1	---	---
Yes	38 (24.1)	49 (31.0)	0.70	0.43 to 1.16	0.167
Major surgeries					
No*	130 (52.4)	118 (47.6)	1	---	---
Yes	28 (41.2)	40 (58.8)	0.64	0.37 to 1.09	0.102
Minor surgeries					
No*	145 (50.2)	144 (49.8)	1	---	---
Yes	13 (48.1)	14 (51.9)	0.92	0.42 to 2.03	0.841
History of dental treatment					
No*	129 (81.6)	124 (78.5)	1	---	---
Yes	29 (18.4)	34 (21.5)	0.82	0.47 to 1.43	0.482
History of blood transfusion					
Never*	110 (69.6)	118 (74.7)	1	---	---
Ever	48 (30.4)	40 (25.3)	1.29	0.79 to 2.11	0.316
Ear piercing					
No*	4 (2.5)	3 (1.9)	1	---	---
Yes	154 (97.5)	155 (98.1)	0.75	0.16 to 3.38	0.703
Nose piercing					
No*	25 (15.8)	21 (13.3)	1	---	---
Yes	133 (84.2)	137 (86.7)	0.82	0.44 to 1.53	0.524
Lived with Jaundice patient in household					
Never*	84 (53.2)	125 (79.1)	1	---	---
Ever	74 (46.8)	33 (20.9)	3.34	2.03 to 5.47	<0.001
Personal jaundice ever					
No*	101 (63.9)	117 (74.1)	1	---	---
Yes	57 (36.1)	41 (25.9)	1.61	0.99 to 2.60	0.052

4.3 Past Obstetrical History

Obstetrical risk factors are shown and compared in Table 4. The mean number of pregnancies was 4.94 (± 3.57) in cases and 4.6 (±3.86) in controls. Increased number of children were seen in case than controls, 4 (±3) and 3 (±3.24).

About 45.6% of the cases and 46.4% of the controls ever had antenatal care.

As we know there are 2 modes of the deliveries, 81.6% of the cases and 84.2 % of the controls had normal vaginal deliveries, while 12.0% of the cases and 12.7% of the controls had caesarian section.

Most of the cases and controls (59.5% and 50.6% respectively) gave birth to their babies at homes, 58.2% of the cases and 49.4% of the controls used hospitals for the birth.

Almost same number of cases and controls had abortion. Only a small number of subjects 15.8% among cases and 17.1% of the controls had D&C ever in their life.

Table 4 Past Obstetrical History

Characteristics	Number (percentage)		Odds ratio	95% CI	*P value*
	Case	Control			
Number of pregnancies			1.03	0.96 to 1.09	0.458
Range	0-20	0-20			
Mean ± SD	4.94 ± 3.57	4.63±3.86			
Parity			1.02	0.95 to 1.10	0.540
Range	0-19	0-17			
Mean ± SD	4.04±3.01	3.83±3.24			
Antenatal care					
Never*	86 (54.4)	85 (53.8)	1	---	---
Ever	72 (45.6)	73 (46.2)	0.98	0.63 to 1.52	0.910
Types of delivery					
Normal vaginal					
No*	22 (13.9)	25 (15.8)	1	---	---
Yes	136 (86.1)	133 (84.2)	1.16	0.63 to 2.16	0.635
Caesarean section					
No*	139 (88.0)	138 (87.3)	1	---	---
Yes	19 (12.0)	20 (12.7)	0.94	0.49 to 1.84	0.864
Place of delivery					
Hospital					
No*	66 (41.8)	80 (50.6)	1	---	---
Yes	92 (58.2)	78 (49.4)	1.43	0.92 to 2.23	.0.115
Maternity home					
No	149 (94.3)	145 (91.8)	1	---	---
Yes	9 (5.7)	13 (8.2)	0.67	0.28 to 1.62	0.379
Subject's Home					
No*	64 (40.5)	78 (49.4)	1	---	---
Yes	94 (59.5)	80 (50.6)	1.43	0.92 to 2.24	0.114
History of abortion					
Never*	119 (75.3)	120 (75.9)	1	---	---
Ever	39 (24.7)	38 (24.1)	1.04	0.62 to 1.73	0.896
History of D&C					
Never*	133 (84.2)	131 (82.9)	1	---	---
Ever	25 (15.8)	27 (17.1)	0.91	0.50 to 1.66	0.762

4.4 Bivariate analysis

In bivariate analysis factors associated with HCV sero-positivity are as follow, age (OR,101 95%CI0.98 to 1.05, p=0.384), living place (OR1.37, 95%CI 0.87 to 2.14, p=0.172), occupation (OR 0.64, 95%CI 0.35 to 1.15, p=0.137), family income (OR 0.69, 95%CI 0.50 to 0.94, p=0.019), History of injection last month (OR 1.44, 95%CI 1.02 to 2.04, p=0.039),History of injection last year (OR 2.71, 95%CI 1.95 to 3.76, p<0.001),History of injection last five year (OR 1.92, 95%CI 1.51 to 2.44, p<0.001), place of injection dispenser (assistant pharmacist trained for one year to help other staff of the hospital dispensing drugs, and routine ward works) (OR 2.14, 95%CI 1.36 to 3.38, p=0.001), place of injection unregistered(OR 4.80, 95%CI 2.75 to 8.39, p<0.001), History of previous surgeries (OR 0.70, 95%CI 0.43 to 1.16, p=0.167), history of surgeries were further categorized in to minor and major (OR 0.64, CI 0.37 to 1.09, p=0.102) and (OR 0.92, CI 0.42 to 2.03, p=0.841) respectively.

History of blood transfusion (OR 1.29, 95%CI 0.79 to 2.11, p= 0.136),Lived with Jaundice patient in household (OR 3.34, 95%CI 2.03 to 5.47, p < 0.001),Personal jaundice ever (OR 1.61, 95%CI 0.99 to 2.60, p=0.52),Number of pregnancies (OR 1.03, 95%CI 0.96 to 1.09, p=0.458),Parity (OR 1.02, 95%CI 0.95 to 1.10, p = 0.540), Types of delivery vaginal (OR 1.16, 95%CI 0.63 to 2.16, p = 0.635), Place of delivery hospital (OR 1.43, 95%CI 0.92 to 2.23, p=0.115), Place of delivery subjects home (OR 1.43, 95%CI 0.92 to 2.24, p=0.114),History of abortion (OR 0.91, 95%CI 0.50 to 1.66, p=0.762).

4.5 The multivariable logistic regression model risk factors associated with Hepatitis virus C infection.

The thirteen variables for which p<0.20 were included in the multivariable logistic model. Model results are given in table 5. Injections in the past year (OR 1.96, CI 1.22 to 3.15, p = 0.005), injections last 5 years (OR 1.61, CI 1.14 to 2.28p= 0.008), injections at unregistered place (OR 3.81, CI 1.98 to 7.34 p< 0.001),, subjects lived with jaundice in house hold (OR 2.50, CI 1.39 to 4.47, 0.002), personal jaundice (OR 1.90, CI 1.05 to 3.42, p = 0.035) were positively associated with being HCV-positive. While family income (OR 0.59, CI 0.39 to 0.87 p = 0.008), history of previous surgeries (OR 0.37 CI 0.19 to 0.72 P = 0.004) were associated negatively.

Table 5. Multivariable assessment of risk factors for Hepatitis C infection: 13-variable logistic model.

Characteristics	Number (percentage)		Odds ratio	95% CI	*P value*
	Case	Control			
Living place					
Urban *	86(54.4)	98(60)	1	---	---
Rural	72(45.6)	60(30)	0.92	0.51 to 1.64	0.766
Occupation					
Housewife*	136 (51.9)	126 (48.1)	1	---	---
Other	22 (40.7)	32 (59.3)	0.84	0.38 to 1.84	0.651
Family Income			0.59	0.39 to 0.87	0.008
<5000	56 (52.8)	50 (47.2)			
5000-10000	83 (56.1)	65 (43.9)			
>10000	19 (30.6)	43 (69.4)			
History of injection					
In last month			1.00	0.63 to 1.59	0.994
Nil	98 (62.0)	112 (70.9)			
1-3	41 (25.9)	37 (23.4)			
> 3	19 (12.0)	9 (5.7)			

In last year			1.96	1.22 to 3.15	0.005
Nil	41 (25.9)	86 (54.4)			
2-5	64 (40.5)	58 (36.7)			
≥ 6	53 (33.5)	14 (8.9)			
In last 5 years			1.61	1.14 to 2.28	0.008
< 5	41 (25.9)	82 (51.9)			
5-10	48 (30.4)	47 (29.7)			
11-20	39 (24.7)	20 (12.7)			
> 20	30 (19.0)	9 (5.7)			
Place of injection					
Dispenser					
No*	77 (48.7)	106 (67.1)	1	---	---
Yes	81 (51.3)	52 (32.9)	1.62	0.92 to 2.86	0.096
Unregistered					
No*	91 (57.6)	137 (86.7)	1	---	---
Yes	67 (42.4)	21 (13.3)	3.81	1.98 to 7.34	<0.001
History of previous surgeries					
No*	120 (75.9)	109 (69.0)	1	---	---
Yes	38 (24.1)	49 (31.0)	0.37	0.19 to 0.72	0.004
Lived with Jaundice patient in household					
Never*	84 (53.2)	125 (79.1)	1	---	---
Ever	74 (46.8)	33 (20.9)	2.50	1.39 to 4.47	0.002
Personal jaundice ever					
No*	101 (63.9)	117 (74.1)	1	---	---
Yes	57 (36.1)	41 (25.9)	1.90	1.05 to 3.42	0.035
Place of delivery					
Hospital					
No*	66 (41.8)	80 (50.6)	1	---	---
Yes	92 (58.2)	78 (49.4)	1.85	1.05 to 3.25	0.032
Subject's Home					
No*	64 (40.5)	78 (49.4)	1	---	---
Yes	94 (59.5)	80 (50.6)	0.74	0.41 to 1.34	0.327

4.6 Comparison of risk factors at Sandeman provincial hospital to those at Bolan medical complex hospital

Regarding comparison of risk factors of hepatitis C in two main hospitals at Quetta 13- variable risk factors model was created to find risk factors in each hospital and compare BMCH Vs SPH. The results showed that history of injections for last month and last one year (OR 2.40, CI 1.00 to 5.75, P = 0.045) and (OR 4.32, CI 1.90 to 9.90, P = <0.001) respectively, lived with jaundice in house hold (OR 3.21, CI 1.09 to 9.56, P = 0.035), delivery at hospitals (OR 1.89, CI 0.89 to 4.57, P =0.097) were positively associated significantly. While risk factors like monthly family income (OR 0.38, CI 0.20 to 0.75, P = 0.005) and history of previous surgeries (OR 0.18, CI 0.05 to 0.61, P = 0.006) showed negative association significantly at SPH.

Considering BMCH, following risk factors showed positive association for HCV infection, history of injections for last 1 year (OR 1.43, CI 0.73 to 2.80, P = 0.294) and for last five years (OR 2.26, CI 1.35 to 3.78, P = 0.002) was associated significantly. Place of injections like dispenser and unregistered place (OR 2.44, CI 1.19 to 5.34, P = 0.025) and (OR 6.60, CI 2.82 to 15.42, P = 0.001) respectively associated significantly. Subjects who lived with jaundice patients in house hold (OR 2.04, CI 0.91 to 4.54, P = 0.081) and personal jaundice (OR 2.00, CI 0.88 to 4.57, P = 0.097).

The model also revealed significant differences for HCV risk factors when BMCH compared with SPH these differences include, Living place (OR 0.26, P = 0.048), occupation (OR 6.30, P = 0.051), history of injections in last month and last 1 year (OR 0.30, P = 0.024) and (OR 0.12, P = 0.035) respectively. While monthly family income (OR 2.07, P = 0.081) was marginally significant.

Table 6 Comparison of risk factors at Sandeman provincial hospital to those at Bolan medical complex hospital

Characteristic	Sandeman provincial hospital			Bolan medical complex hospital			BMCH vs. SPH	
	OR	95% CI	p-value	OR	95% CI	p-value	OR	p-value
Living place								
Urban *	1	---	---	1	---	---		
Rural	2.14	0.73,6.29	0.167	0.56	0.25,1.24	0.151	0.26	0.048
Occupation								
Housewife*	1	---	---					
Other	0.29	0.06,1.30	0.104	1.80	0.61,1.30	0.283	6.30	0.051
Family Monthly Income	0.38	0.20,0.75	0.005	0.79	0.45,1.38	0.404	2.07	0.081
History of injection								
In last month	2.40	1.00,5.75	0.048	0.72	0.39,1.34	0.302	0.30	0.024
In last year	4.32	1.90,9.90	<0.001	1.43	0.73,2.80	0.294	0.12	0.035
In last 5 years	1.19	0.68,2.13	0.535	2.26	1.35,3.78	0.002	1.89	0.107
Place of injection								
Dispenser								
No*	1	---	---	1	---	---		
Yes	1.52	0.49,4.72	0.468	2.44	1.19,5.34	0.025	1.60	0.500
Unregistered								
No*	1	---	---	1	---	---		
Yes	2.24	0.54,9.22	0.264	6.60	2.82,15.42	0.001	2.95	0.200
History of previous surgeries								
No*	1	---	---	1	---	---		
Yes	0.18	0.05,0.61	0.006	0.54	0.21,1.37	0.195	4.14	0.147
Lived with Jaundice patient in household								
Never*	1	---	---	1	---	---		
Ever	3.21	1.09,9.56	0.035	2.04	0.91,4.54	0.081	0.63	0.508
Personal jaundice ever								
No*	1	---	---	1	---	---		
Yes	1.20	0.40,3.60	0.731	2.00	0.88,4.57	0.097	1.66	0.464
Place of delivery								
Hospital								
No*	1	---	---	1	---	---		
Yes	1.89	0.89,4.57	0.097	1.85	0.64,5.33	0.256	0.85	0.802

Home								
No*	1	---	---	1	---	---		
Yes	0.73	0.41,2.16	0.570	0.92	0.41,2.04	0.840	1.26	0.732

CHAPTER V

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

My study identified the association of HCV sero-positivity with the family income, injections last year, injections in the last 5 years, injections at unregistered place/practitioner, subjects lived with jaundice in household, and personal jaundice, while history of previous surgeries was negatively associated among women seeking treatment in hospitals of Quetta. This calls for strengthening patient safety at health care facilities, which requires comprehensive infection control steps, especially use of a new syringe for every injection.

The association of therapeutic injections with HCV seropositivity has been reported consistently in the male population in Pakistan (Bari, 2001; Khan, 2000; Luby, 1997) and elsewhere, such as Taiwan (Sun, 1997; Wang, 1998; Wang, 2002), China (Ho, 1997) and Egypt (Habib, 2001). Transmission of HCV and other blood-borne pathogens is efficient if injection equipment is contaminated with blood of infected patients. Studies in Pakistan have reported that injection overuse is very common in Pakistan and most injections are provided with previously used equipment (Janjua, 2005) as majority of health care workers are not medically qualified or scientifically trained and are unaware of standard sterilization procedures or the importance of safe injection practices in Pakistan (Raja, 2008). Patients are unaware of the risks associated with injections and hold exaggerated beliefs in their efficacy; practitioners, on the other hand, believe that patients want injections and have economic incentives to use them. Hence, a large proportion of health care visits

results in injection prescription (Altaf, 2004; Janjua NZ, 2006). In this study the history of injections showed a significant association for last one and five years. The findings of my study were consistent with another study conducted in Karachi and Peshawar (Khan, 2008; Muhammad, 2008) their study concluded an odds ratio of 2.4 for one or more injections. Women are especially at higher risk of receiving injections because of their greater health care needs.

The data showed that 27% of women had history of surgical interventions among cases and controls. Results of the multivariate logistic regression revealed negative association of surgical interventions with HCV infection. However previous studies among general population in Pakistan, Poland, Italy and Turkey suggest that previous surgeries had a significant positive association with HCV infection (Chlabicz, 2004; Jaffery, 2005; Karaca, 2006; Mele, 2000). The reason for this negative association might be that these hospitals have universal screening programs and patients are screened for diseases like hepatitis and AIDS prior to surgeries and infected patients are treated separately from other patients, and more over the infection may be associated with post surgical consequences like more frequent use of injections at their homes or unregistered places when the patients are discharged from the hospitals. A study conducted recently in Karachi, Pakistan among pregnant women also showed no association with gynecological procedure(Khan, 2008). In this study results showed that caesarean sections and D&C were not risks factors for HCV this might be related to many factors such universal screening programs for infectious diseases prior to surgery and may be related to the income as in my study most of the cases were belonging from low income category. Moreover, women during their reproductive life are more likely to receive therapeutic injections and minor surgical

interventions such as episiotomy (which is performed routinely in primipara women in these hospitals and maternity homes) during their normal vaginal deliveries. There is always a potential of recall bias and imperfect recall in case-control design. We attempted to minimize this problem by enrolling hospital controls to have comparable recall. Furthermore, given the long duration of asymptomatic phase of HCV infection, cases were as likely as controls to recall exposures potentially associated with HCV infection.

Delivery in hospital was associated with significantly increased risk of HCV. Hospitalization for the delivery as a risk factor flags the need for the universal precautions in health care to prevent the nosocomial exposure to HCV. Data from other studies substantiate the findings of poor infection control and non-adherence to universal precautions at first-level care facilities. Furthermore, health care workers are not trained in universal precautions(Janjua, 2007). National regulatory requirements for infection control are badly needed.

Household contact with a member who had jaundice/hepatitis was associated with elevated adjusted odds of HCV seropositivity among cases compared with controls. Other studies confirm these findings (al Nasser, 1992; Chang, 1994; Khan, 2008; Mohamed, 2005; Neal, 1994). The possible explanation could be by infectious blood or saliva, sharing a needle, razor and toothbrush within the household. Another study in Karachi revealed that sharing of toothbrushes was significantly associated with HCV seropositivity among household contacts of HCV positive patients (Akhtar, 2002). Some studies have reported a significant amount of HCV RNA in saliva in a substantial number of patients with chronic hepatitis (Pastore, 2006). Household clustering may have been reported because of similar behaviors and exposure to same

external exposure sources such as therapeutic injections it could be another explanation for interfamilial clustering. Inferences about the causal relationship should be drawn with care because of the case-control design. The implications are relevant to the members of high risk group and to the household members of already infected HCV patients to reduce and to prevent the spread of HCV.

Personal jaundice was also significantly associated with HCV infection among women. Other studies also revealed an odds ratio of 4.11 and 2.5 this may be due to that most of the subjects in my study were not able to get screening before.

To the best of my knowledge, this is the first case-control study of HCV risk factors among women in Quetta district and included exploration of past medical and reproductive specific risk factors. However, case-control studies have an inherent limitation for establishing causality, especially for HCV which is a chronic and mostly asymptomatic disease (CDC staff member, 1998). Even if cases were diagnosed for the first time at enrolment in our study, they may have been harboring the infection for years. While health care needs may increase because of infections, need for surgery and gestations do not get extenuated with HCV infections. Ideally in a case-control study using hospitalized cases should identify the reference population that is the source of the cases so that this reference population can be sampled to select controls. But our study hospitals do not have well-defined catchment areas. Since we selected controls from the same hospitals presenting for same reasons as cases, both were subject to the same selection factors.

Sandeman provincial hospital was built after the devastating earthquake in 1935 and is located in the center of city; it remained the only major hospital of the province for more than 5 decades till 1999 when Bolan medical complex hospital

started working and traditionally patients from the center of the city visit SPH. Bolan medical complex hospital which is located about 8 kilometers from the center of city and more patients are referred from rural areas of province. Result of this study showed interesting differences among patients visiting these hospitals. Respondents visiting Bolan medical complex showed dose-response relationship for history of injections in last one year and for last five years the reason may be that patients from rural areas stay for short time due to economical problems before completing their treatment and go back to their homes and get more exposure to risk factors in the form of injections and household contacts, but in the Sandeman hospital respondents had one month and last one year significance for HCV infection and place of injections was also associated significantly among respondents from Bolan medical complex, this shows that respondents visiting BMCH visit more unregistered places as compare to people living in cities. Cross tabulation showed that more patients were from rural areas visiting to BMCH. In Pakistan where we don't have enough registered medical practitioners in rural areas unregistered medical practitioners are providing services. This difference might be due that respondents living in the center of city have more awareness regarding not to use of injections frequently and have more health care facilities and registered health care providers as compared to the people living in rural areas.

In comparison with income respondents visiting SPH showed negative association for HCV infection the reason may be that people living in city have more income and spend more money for their heath. Vis-à-vis occupation was negatively associated with HCV infection as they also had occupation other than house wife and

may have more awareness about risk factors and less exposure as other study from Karachi showed no association (Khan, 2008).

Concerning surgeries SPH showed considerable negative association for HCV infection as compared to BMCH, as stated above many factors might be influencing like income, post surgical consequences, more awareness and more health care providers in the cities.

As it was assumed that there might be some differences between these two hospitals as they differ from each other in many aspects like location, providing medical facilities, training facilities for medical students and catchment area. When BMCH and SPH were compared with each other results showed significant difference for living place, occupation of the subjects, family monthly income and history of injections for I month and for last 1 year.

BMCH is tertiary care hospitals and is a teaching hospital for undergraduate and postgraduate medical students, equipped with latest technology; most of the patients from outside Quetta city are referred to BMCH as compared to the SPH.
The reason for the significant difference might be due to that patients from the rural areas are referred to BMCH and most of the females are house wives in rural areas. The income is also low in rural areas as compared to the cities and this may be related to the more exposure to the risk factors which lead to HCV infection.

Concerning differences for injections as the subjects visiting BMCH belong from rural areas they might don't have enough opportunities to get screened as well as awareness for screening as compared to the people living in Quetta, as the central city population visit SPH frequently for their medical purposes and screened frequently they may get diagnosis early.

There is always a potential of selection bias and imperfect selection in case-control design. To minimize this problem subjects (cases and controls) were enrolled from the same laboratory on same day. Furthermore, given the long duration of the asymptomatic phase of HCV infection, cases were as likely as controls to recall exposures potentially associated with HCV infection.

Household contact with jaundice/hepatitis has been identified as a risk factor for HCV infection but it was not feasible for us to ascertain the anti-HCV status of the study subjects' household.

5.2 Conclusions

This study showed that some kinds of iatrogenic exposure, number and place of health care injections, hospitalizations for deliveries and household contact with jaundice were the major risk factors for transmission of HCV among women in reproductive age in patients visiting BMCH and SPH Quetta, Pakistan. This calls for strengthening the prevention aspect of the hepatitis control program to focus on behavior change for reducing therapeutic injection reuse and overuse among general population. Policies regarding health care providers and hospitals especially towards use of injections, controlling over use, quality of sterilization of injections used by dispensers and polices for unregistered places should be revised and to be implemented strictly as infusions and injections are easily available at medical stores in Pakistan. Further steps are required to enhance infection control practices at health care facilities. This could start with establishing infection control committees in hospitals, providing training to health care workers and measures to enforce adherence to universal precautions.

Thus, reducing the reuse of injection equipment and overuse of unnecessary injections would be a major step towards reducing transmission of HCV among women. The Government of Pakistan has recently launched a hepatitis prevention and control program. Our results emphasize the need for more concerted efforts towards behavior change among both patients and health care providers.

Also, as there is no nationwide data is available, an important task is to find out the actual prevalence of hepatitis in the whole country.

Public awareness programs targeting risk factors for male like barbers, and general public, with a focus on hygienic issues such as use of disposable blades, are need to reduce this contributor to HCV transmission.

5.3 Suggestions for further studies

Similar studies should be conducted with other individuals and institutions in the community to evaluate injections further as risk factors during reproductive life for females.

Studies should be carried out to investigate and evaluate the performance of public health officials in preventing and controlling of HCV infection.

Studies should also be carried out investigate the risk factors in the general population for severity of the disease, and perceived benefits of action.

Studies related to the knowledge about the risk factors of hepatitis should also be carried out in the community to take further steps for cure and management of the patients.

More studies should be carried to confirm the association of surgery with HCV infection among females for their reproductive health surgical interventions and general surgeries.

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APPENDICES

APPENDIX A Questionnaire

Survey objectives: "Risk factors association of hepatitis among women of reproductive age: a case control study at Quetta, Pakistan"

Code: . . . . . Date: . . . . /...../ 2009

Interviewer: Place an X in the box of the selected answer(s).

Do not read responses unless the directions indicate.

A. Socio demographic information

1. Name: . . . . . .
2. Age: . . . . . .
3. Address: . . . . . .
. . . . . .
4. Living place:
    1. ☐ Urban
    2. ☐ Rural
5. Tribe:
    1. ☐ Baloch
    2. ☐ Pashtoon
    3. ☐ Other.....
6. What is the highest level of education you have completed?
    1. ☐ No schooling
    2. ☐ Primary school
    3. ☐ Secondary school
    4. ☐ High school
    5. ☐ College, University (bachelor)
    6. ☐ Post–graduate education (professional or post-graduate)
7. Marital status?
    1. ☐ Yes (for how many years.....)
    2. ☐ No

8. Occupation?
   1. ☐ Government servant
   2. ☐ Health care provider
   3. ☐ Service provider
   4. ☐ Business
   5. ☐ Non-earning
   6. ☐ Housewife
   7. ☐ Other, please specify.....

9. What is your average family income per month (Rupees)?
   1. ☐ < 5,000
   2. ☐ 5,000 – 10,000
   3. ☐ ≥ 10,000

10. Number of household members?

    .....people

B. Past medical history

11. History of hospitalization?
    1. ☐ Never
    2. ☐ Ever

12. History of injection/infusion? (include both obstetrical and non obstetrical injections)

    a. in last month
    1. ☐ 0
    2. ☐ 2
    3. ☐ >3

    b. in last 1 year
    1. ☐ 0
    2. ☐ 2-5
    3. ☐ 6-10
    4. ☐ >10

    c. in last 5 years
    1. ☐ 0
    2. ☐ 5-10
    3. ☐ 11-20
    4. ☐ > 20

13. Place of injection? May chose more then one

1. ◻ Government hospital
2. ◻ Private hospital
3. ◻ Dispenser
4. ◻ Unregistered health practitioner
5. ◻ other please specify……..

14. History of previous surgeries?

14a. what kind of surgery?

……

……

……

……

1. ◻ Minor
2. ◻ Major

15. History of dental treatment?

If no, skip to 16.

1. ◻ Yes
2. ◻ No

15a. if yes,

About how many times have you had dental treatment in the last 12months?

1. ◻ 0
2. ◻ 1
3. ◻ 2
4. ◻ 3 or more

16. History of blood transfusion?

1. ◻ Never
2. ◻ Ever

17. Ear piercing?

1. ◻ Yes
2. ◻ No

18. Nose piercing?

1. ◻ Yes
2. ◻ No

19. Lived with Jaundice patient in household?

1. ☐ Never
2. ☐ Ever

20. Personal jaundice ever?

1. ☐ Yes
2. ☐ No

C. Past obstetrical history

21. Number of pregnancies?

..... pregnancies

22. Parity?

..... live births

23. Antenatal care visits during pregnancy?

1. ☐ Never
2. ☐ Ever

24. Types of delivery? May chose more than one

1. ☐ Normal vaginal delivery
2. ☐ Caesarean section

25. Place of delivery? May chose more than one

1. ☐ Hospital
2. ☐ Maternity home
3. ☐ Home

26. History of abortion?

1. ☐ Never
2. ☐ Ever

27. History of dilatation & curettage by? May chose more than one

1. ☐ Never
2. ☐ Doctor
3. ☐ Nurse

Thank you very much for participating in our research!

APPENDIX B Schedule activities

RESEARCH PROCESS	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY
LITERATURE REVIEW	→	→						
WRITING PROPOSAL		→	→					
REVISE PROPOSAL			→	→				
DATA COLLECTION				→	→	→		
DATA ANALYSIS						→	→	
DISCUSSION							→	
WRITING REPORT							→	→
SUBMIT FINAL REPORT							→	→

APPENDIX C BUDGET

No	Activities	Unit	Price (baht)	Unit (Number)	Total Budget (Baht)
1.	Air fair: BKK – Karachi – BKK	Trip	15,000/tr	2 trips	30,000
2.	Pre-testing				
	- Photocopy	Quest.	1/page	6 x 40	240
	- Stationery	Set	300/set	1	300
3.	Data Collection:				
	- Interviewer training	Person	100/p/d	5 prs/day	500
	- Assistant interviewer	Person	50/p/d	5 prs x 7days	1.750
	- Photocopy	Quest.	1/page	6 x 350	2,100
	- Interviewers per diem	Person	400/p/d	5 prs x 7days	14,000
	- Gift for interviewees	Person	20/p/d	350 prs	7,000
	- Data Processing	Person	200/p/d	2 prs x 3days	1,200
4.	Document Printing:				
	- Paper + Printing	Page	5/page	800 pages	4,000
	- Photocopy (exam +final submit)	Page	1/page	10 x 400	4,000
	- Stationery	Set	300/set	1 set	300
	- Binding Paper (exam)	Set	100/set	5 sets	500
	- Binding Paper (submit)	Set	200/set	6 sets	1,200
TOTAL					**69690**

APPENDIX D Informed Consent Form

Sample no.....

"RISK FACTORS ASSOCIATION OF HEPATITIS AMONG WOMEN OF REPRODUCTIVE AGE: A CASE CONTROL STUDY

Quetta, Pakistan"

Responsible person(s) and institute:
Dr. Abdul Ghaffar
Masters of Public Health
College of Public health sciences,
Chulalongkorn University Bangkok 10400, Thailand

Date of consent.../...../...

I (Mr. /Mrs. /Ms.) ..... Home address .....
..... Contact No .....

I have read and understood all statements in the **informed consent form**. I have also been explained the objectives and methods of the study, as well as possible risk and benefits that may happen to myself upon the participation in the study. I understand that the information the information will be kept confidential and my name will no be disclosed in any case. I shall be given a copy of the **signed informed consent form.**

I have the right to withdraw from the study at any time without any adverse effects upon myself.

Signature..... (Respondent) (Informant) .....

Signature..... (Researcher) (dr Abdul Ghaffar)

BIOGRAPHY

Mr. dr Abdul Ghaffar was born on 7th April 1973, in Balochistan province, Pakistan. He received a Bachelor of Medicine in Medical Doctor in 1998 from Bolan medical complex Quetta, Pakistan. After graduated he worked as medical officer, trainee medical officer in different hospitals of Pakistan for post graduation. He continued his study for a Master of Public Health in Health Systems Development in College of Public Health Sciences, Chulalongkorn University in 2008 and completed the program in 2009.