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PREVALENCE OF AT-RISK MARRIAGES AMONG COUPLES ATTENDING PREMARITAL SCREENING (PMS) PROGRAMS: A SYSTEMATIC REVIEW AND META-ANALYSIS

BY

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ABSTRACT

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Title: Prevalence of At-Risk Marriages among Couples Attending Premarital Screening (PMS) Programs: A Systematic Review and Meta-Analysis

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Introduction/Background: Hemoglobinopathies are among the most common inherited genetic diseases. The World Health Organization estimates that at least 5% of the world's population are carriers for hemoglobinopathies (2.9% for thalassemia and 2.3% for sickle cell disease). Programs like Premarital Screening (PMS) have been developed in most Middle East countries on a mandatory basis to reduce at-risk marriages by providing counseling after a confirmed "genetic carrier" state for hemoglobinopathies.

Aim/Objective: The aim of this systematic review and meta-analysis was to estimate the prevalence of at-risk marriages globally and see the variation by region, income level, ethnicity, study period, implementation year of PMS program, study design and consanguinity proportion.

Methods**:** Different databases such as PubMed, Science Direct, and Scopus were searched systematically by using key terms and MeSH Terms. Studies from Google Scholar and reference lists of studies were also collected, and the author extracted all relevant data. Two reviewers independently conducted quality assessment by using Hoy et al (2012) risk of bias tool. Quality effects model (QEM) was used due to considerable heterogeneity observed between studies. Subgroup analysis and sensitivity analysis were also performed for assessing the causes of heterogeneity.

Results: a total of 15 studies were included in this meta-analysis. The overall pooled prevalence of at-risk marriages among total couples at-risk was 64% (95% CI: 49%- 78%). Estimates of several subgroups were found to be different as compared to the overall pooled estimate. Funnel plot and Doi plot indicated the presence of publication bias. Sensitivity analysis including only studies with low risk led to a pooled estimate of 52% (CI: 46%, 57%) and indicated absence of publication bias.

Conclusion and Recommendations: The pooled estimates varied widely and there was a substantial heterogeneity among studies, therefore, there is a need for more well-designed studies across different countries. Moreover, the importance of the quality of counseling sessions should be stressed and combined with efforts in other community sectors, such as high schools where students can attain high knowledge regarding genetic diseases before the age of marriage.

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CHAPTER 1: INTRODUCTION

1.1 Background

The Global Report on Birth Defects published in 2006 ranked 193 countries with respect to the rates of highest birth defects. Countries from the Middle East region were among the top with Sudan being the first (82 birth defects per 1000 live births), followed by Saudi Arabia (81.3 birth defects per 1000 live births) then United Arab Emirates, Iraq, Kuwait, Oman and Syria. Qatar was ranked as sixteenth with 73.4 birth defects per 1000 live births. Moreover, the report stated the lowest rate of birth defects in France with 39.7 per 1,000 live births (1). Common birth defects include congenital heart defects, neural tube defects, hemoglobinopathies such as thalassemia and sickle cell disease(SCD), down syndrome and glucose-6-phosphate dehydrogenase (G6PD) deficiency (1).

Globally, the prevalence of some autosomal recessive single gene birth defects such as sickle cell disease, cystic fibrosis and glucose-6-phosphate dehydrogenase (G6PD) deficiency is higher in developing countries, due to high rates of consanguineous marriages (2). As a result, consanguinity increases the chances for children of consanguineous parents to inherit identical copies (being homozygous) for recessive alleles due to the shared ancestor between the parents (autozygosity). In other words, consanguinity is defined as a marriage between individuals who are related to each other as second cousins or closer.

Evidence has shown that children with consanguineous parents are at 2.5 times higher risk of having autosomal-recessive diseases such as thalassemia, sickle-cell anemia and cystic fibrosis as well as congenital diseases/malformations (2).

Due to high burden of several recessive genetic diseases, especially beta-

thalassemia, in the Middle East, several countries have implemented mandatory premarital screening programs (PMS) to reduce at-risk marriages and the prevalence of such diseases and thus their medical and economic burden (3). The PMS mandatory programs started in the Mediterranean region with Cyprus being the first country implementing a PMS program for beta-thalassemia in 1973 (3). In other countries such as Jordan, Saudi Arabia and Bahrain, the PMS program was mandated in the beginning of the 21st century (3). Similarly, in 2009, premarital screening program was implemented in Qatar (3).

Further, for the purpose of this study, "at-risk marriage" refers to a prospective couple carrying an abnormal gene (both carriers) and therefore, transmitting the genetic disease to their future offspring.

In light of the above-mentioned burden of genetic disorders globally and regionally, there is a wide gap in the literature regarding the effect of the PMS programs and whether such programs reduce the incidence of hemoglobinopathies through reducing at-risk marriages. In other words, the effect of PMS programs in reducing at-risk marriages has not been evaluated globally in terms of systematic review and meta-analysis. Therefore, in the context of the paucity of data on at-risk marriages from PMS programs, this study helps to fill the existing scientific gaps with the following aim, objectives and research questions.

1.2 Aim

To assess systematically the prevalence of marriages among at-risk couples undergoing premarital screening program and to explore factors associated with variation in prevalence.

1.3 Objectives

- To systematically review the global proportion/prevalence of marriages among at-risk couples who underwent PMS program and counseling
- To quantify the global prevalence of marriages among at-risk couples using meta-analysis
- To explore the potential factors associated with variation in the prevalence of marriages among at-risk couples with respect to ethnicity (Arab versus non-Arab), geographical region, income-level, availability of prenatal diagnosis (PND) services, consanguinity among at-risk couples, and year of program implementation**.**

1.4 Research Questions

- What is the global prevalence of at-risk marriages among couples who underwent PMS programs and counseling?
- What are the potential factors associated with the prevalence of marriages among at-risk couples globally?

CHAPTER 2: LITERATURE REVIEW

The literature review is divided into eight sections that discuss the epidemiology of genetic diseases globally with focus on hemoglobinopathies, genetic diseases and its association with consanguinity, implementation of premarital screening programs, mandatory or voluntary aspects of these programs, counseling services in PMS programs, knowledge and attitude of PMS and a final section based on studies on PMS.

2.1 Genetic Diseases Epidemiology

Globally, the incidence of genetic diseases was reported to be 5.32% in births based on a followed up study over 25 years (4). This includes common autosomal recessive disorders such as thalassemia, sickle cell disease and glucose 6 phosphate dehydrogenase(G6PD) deficiency and cystic fibrosis. Available data at global level between 1952 and 1983, estimated the prevalence of cystic fibrosis at 232.5 per million births and the prevalence for thalassemia at 32.5 per million births (4). Additionally, based on the World Health Organization (WHO) estimates, 2.9% of the world's population is affected by beta-thalassemia, which affects the health of the population worldwide negatively and gives rise to many challenges on health care system and resources globally (5).

Specifically, in the Middle East region, the prevalence of beta-thalassemia is considered high; 1-15% of the population are carries of the trait (i.e. have a single copy of the disease allele) (6)(7). However, in the Arab World, studies suggesting the percentages of beta-thalassemia carriers vary from one country to another but in general, the range is between 2.0% to 10.0% (7). For example, in the Gulf region, a study carried out in Saudi

Arabia (5) on 15,72,140 men and women found that the prevalence of sickle cell was 45.1 per 1000 persons examined, and this included 42.4 carriers and 2.7 cases. In case of beta-thalassemia, the prevalence of positive beta-thalassemia among couples was 18.5 per 1000 persons examined (including 18.0 carriers and 0.5 cases per 1000 persons examined).

Another study from Gulf i.e. Oman reported the prevalence of sickle-cell and beta-thalassemia among Omani children under age five years was 6% and 2% respectively (8). It has been reported that patients with hereditary blood disorders usually experience decreased academic performance and higher rates of absenteeism due to frequent hospitalization needs and follow up appointments(8).

In addition, a study conducted in Qatar analyzed data of a sample of 3,275 patients from Hamad Medical Corporation that was collected between 1994-2000(9). The study showed that 30.4% of Qataris and 42.3% of non-Qataris had beta-thalassemia (trait, intermediate or major).

It is evident from aforementioned percentages that not only genetic diseases are widely studied in the Middle East region, but such genetic diseases could be life-threatening and expensive to treat as well. For example, it is estimated that the treatment for beta-thalassemia costs more than U$ 400,000 per person over a lifetime and cystic fibrosis costs U$ 9,400 per person per year. In the Arab world specifically, it has been estimated that the overall cost of almost all common genetic disorders is US$ 13 billion per year (2).

2.1.1 Hemoglobinopathies

Among the many types of genetic diseases, beta-thalassemia and sickle-cell disease are the two examples of hemoglobinopathies. The prevalence of beta-thalassemia

mutations ranges from <1% to 16%; it is 3.5%- 3.8% in South China, 3-4% in India, 1.5-3% in North Africa and 4-11% in Middle East countries.

Countries in the Mediterranean region also have high prevalence of beta-thalassemia; For example, Cyprus was reported to have 17.2% prevalence, but the proportion dropped to 12.1% in the last two decades mainly due to implementation of prenatal screening (10). This is because, prenatal screening helps in identifying if the baby experiences any birth defects that could be a result of genetic disorder and therefore, prenatal screening might act as a preventative strategy due to termination of pregnancy(10). In addition, in Cyprus, the mean frequency of heterozygous thalassemia all over the country was reported to be 7.4% with extremely uneven distribution between low altitude (up to 15%) fertile areas as compared to high altitude areas (11).

As stated earlier, genetic diseases are known to be life-threatening for patients affected with it. For example, in developed countries, it has been estimated that thalassemia patients can survive for 25-55 years depending on the medical treatment's compliance by the patient. On the other hand, in developing countries, due to insufficient medical treatment, most of the thalassemia-affected patients die before the age of 20 (12).

Another common example of Hemoglobinopathies is Sickle Cell Disease. SCD is defined as a group of conditions that results from the inheritance of hemoglobin S (HbS). It is reported (13) that HbS allele was originally found to be distributed throughout the region of Sub-Saharan Africa, the Middle East, the Mediterranean area and India. The carrier rates for this disease range from 5% to greater than 40% in above mentioned regions (13).

2.1.1.2 Genetic Diseases and Consanguinity

The high prevalence of beta-thalassemia in the Middle East is usually attributed to the high proportion of consanguineous marriages which is estimated as 25 to 60% among first degree cousins (14). It has been reported that consanguineous marriages are favored in many Arab majority countries (14). There can be numerous factors that explains the attitude of several populations towards supporting consanguineous marriages. For example, previous sociological studies showed that consanguineous marriages increase stability, compatibility and strengthen family ties between the husband, the wife and the in-laws as well as help in allowing wealth and property to stay within the family (15).

A study in Egypt indicated that men who had good educational and occupational status were considered as 'valuable assets' and were usually pressured to marry within the family (16). Moreover, evidence from a developing country like Pakistan shows that sociocultural factors rather than religion or economic reasons seem to be the major determinants of consanguineous marriages(17). These factors are regarded as advantages of consanguineous marriages and they include; "relatives more caring", "relatives-known entity", "easy adjustment for girls", "tradition" and "strengthen family ties". Furthermore, it has been estimated that such factors are acting as resistant to change (17).

In terms of consanguinity rate in Qatar, a study conducted among 876 women (between 2004 and 2005) indicated that the rate of consanguinity among the study population was 51.0% while the rate of first cousin marriages was 26.7% (18). In another study(19) conducted in 2006 among Qatari females, the proportion of consanguineous marriages was reported to be 54% whereas the proportion of first-cousin marriages was 34.8%. Similarly, in Saudi Arabia, the overall prevalence of consanguineous marriage is

56% while it is reported to be 33.6% between first cousins (20).

In the Middle East countries, a more recent study in 2018 reported the percentages of consanguineous marriages as above 20%. For example, the prevalence of consanguineous marriages are: 42-67% in Saudi Arabia, 54% in Qatar, 40-54% in United Arab Emirates, 29-64% in Jordan, 21-33% in Egypt, 44-63% in Sudan, and 40-45% in Yemen (2). Consequently, due to high rates of consanguinity, findings from different studies in Arab countries suggest that percentages of beta-thalassemia carriers vary from one country to another but in general, the range is between 2.0 to 10.0% (7).

2.1.1.3 Implementation of Premarital Screening (PMS) Programs

Due to high rates of autosomal recessive disorders in the Middle East countries, various countries implemented mandatory premarital screening programs in order to reduce the incidence of such diseases, improve the population health and reduce the burden on the health service systems in these countries.

First of all, Cyprus (a country in the Mediterranean region) implemented beta-thalassemia Premarital Screening (PMS) program in 1973. This program proved to be very successful against reducing the beta-thalassemia rates from 51 in 1974 to 8 in 1979. The incidence further decreased after the program was mandated in early 1980s with zero affected births occurring between 2002 and 2007 (21). Later, in Turkey (Denizli), Iran and Jordan, PMS was first implemented in 1995, 1997 and 2004 respectively(3). In Turkey, written regulations for the Fight against Hereditary Blood disease were established in 2002. Ministry of Health and Turkish National Hemoglobinopathy Council (TNHC) selected a total of 33 provinces that had high prevalence of severe hemoglobinopathies. A premarital screening program for hemoglobinopathies called the Hemoglobinopathy Control Program

(HCP) started in 2003 in these 33 provinces and it is running successfully since then (22).

Considering the Gulf countries, the PMS was implemented in Oman in 1999, in Saudi Arabia in 2004, in Bahrain in 2005, and in Kuwait in 2008. Similarly, PMS was implemented in Qatar and UAE in 2009 and 2011 respectively. It is worthy to highlight that in all Gulf countries, the program is provided on a mandatory basis except in Oman, where it is still provided as an optional free of charge service (3). One of the main uses of Premarital screening programs is that it can help prevent affected births if the couples at-risk could be identified beforehand (23). Such programs gives the at-risk couples the wide range of possible options even if they proceeded to marry. This is because no single screening strategy provided in premarital counseling can work effectively to meet the needs of an entire population (24).

2.1.1.3.1 Voluntary vs. Mandatory PMS

Some of the countries in Mediterranean region (Cyprus, Italy and Greece) and Middle East (Turkey, Iran, Jordan, Palestine and most GCC countries) have made it mandatory for all couples to undergo premarital screening (25). The main aim of conducting such programs on a mandatory basis is due to high rates of consanguinity prevailing in middle east region that contributes to various genetic diseases particularly hemoglobinopathies (25).

In China, the program is offered on a voluntary basis and some studies have investigated the participation rate among the general population in China. For example, a study conducted in China in 2013 (26) reported that the participation rate in the PMS program was 34.8%. Factors like higher age, residency and profession were found to be

associated with the participation of program (26).

In Bahrain the screening was introduced as an optional service in 1993. A ten-year evaluation of the Bahrain's PMS program in 2003 indicated that only 25% of the couples approached the screening service offered. Consequently, in 2005, a Royal decree of compulsory testing before providing marriage certificate was introduced that aimed to reduce the prevalence of hereditary diseases (27).

On the other hand, in most other countries globally like Egypt, Canada, China, UK, India and Indonesia, these type of screening programs are done on a voluntary basis (25).

2.1.1.3.2 Premarital Counseling Services

Genetic counselling services are usually offered to those planning to marry and might be at risk of carrying some genetic diseases. These sessions are provided on a one-to-one basis after a couple receives a "mismatch certificate". The counselors usually provide information regarding carrier status for at-risk couples, also they provide counseling regarding preventative reproductive options such as prenatal diagnosis and pre-implantation genetic diagnosis (PGD). Such services are useful for various defects in genes, and chromosomal abnormalities (28).

However, there are certain challenges that may be faced by genetic counsellors while providing counseling to at-risk couples. These include high consanguinity rate, preventative reproductive options or termination of pregnancy, emotional and social impact on carrier women as well as complex molecular test findings (28).

Evidence from Iran (24) shows that at the outset, the options for at-risk couples were limited to either marrying as planned, separating and finding a non-carrier partner, or favoring to postpone marriage or childbearing in the hope that there will be a better solution

in the future. However, it soon became evident from the reported experiences of the counselors that the population wanted to seek prenatal diagnosis option. This led to widespread ethical discussions which ended with a government decision in 1998 to allow the abortion of fetus before 16 weeks from the last menstrual period only if the fetus is known to be genetically affected (24).

2.1.1.4 Knowledge and Attitudes of PMS:

One of the aspects to understand the success or failure of PMS programs is through the knowledge and attitude among general population. This can be explained by the fact that knowledge and attitudes could be regarded as important attributes of decision-making among at-risk couples who underwent premarital counseling.

There are many studies conducted regarding the knowledge and attitude of PMS especially in the Middle East region. For example, a study in Turkey (29) indicated that 91% of the study participants were aware of the positive effects of PMS programs and that such screening can contribute to reduction of genetic diseases. However, the level of knowledge was inadequate when assessed for beta-thalassemia. Hence, 17.5% of the participants thought that thalassemia is contagious while 82.5% of participants had no idea or thought thalassemia is not contagious.

Another study in Egypt assessed the knowledge level and attitude of medical and non-medical female students(30). It indicated that, a level of knowledge among medical female students categorized as ‘high’ was 32.3% as compared to non-medical students (21.0%). In addition, 54.7% of the medical students had positive knowledge regarding PMS as compared to 49.0% of non-medical students(30).

Similarly, due to recent implementation of PMS programs (where screening is either mandatory or voluntary) in the Gulf region, several studies were conducted in the region assessing the knowledge and attitude of subpopulations regarding PMS(8–11). Most of these studies were conducted in the Gulf region (mostly in Saudi Arabia), where knowledge and attitude of PMS was assessed among university students(33,35–44) as well as local population accessing health care in hospitals(32,34,45). For instance, a study conducted in Saudi Arabia in 2013 assessed the knowledge, attitude and satisfaction level of Premarital Screening and Genetic Counselling (PMSGC) program among participants attending governmental outpatient clinics. It showed that 80.5% of subjects had a low knowledge score (<15 score out of 40) while only 5.5% of the participants had satisfactory scores i.e. ≥20 out of 40(32).

Another study in Oman, where the program is not mandatory at national level, explored the knowledge and attitude of university students towards PMS. It showed that most of the students (n=469; 79%) were aware about the availability of PMS program. Similarly, regarding the students' attitude, the majority (n=540; 92%) thought that it is important to undergo screening and they were willing to do it. However, only 53% of the participants were in favor of making the PMS program mandatory for all pre-couples(38).

Another study in Saudi Arabia assessed the knowledge and attitude of PMS among university students through self-administered questionnaire (n= 1000 students)(33). The results indicated that 94.0% (n=922) of participants believed that PMS program was a preventive measure and 77.1% of participants reported that these tests should be held before engagement(33).

In terms of general population, Binshihon et al (2018) has conducted a study (34) on 1039 unmarried males and females from the general population to assess the knowledge and attitude of PMS programs in Saudi Arabia. The results revealed that 51.1% of the participants had poor knowledge scores (≤14 points out of 23) while only 20.5% had good scores (≥19 points out of 23). In addition, educational level was significantly associated with the knowledge score, where people with higher education were having better knowledge scores. Moreover, participants aged ≥ 25 years had higher knowledge score compared to those less than 25 years (p-value=0.005). In addition, participants with a family history of genetic disease had significantly higher knowledge scores compared to their counterparts (*p-value<0.001*). Regarding the attitude, 60.4% of participants had positive attitude towards PMS. Almost half of participants strongly agreed that getting incompatible results and completing the marriage is a wrong decision. Similarly, 40.0% of participants strongly agreed that PMS does not violate the personal privacy (34).

A study done in Qatar (46) collected data between January 2013-May 2014 from hospital and primary health care centers chosen randomly with a total of 873 respondents (70%) out of 1246 initiated surveys. The questionnaire assessed the knowledge, attitude and practice of PMS program among married adults (Qatari nationals and Arab women) aged 18-40 years. The results indicated that there was no statistically significant difference between males and females regarding knowledge score of hemoglobinopathies (sickle cell anemia and thalassemia), homocystinuria, Human Immunodeficiency Virus (HIV), cystic fibrosis and hepatitis.

Regarding the attitude of respondents towards PMS program, there was statistically

significant differences between males and females (*p-value* >0.05). Women were more aware of genetic diseases and the risks associated with genetics, and Sexually Transmitted Diseases (STDs). In addition, through stepwise multivariate regression analyses, the predictors for knowledge of PMS and some associated covariates were; social, religious, family or parental interventions, consanguinity, educational level, knowledge of genetic counseling, and love after adjusting for demographic variables such as age and gender (46).

2.1.1.5 Studies on PMS:

To sum up, there is a lack of research on prevalence of high-risk marriages regionally and globally. Most of the previous studies in the Middle East region focused on exploring the level of knowledge and attitude of PMS and the factors associated with it. Furthermore, a large body of research has been done on reporting the proportion of marriage cancellation rates among at-risk couples undergoing PMS counseling rather than the prevalence of high-risk marriage. These studies were mostly conducted in the Eastern Mediterranean region for the purpose of collecting data on the success of PMS programs. However, from a public health perspective, prevalence of at high-risk marriage should be addressed and flagged as a serious public health issue due to the high economic costs and human suffer of chronic and genetic diseases.

There is a lack of research on the global prevalence of at-risk marriages and to the best of our knowledge, there is no study that addressed this issue at the global or regional levels. Therefore, the current study aims to assess systematically the prevalence of marriages among at-risk couples undergoing Premarital Screening (PMS) program and to explore factors associated with variation in prevalence.

CHAPTER 3: METHODS

This is a systematic review and meta-analysis study design to assess systematically the prevalence of marriages among at-risk couples and quantitatively pool its prevalence while exploring factors associated with its variation.

The aim of systematic reviews is to identify the eligible studies and quantitatively synthesize findings in a narrative manner. Moreover, it also includes the risk of bias assessment to assess internal and external validity of the included studies.

On the other hand, meta-analysis is a quantitative, statistical method that pools the effect sizes of papers identified through systematic review. By calculating the pooled estimate of those couples approaching marriage, a critical outcome is obtained. A well-conducted meta-analysis also helps to investigate the sources of heterogeneity or variability of results among the studies. Heterogeneity might include both statistical and clinical sources. To explain the sources of variability in the included studies, sub-group analysis and sensitivity analysis can be conducted (47).

In this study, the quality of identified studies were assessed using Hoy et al (2012) risk of bias (ROB) tool, developed specifically for evaluation of risk of bias associated with prevalence studies (48). For the meta-analysis, MetaXL software (version 5.3) (49) was used to estimate the overall pooled estimate as well as to conduct the sensitivity and sub-group analysis.

The scores obtained from ROB assessment tool, used for pooling the prevalence (proportion of marriage among at-risk couples), were also used as inputs for Quality Effects Model (QEM) invented by Doi & Thalib (50).

The recommendations from the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) were followed and used as a guide to report this systematic review and meta-analysis (51). In addition, the reporting guidelines from Meta-analysis of Observational Studies in Epidemiology (MOOSE) were also followed as guidance for this meta-analysis (52).

3.1 Search Databases

The electronic databases that were searched systematically included PubMed, Science Direct, Scopus and Google scholar. These databases were chosen as they are the most relevant in terms of publishing on health and social issues. All relevant studies starting from 2000 up to September 15, 2019 were identified.

3.2 Search Strategy

The search strategy was developed including a comprehensive database search using key terms: Premarital examination* OR pre-marriage counsel* OR Pre-marriage counseling program* AND evaluation OR outcome* OR result*. In addition, relevant Medical Subject Heading (MeSH) terms were included in the search strategy. We used the above mentioned terms and combined them with ‘AND’ and ‘OR’ in accordance with search engine specifications. Additional studies were identified through manual search of the reference lists of the relevant articles. There has not been any systematic review or meta-analysis published on the prevalence of marriages among at-risk couples; hence, searching included review articles to identify any related studies. Furthermore, the search was limited to articles in English language. All duplicate studies, obtained from different databases, were removed by Zotero reference manager program.

3.3 Eligibility Criteria

The following inclusion criteria were developed to identify and select the relevant or eligible research articles:

- Published in English language
- Peer-reviewed studies
- Cohort, cross-sectional, case-control and case series
- Both prospective and retrospective studies were included
- Studies with prevalence/proportion of at-risk marriages in cohort or population-based studies of couples screened.

3.4 Exclusion Criteria

The excluded studies were:

- Experimental studies and narrative reviews
- Studies that reported the prevalence or incidence of beta-thalassemia trait or disease among the children of at-risk couples
- Studies where cancellation rates of marriages were not reported

3.5 Identification of Publications

A total of 15 published studies were identified based on our inclusion and exclusion criteria. The 15 studies which reported on the prevalence of at-risk marriages, were peer-reviewed, and were published between 2000 and 2016. Overlapping or duplicate studies were removed and a PRISMA flowchart was generated (51). (Figure 1)

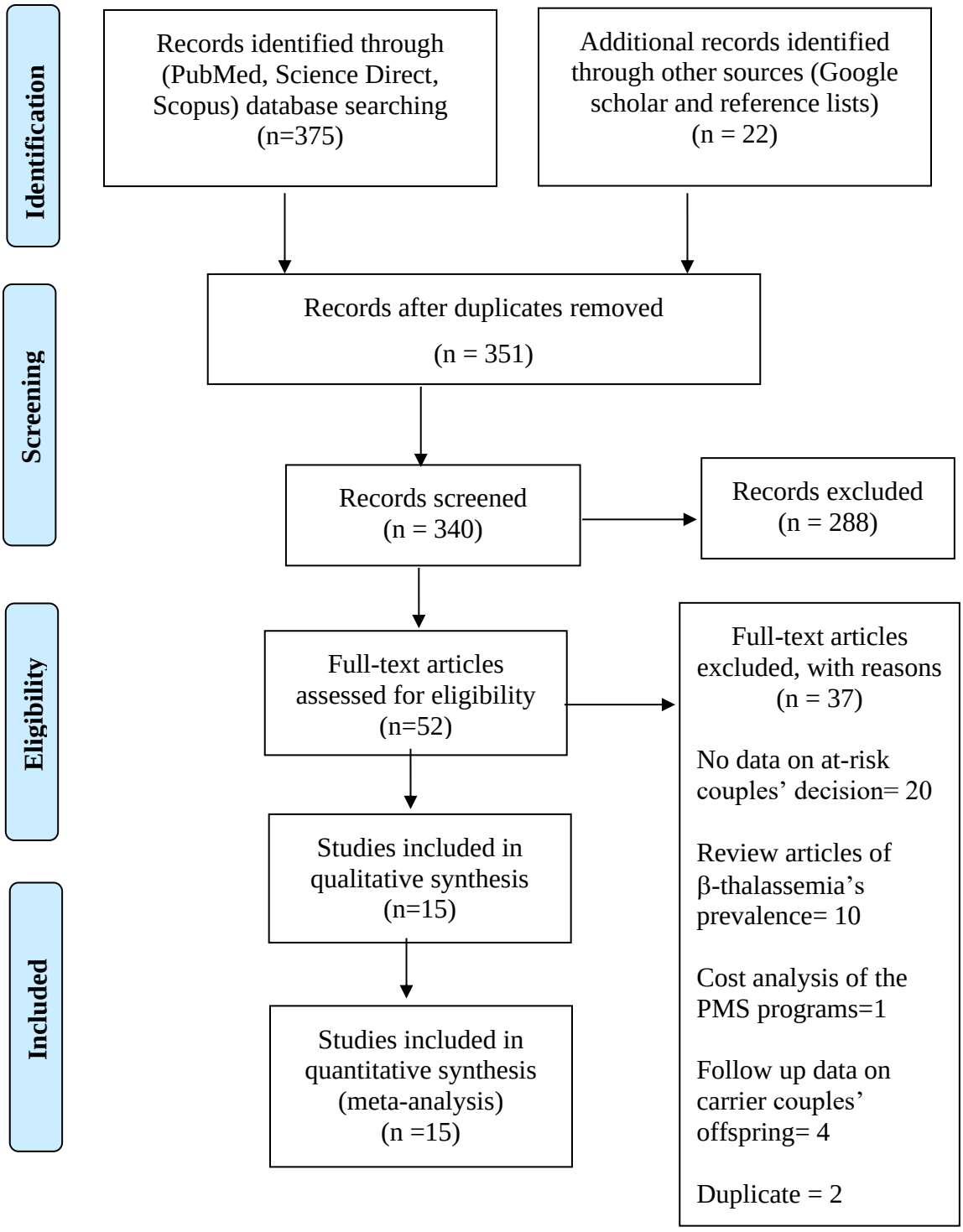

Figure 1: PRISMA Flowchart for Search Strategy

3.6 Data Extraction

Data was extracted from the 15 studies by using a standardized form including: author, year, city (country), study design, total couples screened, couples found at risk and carrier couples who married despite receiving counseling. Furthermore, data on some prespecified characteristics from included studies were also extracted in case of possible detection of heterogeneity among studies. A qualitative narrative summary of the 15 included studies is presented in the result section,

3.7 Risk of Bias Assessment

The quality of each of the included studies was assessed independently by the researcher and a second reviewer. The two reviewers also assessed independently and in a blinded fashion the risk of bias of the 15 studies, to ensure that the criteria were applied consistently, and a consensus reached according to the recommendations provided by Cochrane Handbook for Systematic Reviews of Interventions (53).

The two reviewers independently used Hoy's criteria (2012) (48) that is explicitly designed to assess risk of bias in prevalence studies. The tool consists of two sections, internal validity and external validity. Internal validity refers to the degree to which the design and conduct of a study have used methodologically sound steps to obtain minimum possible bias. On the other hand, external validity refers to the ability of the study to generalize its results to a larger population (54).

The Hoy's risk of bias tool consists of a total of 10 components (Figure 2). The first three components assess the risk of selection bias, item 4 assesses non-response bias and items from 5 to 9 assess measurement bias in a study. In addition, the last component of Hoy's tool is related to the analysis which implies that the numerator(s) and denominator(s)

related to parameter of interest should be appropriate(55). For each criterion, the risk of bias was assessed either “low risk” or “high risk” based on “yes” and “no”. Low risk was classified as “yes” and was given a score of 1, while high risk was classified as “no” and was given a score of 0. If the particular criteria mentioned in Hoy’s tool was unclear in a study, it was reported as “high risk” (55). The scores, across all components were summed up to get a final score out of 10 for each study. Furthermore, Hoy et al.’s (2012) classification was used to classify the 15 studies as high, moderate or low risk of bias. Scoring of 9 and above means having a low risk of bias, while a score of 6 to 8 is moderate risk of bias and having a score of 5 or less is categorized as having high risk of bias.

External validity

1. Was the study's target population a close representation of the national population in relation to relevant variables?
2. Was the sampling frame a true or close representation of the target population?
3. Was some form of random selection used to select the sample, OR was a census undertaken?
4. Was the likelihood of nonresponse bias minimal?

Internal validity

5. Were data collected directly from the subjects (as opposed to a proxy)?
6. Was an acceptable case definition used in the study?
7. Was the study instrument that measured the parameter of interest shown to have validity and reliability?
8. Was the same mode of data collection used for all subjects?
9. Was the length of the shortest prevalence period for the parameter of interest appropriate?
10. Were the numerator(s) and denominator(s) for the parameter of interest appropriate?
11. Summary item on the overall risk of study bias

Overall agreement for the 11 items

Figure 2: Hoy et al.'s (2012) Items of Risk Assessment Bias

The agreement between the two raters was assessed using proportion of agreement (p_o) and the Kappa statistic. The proportion of agreement (p_o) is the raw percentage of the number of times the two raters agree. However, there is disadvantage of using p_o because it does not take into account chance agreement, the distribution of agreement as well as ordering of agreement in terms of ordinal data(56). On the other hand, the advantage of using Kappa statistic is that it takes into account chance agreement. Kappa values range from -1 to +1, where 0 or less is regarded as poor agreement, 0.01 to 0.20 as slight, 0.21 to 0.40 as fair, 0.41 to 0.60 as moderate, 0.61 to 0.80 as substantial, and 0.81 to 0.99 as almost

perfect agreement (57).

An agreement of 80% was achieved between the scores of the two reviewers, using STATA. Such high percentage of agreement shows that there is substantial agreement between the reviewers in terms of rating. Moreover, the Kappa statistic was used to assess agreement where a Kappa coefficient of 0.71 (CI: 0.67, 0.78) was obtained (Table 1). This showed substantial agreement because kappa value (0.71) lied between 0.61 and 0.80(53). Discrepancies between the two reviewers were resolved by discussion. The final quality assessment results for each included study are reported in Table 2.

Table 1: Kappa Agreement between the Two Raters for Quality Assessment

Agreement	Expected Agreement	Kappa	Standard Error	Z	P-value	95% CI
80%	30.22%	0.7134	0.1508	4.73	<0.001	(0.672, 0.783)

Table 2: Methodological Quality Assessment Using Hoy et al (2012) Tool

	External Validity				Internal Validity							
Author & Year	1	2	3	4	5	6	7	8	9	10	Score	Risk of bias
Tamhankar et al (2009)	No	No	No	Yes	Yes	Yes	Yes	No	Yes	Yes	6	High
Memish et al (2011)	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	8	Intermediate
Karimi et al (2007)	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9	Low
Zeinalian et al (2013)	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	8	Intermediate
Al Sulaiman et al (2010)	No	No	No	No	Yes	Yes	No	Yes	No	Yes	4	High
AlHamdan et al (2007)	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	8	Intermediate
Al-Allawi et al (2013)	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	8	Intermediate
Samavat et al (2004)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	9	Low
Salama et al (2016)	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	7	Intermediate
Almutawa et al (2009)	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9	Low
Tarazi et al (2007)	No	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	8	Intermediate
Khorasani et al (2008)	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9	Low
Alsuwaidi et al (2012)	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	8	Intermediate
Keskin et al (2000)	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9	Low
Guler et al (2010)	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9	Low

3.8 Statistical Analysis

For statistical analysis purposes, MetaXL software version 5.3 (50) was used to calculate the overall pooled effect size via quality effects model (QEM).

QEM was developed by Doi & Thalib (2008) and is used, especially, when significant heterogeneity is detected (50). The main difference between the random-effects and quality-effects weights is that the former lacks to provide assessment of differences in study quality (50). In QEM, bias adjustment is based on measured methodological heterogeneity between studies, which results in a greater clinical relevance as well as helps to avoid the artificial inflation in variance which is otherwise seen in the random effects model (REM).

To evaluate the statistical heterogeneity, Cochrane Q statistic (chi-square test) and Higgin's I-squared (I^2) statistics were used (58). Cochran's chi-squared test is used to assess if the variation found across studies is solely due to chance; .a *p-value* less than 0.05 is used to represent statistically significant heterogeneity (53). On the other hand, Higgin's I-squared (I^2) statistics is a quantitative indicator which is used to estimate the proportion of variance between studies that could be due to statistical heterogeneity rather than chance alone(53)(58). An I^2 value of ≤25% represents low heterogeneity, 26-50% represents moderate heterogeneity, 51-75% represents substantial heterogeneity while an I^2 value of 76-100% represents high heterogeneity (53)(58).

Additionally, based on the above-mentioned tests and percentages, the detection of high heterogeneity would imply the uncertainty in our estimates or a risk of bias in study meta-analysis results.

In estimating the pooled effect size, prevalence (proportion) of at-risk marriages from each study and its quality score were considered. In addition to QEM, random effect models were also used for sub-group analysis based on Cochrane group recommendations of using REM when a substantial heterogeneity across studies is detected. Sensitivity analysis and sub-group analysis were also conducted in order to investigate the causes of heterogeneity. For sub-group analysis, specifically for income level of countries, the included studies were divided into World Bank specified income levels (59). In order to test for statistical significance differences between study subgroups, their confidence intervals were drawn against each other and compared for any overlapping.

Further analyses were performed to examine publication bias through Funnel plot and Doi plot. A Doi plot is a new method of detecting publication bias and was developed due to subjective nature of scatter plots in funnel plots. In order to quantify the extent of asymmetry, an *LFK* index is used and the closer the value is to zero, the more symmetrical would be the plot (60).

3.9 Ethical Considerations

This study was approached by Qatar University Institutional Review Board (IRB) under approval number QU-IRB 1113-E/19.

3.10 Source of Funding

This project has not been funded by any partner or organization.

3.11 Registration

The details of this study were registered in PROSPERO and the preliminary reference number provided was, 151490.

CHAPTER 4: RESULTS

4.1 Qualitative Summary

The search strategy yielded a total of 375 articles whereas another 22 articles were identified through Google Scholar and reference lists of related articles. Based on the eligibility and inclusion/exclusion criteria, a total of 15 studies were included in the meta-analysis as shown in Figure 1. Of these, 4 were conducted in Saudi Arabia, 4 in Iran, and 2 in Turkey. In addition, one study was found in each of the following countries: Iraq, India, Bahrain, United Arab Emirates (UAE), and Palestine (Figure 3). Premarital screening programs (PMS) are provided on a voluntary basis in India, while it is mandatory for all other countries stated above.

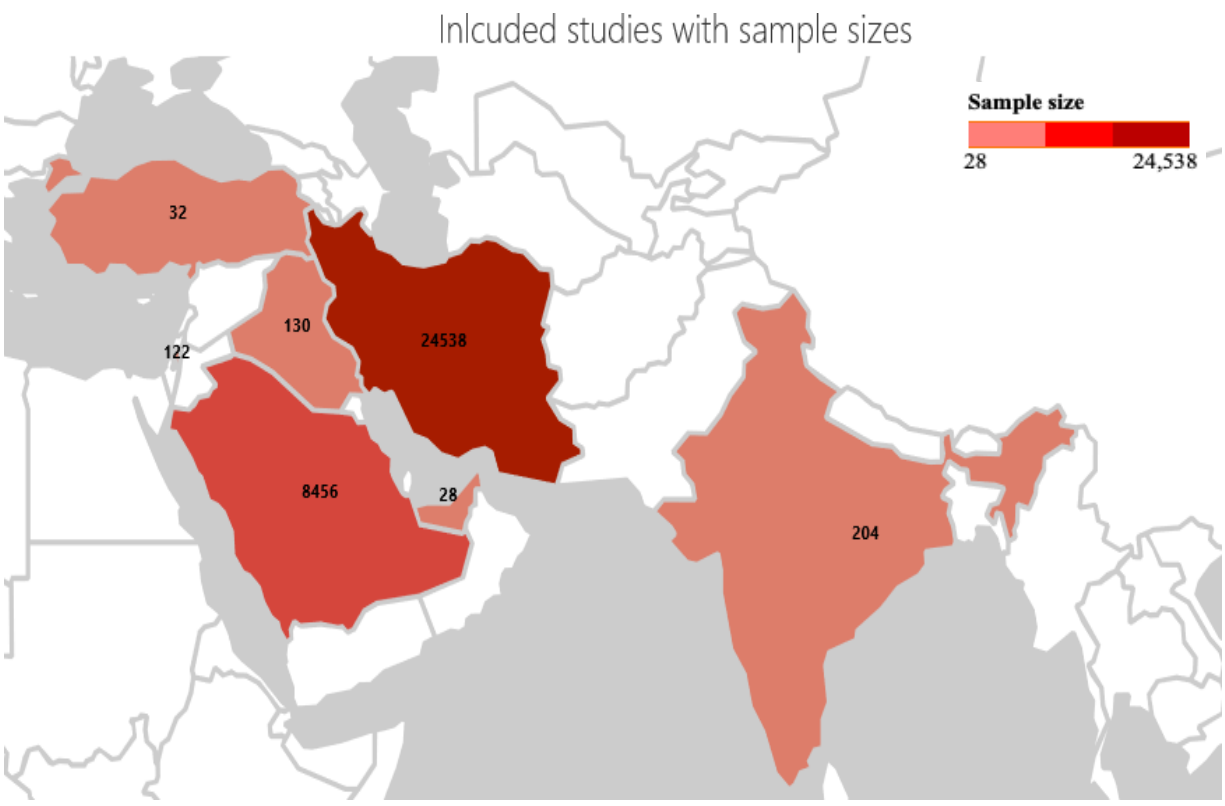

Figure 3: Geographical Location of the Studies Included for Meta-analysis

In terms of study designs, four studies used a cross-sectional design (reporting on point period), while one study used an unmatched case-control design. Moreover, the remaining 10 studies were retrospective population-based study designs reporting on period prevalence.

The 15 studies varied in the number of participants included (couples screened), which ranged from 15 to 10,298 couples. The total sample size of our meta-analysis was 33,540 couples obtained by pooling the 15 studies' participants. Table 3 highlights the characteristics of the included studies in terms of study design, total couples screened, study period, diseases screened, PND data as well as implementation year of program.

Table 3: Characteristics of the Included Studies

Author & Year	City/Country	Study Period	Study Design	Couples screened	Diseases screened	Prenatal Diagnosis data	Implementation year
Alsuwaidi et al (2012)	Saudi Arabia	2005-2006	Unmatched Case control	4,444	SCD, β-Thalassemia & G6PD	-	2004
Memish et al (2011)	Saudi Arabia	2004-2009	Retrospective	786,070	SCD & β-Thalassemia	-	2004
Al Sulaiman et al (2010)	Saudi Arabia	2007	Cross-sectional	129	SCD & β-Thalassemia	-	2004
AlHamdan et al (2007)	Saudi Arabia	2004-2006	Cross-sectional	207,333	SCD & β-Thalassemia	-	2004
Zeinalian et al (2013)	Isfahan, Iran	1992-2010	Retrospective	703,082	β-thalassemia	Available	1997
Khorasani et al (2008)	Mazandaran, Iran	1993-2006	Retrospective	279,936	β-thalassemia	Available	1997
Karimi et al (2007)	Southern Iran	1995-2004	Retrospective	1,038,371	β-thalassemia	Available	1997

Author & Year	City/Country	Study Period	Study Design	Couples screened	Diseases screened	Prenatal Diagnosis data	Implementation year
Samavat et al (2004)	Iran	1997-2001	Retrospective	2,729,000	β-thalassemia	Available	1997
Salama et al (2016)	Ras Al-Khaimah, UAE	2008-2015	Retrospective	8913	SCD & β-Thalassemia	-	2009
Almutawa et al (2009)	Bahrain	2006	Cross-sectional	1,070	SCD, β-Thalassemia & G6PD	-	2005
Al-Allawi et al (2013)	Northeastern Iraq	2008-2012	Retrospective	54,132	SCD & β-Thalassemia	Available	2008
Tarazi et al (2007)	Gaza strip, Palestine	2001-2005	Retrospective	19,712	β-thalassemia	-	2000
Guler et al (2010)	Kahramanmaras, Turkey	2006-2009	Retrospective	24,062	SCD & β-Thalassemia	-	2002
Keskin et al (2000)	Denizli, Turkey	1995-1999	Retrospective	9,902	SCD & β-Thalassemia	Available	1995
Tamhankar et al (2009)	India	2003-2007	Cross-sectional	334	β-thalassemia	Available	-

Abbreviations**:** SCD: Sickle Cell Disease; UAE: United Arab Emirates
-: Not available

4.2 Study Characteristics

A narrative review describing 15 studies included in the meta-analysis is provided below. All the included studies were from the Middle East region except two studies from Turkey and one study from India.

4.2.1 Studies in the Gulf Region

From the GCC region, studies reporting the prevalence of at-risk marriages were from Saudi Arabia, United Arab Emirates (UAE) and Bahrain.

4.2.1.1 Saudi Arabia

In Saudi Arabia, a case-control study was conducted (61) using the results of the first two years of program implementation (i.e. 2004 to 2006). A total of 4,444 at risk couples were identified during the 2-year period and marriage decision was known for 2,375 while it was unknown for 2,069 couples. Out of 4444, around 2375 couples had their marriage decision known to Ministry of Health (MOH); on the other hand, 1135 couples were untraceable while the remaining 934 couples (sampling population) were categorized as with 'unknown marriage decision'. The last category of 934 couples was either categorized as "did not marry" with 110 couples or "did-marry" with 824 couples. However, a required sample size of 582 couples (out of 934) was calculated for this case-control study; 104 cases, who agreed to participate, and 478 controls, who were chosen randomly.

Cases and controls were at-high risk marriages, but cases were defined as those "who did not marry" while controls were defined as "those who did marry". The study showed that 37.2% of the control group got married due to "wedding already arranged/non-cancelable", while 12.1% due to "familial commitment/pressure" (61). In addition, family

dependent individuals were more likely to marry than those who were financially independent (OR=1.1). Similarly, low-income participants were also less likely to marry than high-income participants with OR=1.26 (61).

Another Saudi Arabian study, a retrospective population-based study, (5) assessed the six-year (2004-2009) outcome of the national premarital screening program for hemoglobinopathies (β-thalassemia and Sickle cell disease). Of 1,572,140 couples, 70,962 (4.5%) were found to be carriers or cases for sickle cell disease; while 29, 006 (1.8%) were positive (carriers or cases) for β-thalassemia. It was also estimated that the annual prevalence of β-thalassemia was 18.5 per 1000 examined persons while for sickle cell disease, the prevalence was 45.1 (42.4 for carriers and 2.7 cases) per 1000 persons examined. Among 5,370 at-risk couples, 3,945 couples(73.5%) got married despite undergoing the screening and counseling (5).

Another follow-up survey (62) on at-risk couples was conducted in Saudi Arabia that involved a structured questionnaire for couples who had underwent premarital screening two years ago and were found to be at-risk for sickle cell disease or β-thalassemia. Out of the129 couples at-risk, 127 (98%) had proceeded with the marriage. Additionally, culture pressure was reported as the main reason in more than 48% of the couples while around 83% (107) of these couples appreciated the concept of genetic counseling.

Another cross-sectional population-based study(63) evaluated the screening program of β-thalassemia in Saudi Arabia between 2004 and 2005. During this two-year period, 207,333 couples were screened for hemoglobinopathy (β-thalassemia & sickle cell

disease) and 4444 were reported as “high-risk”. Further, 2,375 of at-risk couples were followed up to collect data via a telephone survey. The results showed that 2,128 (89.6%) of those at-risk couples got married with their partners though the option of prenatal diagnosis was practically unavailable in the “existing” healthcare system at that time. On the other hand, if this option was available, the option of abortion would either be unavailable or unacceptable to families due to social and/or religious reasons (63).

4.2.1.2 United Arab Emirates (UAE)

A retrospective population-based study (64) was conducted in Ras Al-Khaimah, in the United Arab Emirates, based on the premarital screening records of all couples who applied for marriage during the period 2005 to 2008 . Out of 17,826 individuals who were screened for hemoglobinopathies, 4.02% (717 individuals) were tested positive. In addition, the hemoglobinopathy trait was found to be more prevalent than the disease. Among the 8,913 couples who were issued incompatibility certificate, 28 (0.31%) were declared high risk. It was also reported that the rate of consanguinity among those at-risk couples was 70%. All the couples who were at risk (100%) continued for this hemoglobinopathy-risky marriage (64).

4.2.1.3 Bahrain

A cross-sectional study in Bahrain (27) explored the results of premarital counseling of hemoglobinopathy carrier couples, who received premarital services. Data was collected from 1,070 individuals between 1st April and 15th May 2006. The study showed that there were 30 couples with mean age of 25.7 years, and carriers to hemoglobinopathy, who were referred to genetic counseling. However, 17 couples (56.7%) continued with marriage despite the counseling and knowing their at-risk status. The study

concluded that the current policy of premarital screening is not successful in discouraging the at-risk couples (27).

4.2.2 Levant Countries

Levant countries is a term referred to a large geographical area situated in the Eastern Mediterranean region consisting of Iraq, Syria, Lebanon, Cyprus, Turkey (Hatay province), Israel, Jordan ad Palestine(65). From the Levant countries, the data on prevalence of at-risk marriages was reported from Iraq and Palestine.

4.2.2.1 Iraq

In Iraq, Northeastern part, (66) a total of 108, 264 individuals (54,132 couples) were screened during a 5-year period for β-thalassemia and SCD. There were 130 couples at risk and most of them (n=105, 80.7%) proceeded with the marriage despite the counseling received. PND services were available for the couples; and the study showed that selective termination was chosen in 10 of the affected fetuses (66).

4.2.2.2 Palestine

In Palestine, a study examined the impact of a mandatory premarital screening test for β-thalassemia before issuing a certificate marriage, between 2001and 2005 (67). During this period, a total of 122 couples were carriers for β-thalassemia; however, 45.9% (56) of them continued with the marriage in spite of the counseling received. Moreover, 3 thalassemia births occurred among those who had proceeded to marry.

4.2.3 Iran

In Iran, a total of 703,082 couples were screened for β-thalassemia during 1992-

2010 (68). Using these data, a retrospective population-based study showed that 1,317 (0.19%) couples were classified as high-risk for β-thalassemia and 4,412 were classified as "suspected couples". Out of these two at-risk groups (a total of 5729 couples), 78.5% (n= 4497 couples) proceeded to marry their partners despite known status. This means that only 21.5% (n= 1232) of the couples cancelled their planned marriages. According to the study, prediction of prevalence of β-thalassemia trait has becomes more complex due to high rate of consanguineous marriages (30-80%) among Iranians (68).

Another retrospective population-based study in Iran (69) evaluated the 13-year (1993-2005) premarital screening program for β-thalassemia in the province of Mazandaran. A total of 279,936 couples were screened, 3,329 of these couples were at-risk for β-thalassemia, of whom, 1826 couples (55%) decided not to marry. Those who married received special advice for the prevention of unplanned pregnancy as well as for prenatal diagnosis during pregnancy. It has been observed that the number of at-risk couples have increased over the years; from 12 in 1993 to 453 in 2005. This increase could be due to the mandatory nature of the program in 1998; since the program screens for more people now, the number of captured cases increases compared to the past.

An Iranian study (70) assessed the 10-year outcome of β-thalassemia screening program in Southern Iran. A total of 1,038,371 couples were screened, of whom 5,182 (0.4%) couples found to be "carriers" for β-thalassemia trait. More than half (54%) of these carriers (at-risk couples) continued with marriage.

Another study (24) from Iran investigated the national thalassemia screening program over a period of five years (1997-2001), where a total of 2.7 million prospective

couples were screened. Of these 10,298 couples were at-risk and 5431 (53%) proceeded to marry despite knowing their carrier status. The study also stated that since the development of the DNA laboratory network in Iran, there has been a dramatic increase in the number of couples seeking PND (69).

4.2.4 Turkey

In Turkey a study was conducted in the province of Kahramanmaras (71), where the mandatory screening program for β-thalassemia and sickle-cell anemia (SCA) trait took place in 2002. The study presented the results of the 3-year program (2006 to 2009). A total of 24,062 couples were screened during this period, where 17 were found to be carriers (at-risk couples) for β-thalassemia. The decision to marry or not marry was left to the couples themselves after receiving counseling. The results indicated that 14 (82%) out of 17 couples did not marry their partners. Moreover, the other three couples were informed about the prenatal diagnosis.

Another study in Turkey(72) was conducted in Denizli, which is the first province that had implemented PMS program since 1995, evaluated the 4-year (1995-1999) program results of screening β-thalassemia and SCD. The couples who were found at high-risk were also offered prenatal diagnosis and termination of pregnancy services. Among 9,902 couples (19,804 individuals) tested during the study period, 15 couples were carriers (both partners) of β-thalassemia trait. After the couples received counseling, only two (13.3%) of the 15 planned marriages were cancelled; prenatal diagnosis was sought by six (46.1%) of the 13 couples (72).

4.2.5 India

In India, the premarital screening program is not implemented as a mandatory program but rather on a voluntary basis. A study (73) recruited samples in three steps; first group were prospective couples of the extended family member (EFM) of patients who were affected with thalassemia or hemoglobinopathy. The prospective couples who were planning to marry were given counseling and screened. The second group was recruited from the anemia cases seen at the Out-Patient Department (OPD) of the hospital as well as cases of anemia amongst university students. Carriers were counseled and were given the chance of getting their prospective couples screened on a voluntary basis.

The third group included adults from districts with the highest number of cases/carriers and were selected for the College Group (CG) screening component. Single adults were counseled and screened for thalassemia, during a week-long camp in the vicinity of a university. Carriers were called for a second visit and informed about the need of getting their prospective couples screened before marriage. Carriers were also informed to report any cancellation of marriages, or other issues faced at the social level.

The results indicated that a total of 204 couples were at-risk of β-thalassemia out of the three groups. The prospective couples were given counseling and informed about the risk of having children born with thalassemia. However, almost all i.e. 202 (99%) of the 204 couples proceeded with the marriage plans (73).

4.3 Overall Summary of Studies:

It was evident that most of the large studies (with big sample size) were conducted in Iran, i.e. 4 studies (24,68–70), and 2 studies were carried out in Saudi Arabia (5,63).

Among the demographic variables, age was reported in the included studies, e.g. mean or median age of the overall study sample or mean or median age for males and females separately. Mean age of the participants in two studies (70,72) for males ranged from 22.5 to 24.5 years while for females, it ranged from 18.2 to 20.8 years. Similarly, in a study from Turkey(71) and Bahrain (27), the mean age of study sample was 24.9 and 25.9, respectively. Only one study (62) reported the age range for males as 21 to 52 (median 26 years) and for females as 17 to 31 (median 23 years). In addition, very limited data was provided based on the educational level of study participants. In a case control study (61) done on at-risk couples during 2005-2006, 64.6% (n=309) of at-risk couples who married each other had secondary education or lower. Interestingly, the prevalence of at-risk marriages was high: 82%. On the contrary, a post marital follow up survey (62) reported the prevalence of at-risk marriages as 98%, and the study showed that 72% (n=93) had educational level higher than 12 years.

There were variations among studies in terms of length (years) of outcome assessment and thus, the study sample varied accordingly. The study period was two years or less for four studies, three to five years for six studies, 6-10 years for three studies, and 11-20 years for the remaining two studies. Considering the premarital screening- related diseases in the included studies, β-thalassemia was reported in six studies, both β-thalassemia and sickle cell disease (SCD) were reported in seven studies, β-thalassemia, sickle cell disease and G6PD were reported in the remaining two studies

In most of the studies, the data reported the outcomes of national PMS outcomes and thus, it was obtained through longer follow-up period. Additionally, the program's

outcomes in other studies were assessed in specific provinces. Examples of such countries included; UAE, Palestine, Iraq, Turkey and India. Furthermore, the studies also differed widely in terms of the implementation year of PMS program. In countries like Iran, Turkey and Palestine, mandatory PMS was implemented before 2004. On the other hand, in the countries of the Gulf region such as Saudi Arabia, Bahrain, UAE as well as Iraq, the program was mandated in 2004 or after.

In conclusion, all the above-mentioned factors showed variations and weaknesses of the current available evidence, therefore; this variation should be taken into consideration while generalizing results to wider populations. Further, this should call for more well-designed research in this area that could address factors leading for high risk marriages and strategies to prevent it.

4.3 Risk of Bias

Based on Hoy's risk of bias (ROB) assessment criteria, of the 15 articles reviewed, six studies had low risk of bias (24,27,69–72) while seven had moderate risk of bias (5,25,63,64,67,68,74). On the other hand, two studies had high risk of bias (62,73). In terms of external validity, 12 out of 15 studies were rated good as 3 of the 4 external validity criteria were met; however, none of the 15 studies got a full score of 4 on Hoy's external validity assessment. In addition, none of the studies had a summary score of 10 on the risk of assessment tool (Figure 4).

Nine studies (60%) had issues in the first domain that states whether a study's target population is a close representation of national population in terms of relevant variables. Moreover, 3 studies (20%) had done poorly on the second domain of Hoy's ROB tool, in terms of sampling frame as not being a true or close representative of the target population. In terms of random selection of sample or census data, three studies (20%) did not do well. In addition, five studies (33.3%) had done poorly in terms of minimizing non-response bias.

On the other hand, all studies scored 100% in terms of fulfilling the first domain of internal validity i.e. data collected directly from the subjects. This is because the carrier status of the participants can be known only by taking blood sample directly from the patient and then sent to the laboratory. Furthermore, four studies (26.6%) had done poorly in terms of absence of an acceptable case definition.

With regards to a valid and reliable study instrument, only two studies (13.3%) had issues, i.e. either the studies did not state it in the text, or it was unclear. Similarly, for

domain 8, the results showed that three studies (20%) did not use same data collection mode; some studies collected data from male patients while female partners were only invited if the males had a suspected testing result.

As for the domain nine that assesses if length of the shortest prevalence period was used, only one study (6.67%) rated poorly. The participants of the study (62) were required to recall events from their screening tests done two years ago. Lastly, in domain 10 of analysis, only one study (6.67%) did not do well where the numerator and denominator of interest were found unclear or inappropriate (Figure 5).

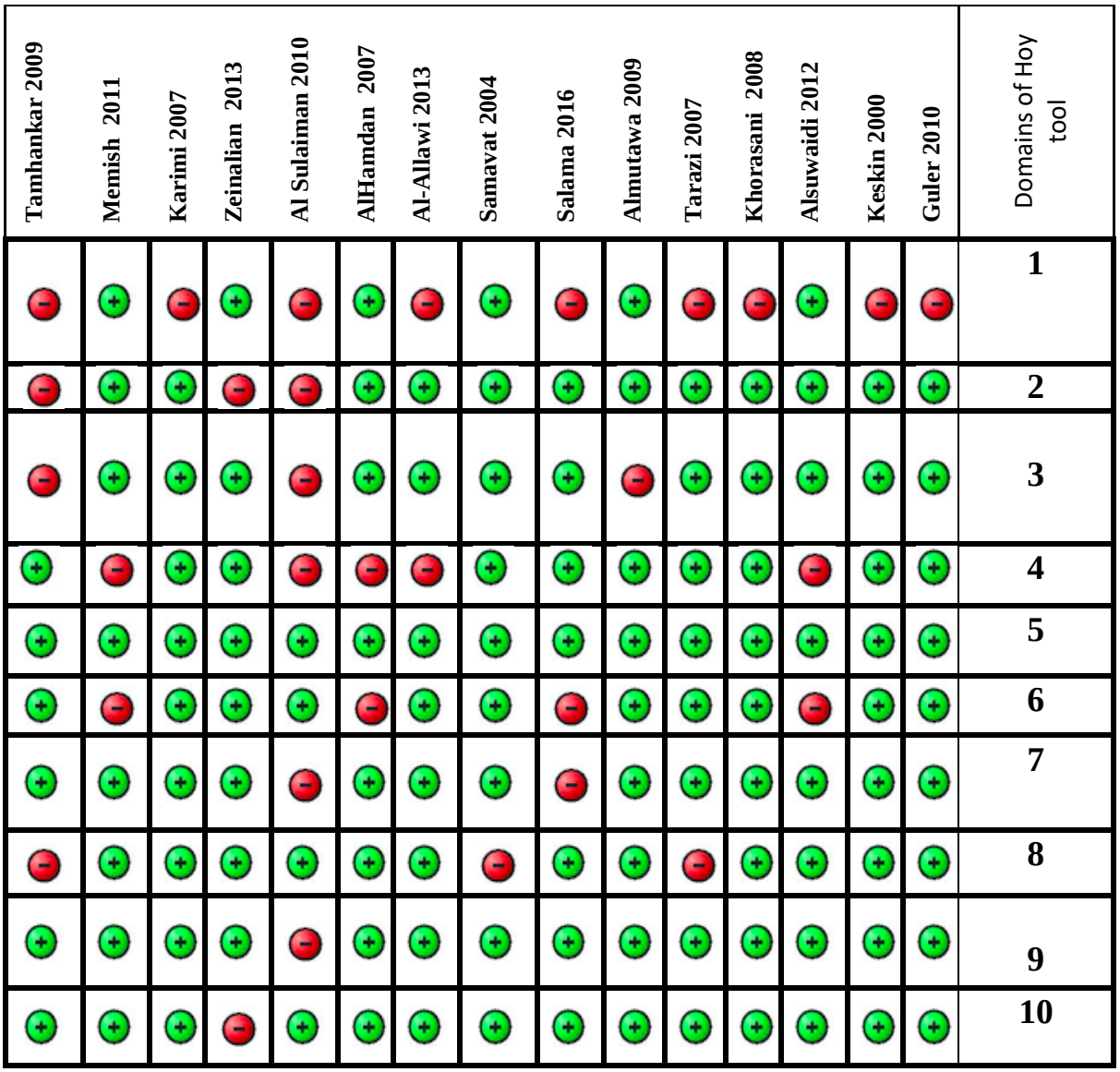

Figure 4: Summary of Risk of Bias for Each of the 15 Studies

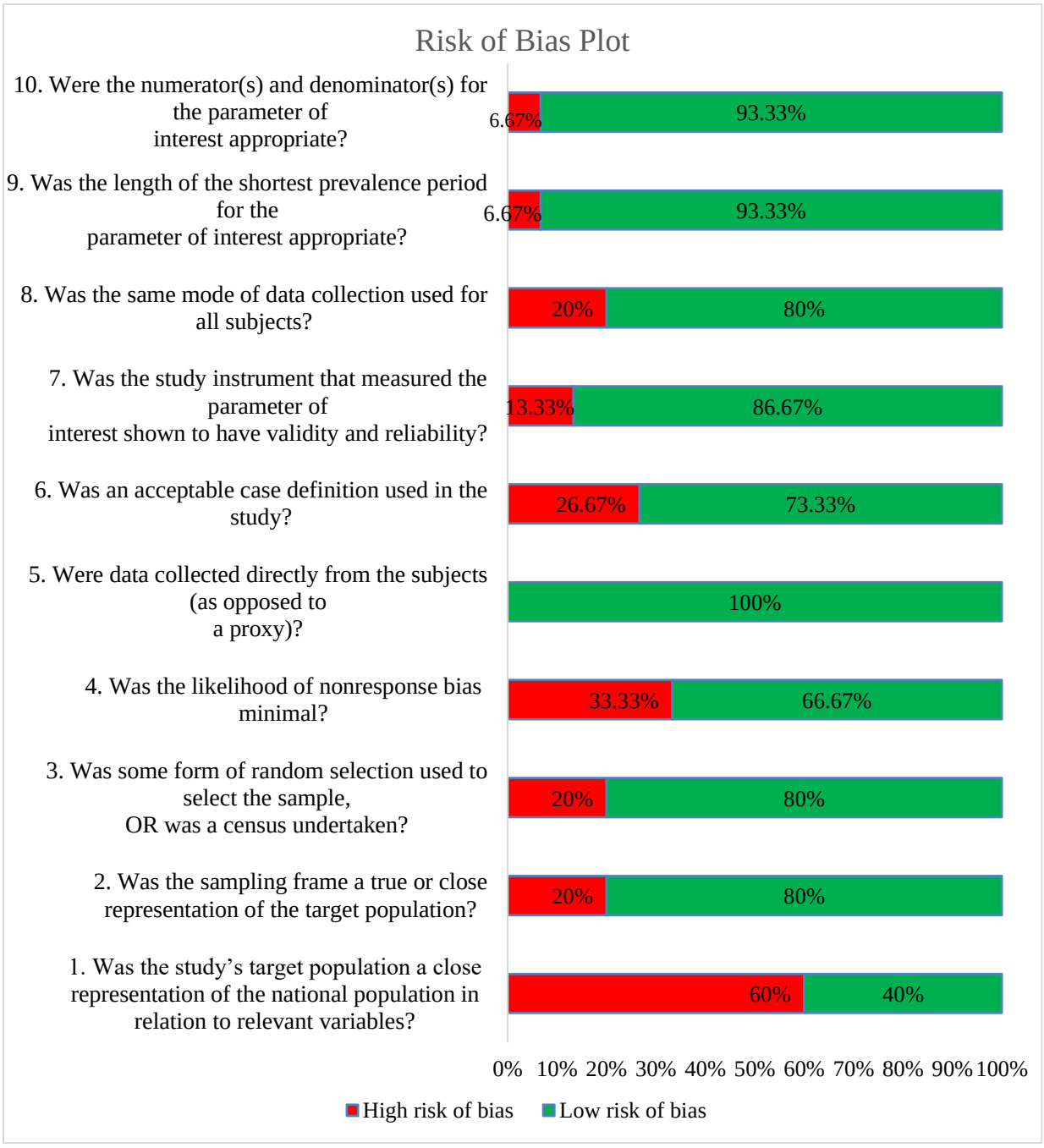

Figure 5: Percentages of Risk (low or high) across the Included Studies

4.4 Quantitative Synthesis

The 15 studies that were included in the systematic review were also used for the meta-analysis. The studies reported different proportion of at-risk marriages among couples who underwent premarital screening program. From these 15 studies, the total population (couples) screened were 5,866,490 and there were 33,540 couples at risk. Out of these at-risk couples, 21,331 couples were cases who married their partners despite undergoing counseling sessions.

Table 4 shows the prevalence of at-risk marriages among “incompatible couples” from each study included in the systematic review and meta-analysis.

Table 4: Prevalence of At-risk Marriages among "Incompatible Couples"

Author (year)	Country	Total couples screened (N)	No. of couples at-risk, n (%)	Couples at risk who married, n (%)	Couples at risk who cancelled marriage, n (%)
Alsuwaidi et al (2012)	Saudi Arabia	4,444	582	478 (82.13%)	104 (17.87%)
Memish et al (2011)	Saudi Arabia	78,6070	5370	3945 (73.46%)	1425 (26.54%)
Al Sulaiman et al (2010)	Saudi Arabia	129	129	127 (98.45%)	2 (1.55%)
AlHamdan et al (2007)	Saudi Arabia	207,333	2375	2128 (89.60%)	247 (10.40%)
Zeinalian et al (2013)	Iran	703,082	5729	4497 (78.50%)	1232 (21.50%)
Khorasani et al (2008)	Iran	279,936	3329	1503 (45.15%)	1826 (54.85%)
Karimi et al (2007)	Iran	1,038,371	5182	2798 (54%)	2384 (46%)
Samavat et al (2004)	Iran	2,729,000	10,298	5,431 (52.74%)	4867 (47.26%)
Salama et al (2016)	United Arab Emirates	8,913	28	28 (100%)	0
Almutawa et al (2009)	Bahrain	1,070	30	17 (56.67%)	13 (43.33%)
Al-Allawi et al (2013)	Iraq	54,132	130	105 (80.77%)	25 (19.23%)
Tarazi et al (2007)	Palestine	19,712	122	56 (45.90%)	66 (54.10%)
Guler et al (2010)	Turkey	24,062	17	3 (17.65%)	14 (82.35%)
Keskin et al (2000)	Turkey	9,902	15	13 (86.67%)	2 (13.33%)
Tamhankar et al (2009)	India	334	204	202 (99.02%)	2 (0.98%)

Using Quality Effects Model (QEM), results of forest plot indicated an overall pooled prevalence of marriages among couples at-risk of 64% (95% CI: 49%, 78%) with significant heterogeneity (Cochran's Q=3550, *p-value* <0.001, and I^2=100%). We can see that the percentage of Higgin's I^2 value is very high and it reflects the proportion of variance(heterogeneity) observed between studies due to the presence of real differences in effect sizes. Forest plot in Figure 6 shows that the prevalence estimates of some individual studies were much higher than the overall pooled estimate. For example, UAE had the highest prevalence of 100% (94%, 100%), followed by India and Saudi Arabia with a prevalence of 99% (CI: 97%, 100%) and 98% (CI: 95%, 100%) respectively.
On the contrary, the lowest prevalence was reported in Turkey which was 18% (CI: 3%, 40%) as shown in Figure 6.

Additionally, it was evident that the larger weights associated with some of the studies in the forest plot could be due to the method (QEM) used as well as large sample sizes from studies included in our meta-analysis. These large studies were conducted in Iran i.e. four studies (24,68–70) and two studies were carried out in Saudi Arabia (5,63).

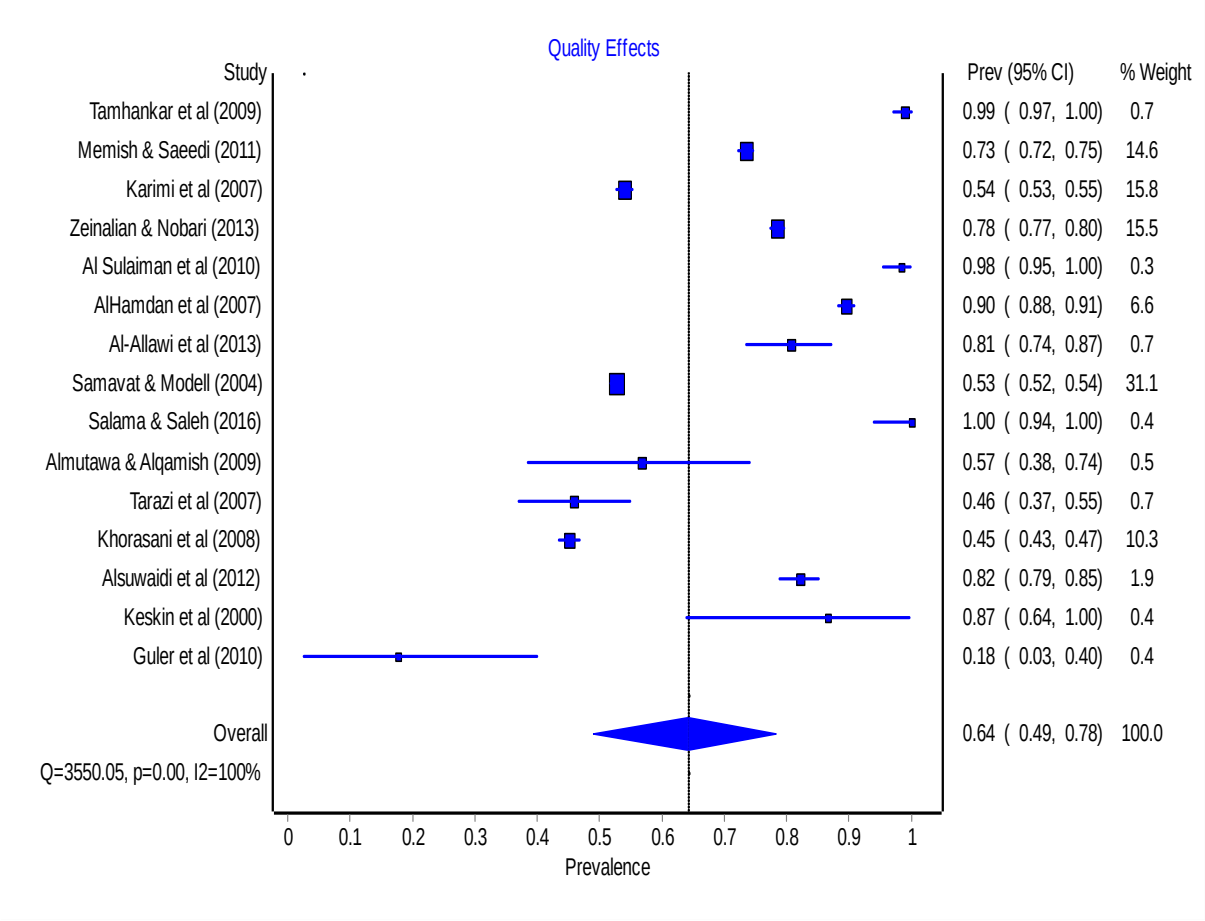

Figure 6: Forest Plot for Pooled Estimate Using Quality Effect Model

4.5 Heterogeneity Assessments

As stated earlier, Cochran's Q value and I^2 percentage were very high; therefore, sub-group analysis was conducted to investigate the sources of heterogeneity and to investigate if Cochran's Q value drops substantially and I^2 value decreases.

4.5.1 Sub-group Analysis

Subgroup analysis was conducted to investigate any possible sources of heterogeneity that might contribute to considerable amount of heterogeneity in terms of

Cochran's Q and Higgin's I^2 value. Included studies were separated through specific characteristics which were pre-specified in the protocol of review before inspecting the results of our meta-analysis. Studies were divided based on different categories such as risk of bias (low or intermediate), region, income level (high or middle), ethnicity (Arab vs non-Arab), sample size, study design, consanguinity proportion, diseases screened (β-thalassemia or SCD and β-thalassemia both), prenatal diagnosis data, and year of implementation of the program.

First, subgroup analysis was conducted between low risk and intermediate risk studies. The pooled prevalence estimate for low risk studies was 52% (CI: 46%, 57%) as compared to 79% (CI: 69%, 88%) for intermediate risk of bias studies. The Cochran's Q dropped to 89 (*p-value*<0.001) and an I^2 value of 94% for low risk studies while it was 378.5 (*p-value*<0.001) for intermediate risk studies with an I^2 value of 98%. Both numbers of Cochran's Q were less than the main pooled estimate's Q value (Q=3550, *p-value*<0.001).

Secondly, subgroup analysis was conducted according to region/country. The prevalence was highest for Gulf countries; it was 80% (CI: 64%, 94%) with a Q-value of 382 (*p-value*<0.001) and I^2 value of 99%. On the other hand, for Levant countries, the pooled prevalence was 65% (CI: 27%, 97%) with a drastic reduction in Cochran's Q value (Q=34.45, *p-value*<0.001) and I^2 value 97%. For Iran, the pooled prevalence was 58% (CI: 42%, 73%) with the highest Cochran's Q value (Q=1506.7, *p-value*<0.001; I^2 value =100%) compared to other regions. The lowest pooled prevalence was reported for Turkey i.e. 50% (CI:0%, 100%) with a substantial drop in Cochran's Q (Q=16.6, *p-value*<0.001)

and I^2 value of 94%. Lastly, for India, the pooled prevalence was 99% (CI: 97%, 100%).

For study design subgroup, there was a substantial drop in Cochran's Q in the subgroup of cross-sectional i.e. it was 71.75 (*p-value*<0.001) with I^2 value of 96% and pooled prevalence of 90% (CI: 71%, 100%). However, for retrospective population studies, the pooled estimate was 61% (CI: 47%, 74%) with high Cochran's Q value of 2050 (*p-value*<0.001) and I^2 value 100% which indicates that the source of such high heterogeneity was possibly due to studies with long term outcome assessment of the program.

Moreover, studies where the program was implemented before 2004 had a lower pooled prevalence of at-risk marriages i.e. 58% (CI: 42%, 73%) and I^2 value of 100% compared to countries where the program was implemented in 2004 or after with a pooled prevalence of 80%(CI: 65%, 93%). For the second subgroup (≥2004), there was a substantial drop in Cochran's Q, which was 382 (*p-value*<0.001) and I^2 value of 98%.

When studies were categorized based on income level, the pooled prevalence of at-risk marriages among high-income countries was 80% (CI: 64%, 94%) with a considerable reduction in Cochran's Q (Q=382, *p-value* <0.001) and I^2 value 99%. On the other hand, middle-income countries had lesser pooled estimate as compared to high income, i.e. 58% (CI: 42%, 75%) with a Cochran's Q of 1846 (*p-value*<0.001) and I^2 value 100%.

Furthermore, four of the included studies also reported consanguinity among couples at-risk. Two of those studies (66,72) reported consanguinity rate of 13.33% and 39.2% respectively. While the remaining two studies (61,64) had consanguinity rate of 55.67% and 70% respectively. Thus, studies were categorized into less than 50% consanguinity rate and those with 50% or more. The results indicated that those with less

than 50% consanguinity proportion had a pooled estimate of 81% (CI: 75%, 87%) with a Cochran Q value of 0.15 (*p-value*=0.70) and I^2 of 0%. Consequently, no heterogeneity was detected between studies of less than 50% consanguinity proportion. On the other hand, studies with consanguinity rate of 50% or greater had an overall pooled prevalence of 83% (CI: 41%, 100%), with a Cochran's Q value of 12.8 (*p-value*<0.001) and I^2 value 92%.

Studies were also categorized based on sample sizes (<2000 and ≥2000 couples at-risk). The prevalence was higher in studies with a sample size less than 2000 i.e. 81% (CI: 59%, 98%) compared to those with ≥2000 couples, where the prevalence of at-risk marriages was 63% (CI: 48%, 77%). There was a substantial drop in Cochran's Q value for studies with less than 2000 sample size i.e. it was 257.1 (*p-value*<0.001) and I^2 value of 97% indicating that most of the heterogeneity is coming from large studies with sample size 2000 or greater (Cochran's Q value= 2999, *p-value*<0.001 and I^2 value= 100%).

In terms of diseases screened among the study population, there were two categories i.e. those screened only for β-thalassemia and those that screened for both β-thalassemia along with SCD. The results from sub-group analysis showed a prevalence of 58% (CI: 41%, 75%) among couples screened for only β-thalassemia compared to 79% (CI: 63%, 93%) for studies that screened both β-thalassemia and SCD. There was a substantial drop in Cochran's Q value in the second subgroup that assessed SCD and β-thalassemia i.e. the value was 411(*p-value*<0.001) and I^2 value of 98%.

Furthermore, studies were also grouped according to ethnicity i.e. Arab vs non-Arab. Pooled prevalence estimate of Arab countries was found to be 79% (CI: 63%, 93%) which was higher than non-Arab countries where it was 58% (CI: 41%, 75%). Furthermore,

there was a substantial drop in Cochran's Q value for Arab subgroup with a Q value of 444 (*p-value*<0.001) and I^2 value 98%.

With regards to prenatal diagnosis data availability, studies were group into those where PND was available, and those where PND was not present or prenatal diagnosis was not done. The pooled prevalence for PND data subgroup was 59% (CI: 42%, 75%) while it was higher for studies where PND was not available with a pooled prevalence of 78% (CI: 60%, 93%). It could probably be explained by the presence of efficient PMS system as well as more utilization of PND services by the general public. There was a substantial drop in Cochran's Q value for second subgroup (Q=473, *p-value*<0.001) and I^2 value 99%.

Similarly, studies were also categorized into four levels based on outcome assessment years or length of study period. For countries with less than or equal to two years outcome assessment (or study period), the prevalence was highest compared to other categories i.e. 86% (CI: 70%, 98%), while for studies with three to five years outcome assessment, it was 54% (CI: 0%, 100%). The other categories were 6-10 years with pooled prevalence of 65% (CI: 44%, 84%) and 11 to 20 years with pooled prevalence of 66% (CI: 29%, 97%). There was a substantial drop in Cochran's Q value in the first three sub-categories. For first category, it dropped to 103 with *p-value*<0.001 with I^2 value 96%.; for second category it dropped to 390 (*p-value*<0.001) with I^2 value 99%; whereas for third category, it dropped to 474 (*p-value*<0.001) and I^2 value 100%..

4.5.1.2 Testing Significance between Subgroups:

In order to observe the statistically significant differences between subgroups, overlapping of confidence intervals were compared. A study (75) conducted by Cumming (2009) stressed the importance of accurate reporting of 95% confidence interval (CI) bars

of two independent means as researchers misinterpret the overlapping results. In other words, the author clarified that if two confidence intervals nearly touch, *p-value* can be about 0.01. Additionally, *p-value* can only reach to 0.05 when about half the length of one confidence interval arm overlaps with the arm of other CI (75).

Figures 7-18 below show the confidence intervals for all subgroups mentioned above to inspect statistically significant differences between the subgroups. This helps in understanding factors that could be associated with the heterogeneity observed in our study. The risk of bias groups were compared (Figure 7), and there was statistically significant difference between studies with low risk and intermediate risk of bias.

With respect to region, India was statistically significantly different than other regions (Figure 8). Additionally, subgroups like ethnicity (Arab/non-Arab), income level and diseases screened were marginally significant (Figures 9, 10 &15). Confidence intervals of sample size and PND data availability showed overlapping (Figures 11 &12), i.e. overlapping by more than half the length of one CI arm; therefore, there was no significant difference between subgroups of these factors. In contrast, in subgroups pertaining to length of outcome assessment, studies with period 2 years or less were significantly different than other categories (Figure 13).

Similarly, there was statistically significant difference in studies with less than 50% consanguinity compared to those with 50% or greater (Figure 14). The remaining subgroups significantly different were; study design (Figure 16) and implementation year (Figure 17).

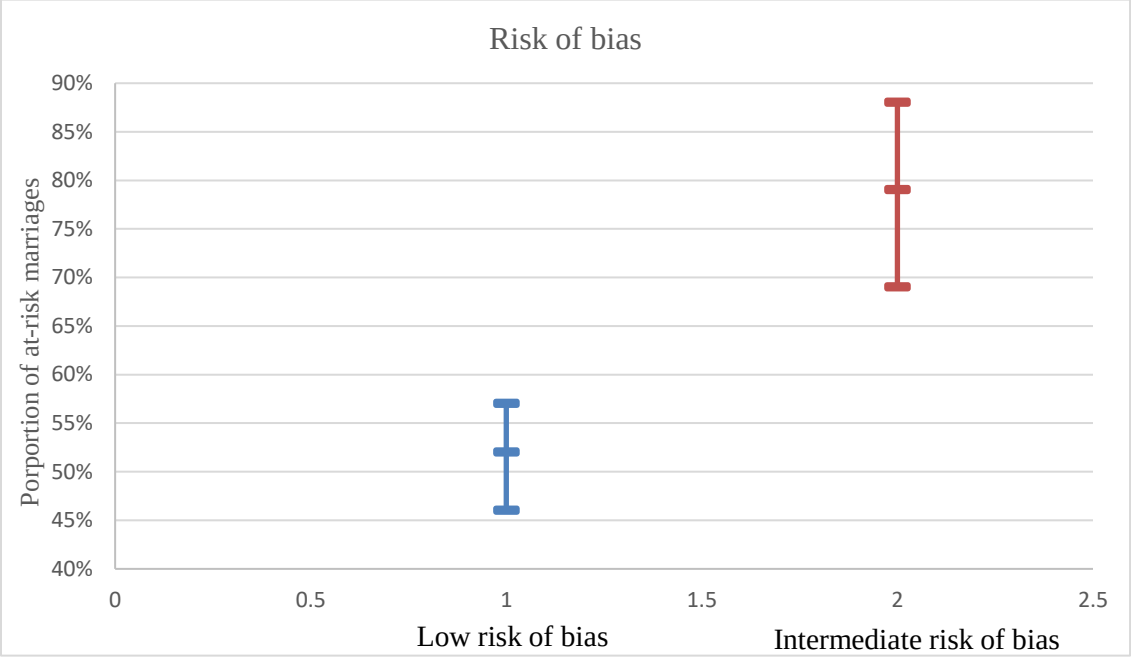

Figure 7: Confidence Interval Plot for Risk of Bias Subgroup

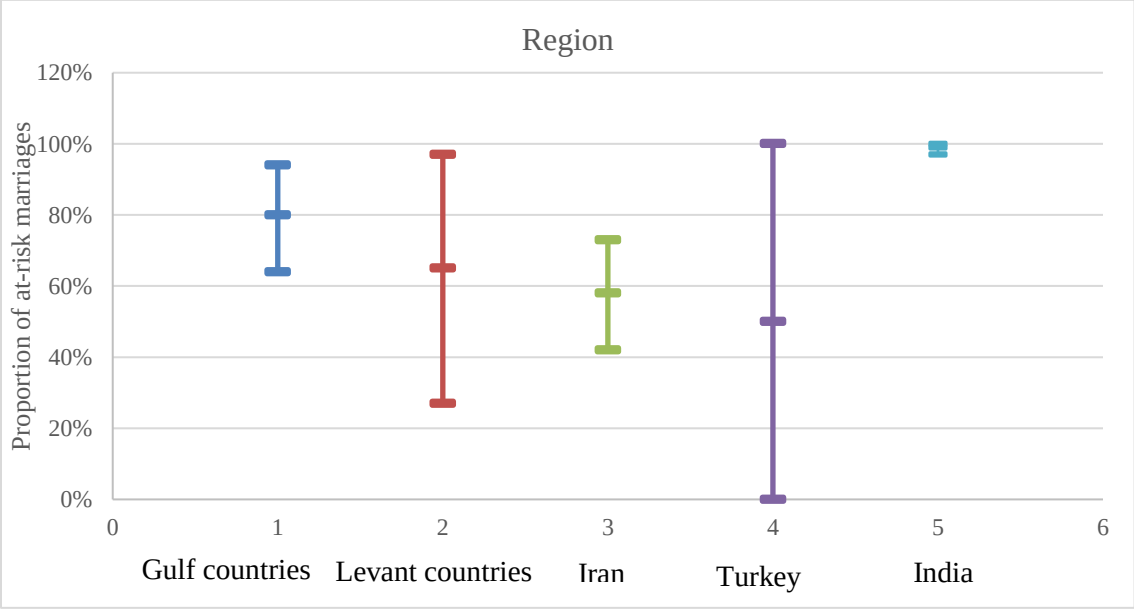

Figure 8: Confidence Interval Plot for Region/country Subgroup

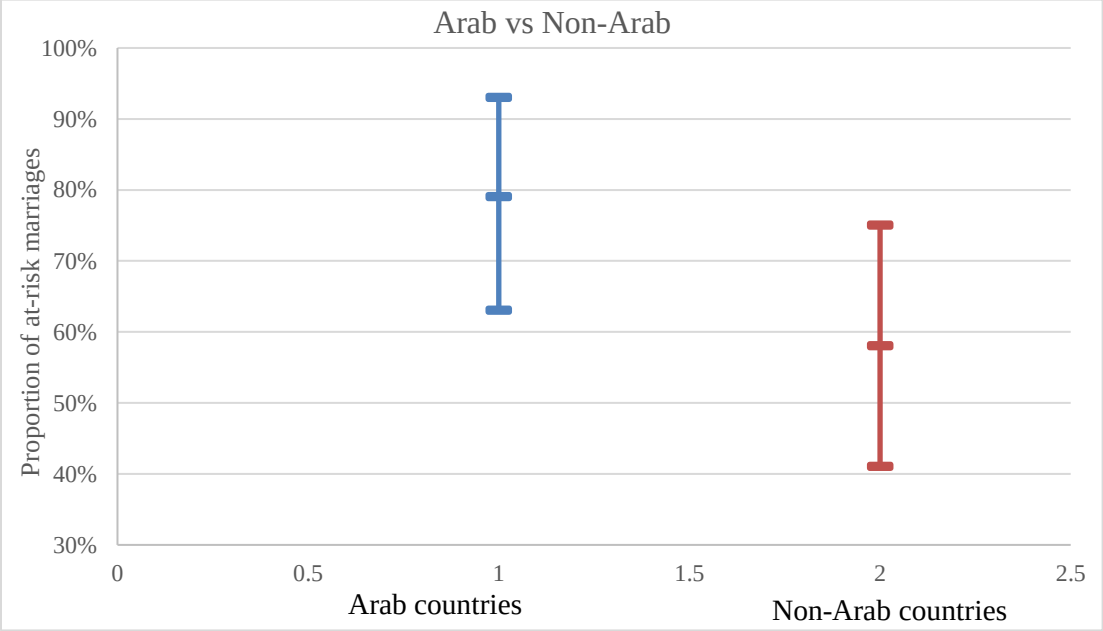

Figure 9: Confidence Interval Plot for Ethnicity Subgroup

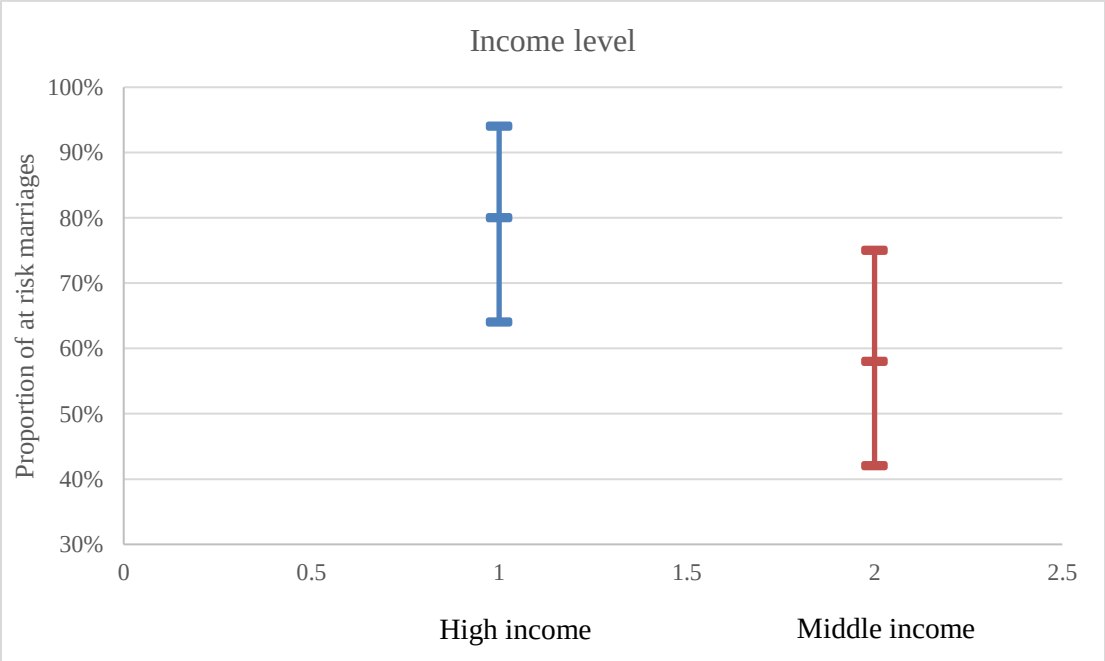

Figure 10: Confidence Interval Plot for Income Level Subgroup

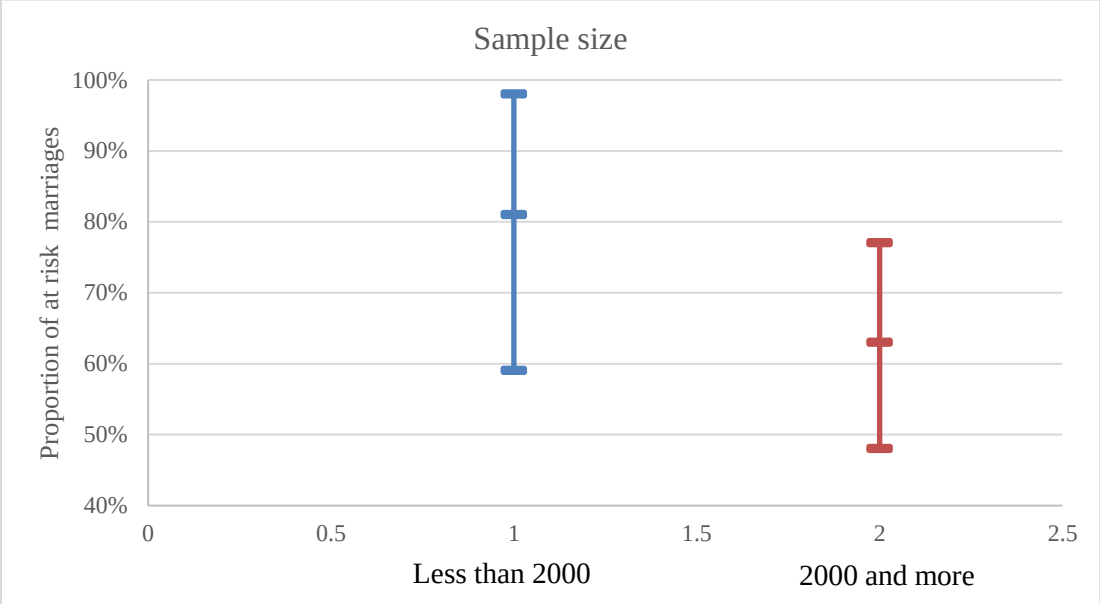

Figure 11: Confidence Interval Plot for Sample Size Subgroup

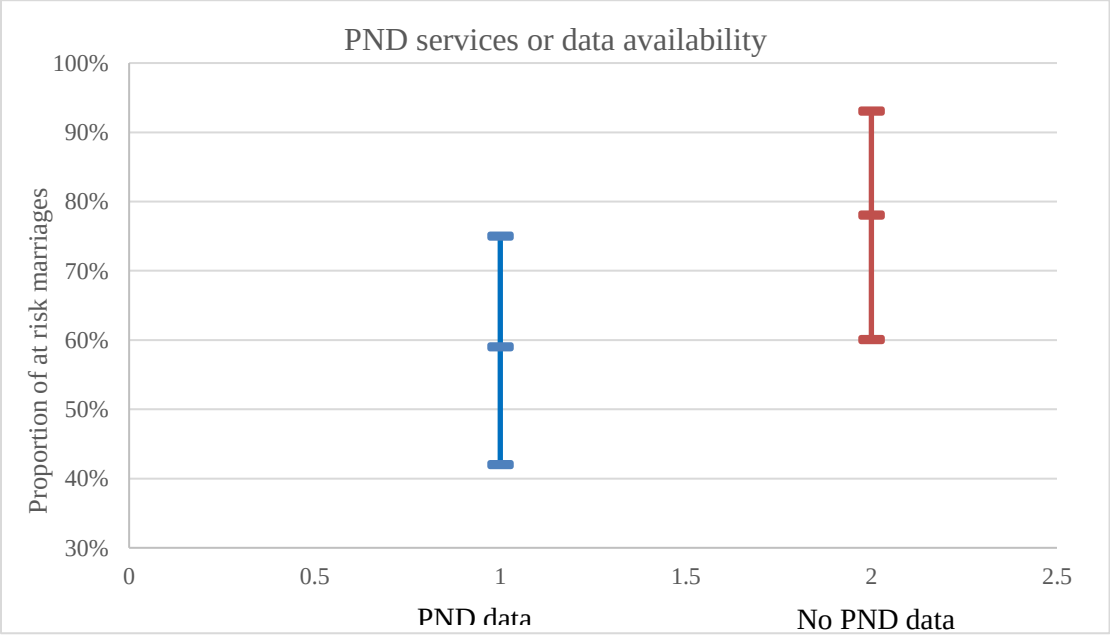

Figure 12: Confidence Interval Plot for PND Subgroup

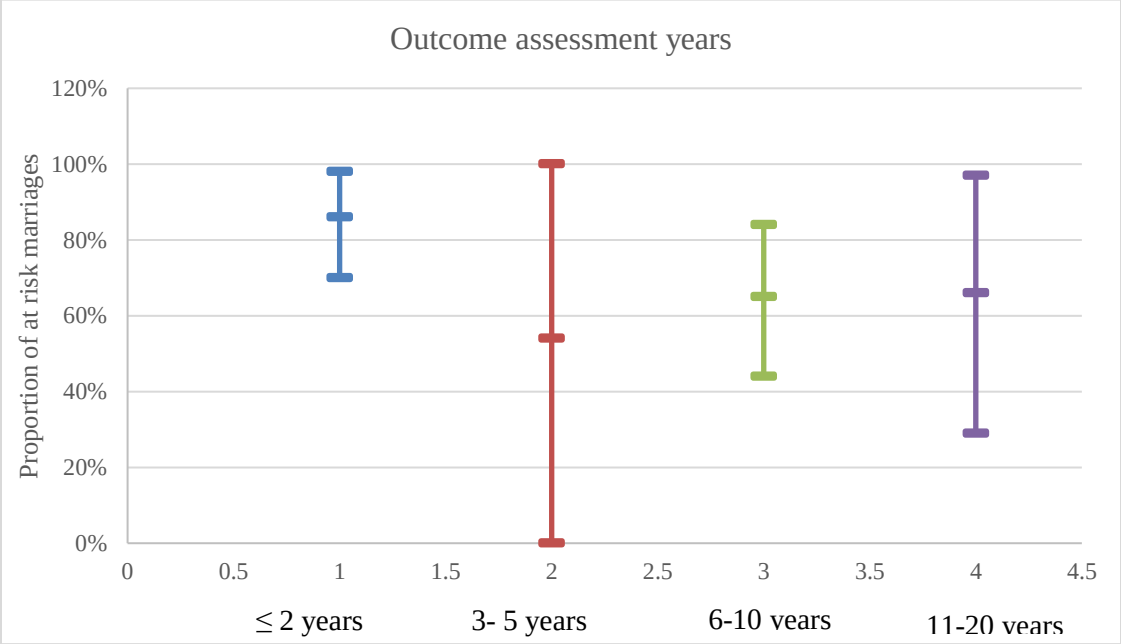

Figure 13: Confidence Interval Plot for Length of Outcome Assessment (years) Subgroup

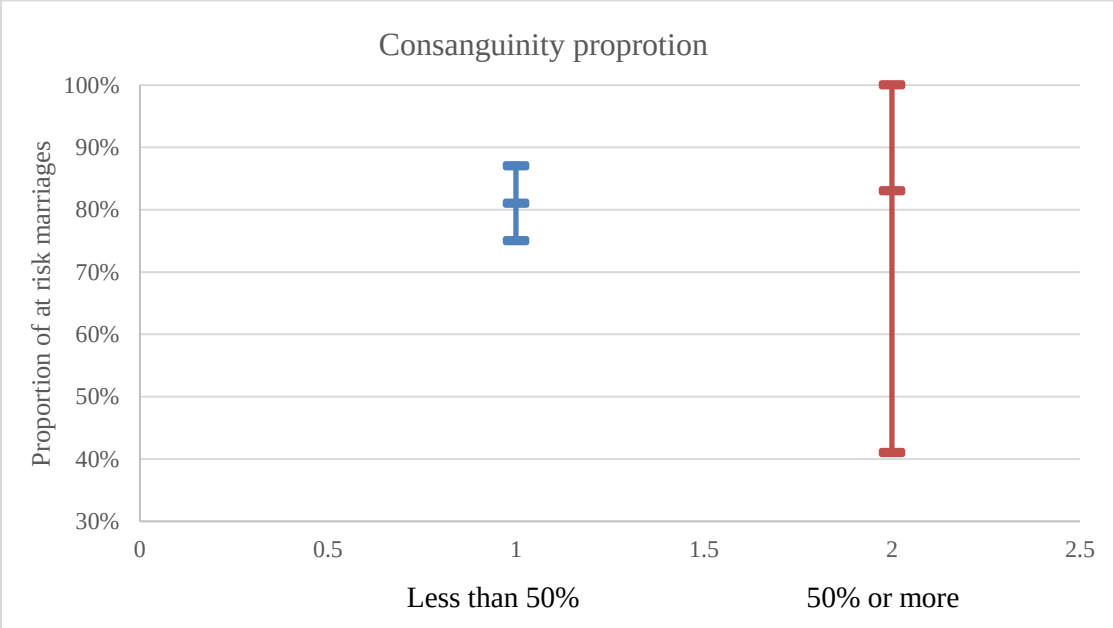

Figure 14: Confidence Interval Plot for Consanguinity Proportion Subgroup

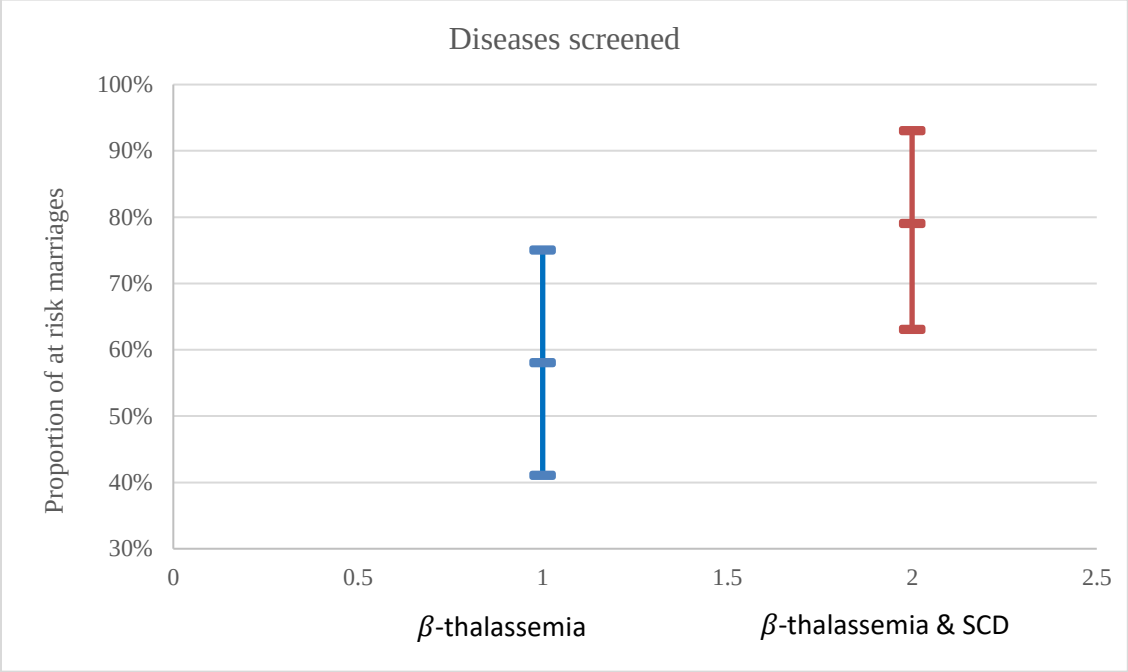

Figure 15: Confidence Interval Plot for Diseases Screened Subgroup

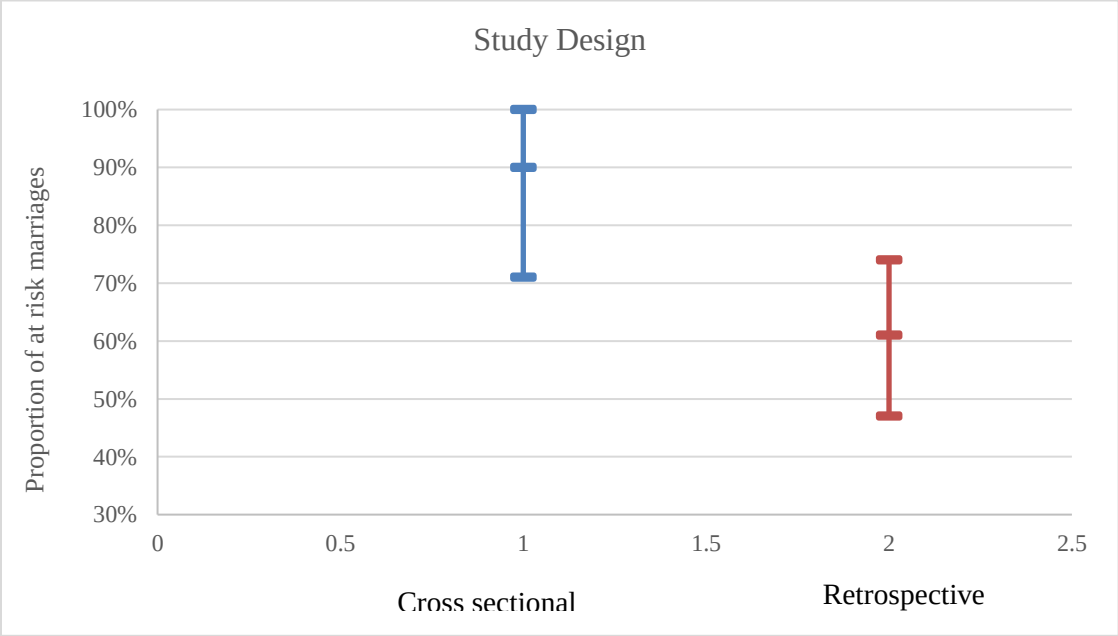

Figure 16: Confidence Interval Plot for Study Design Subgroup

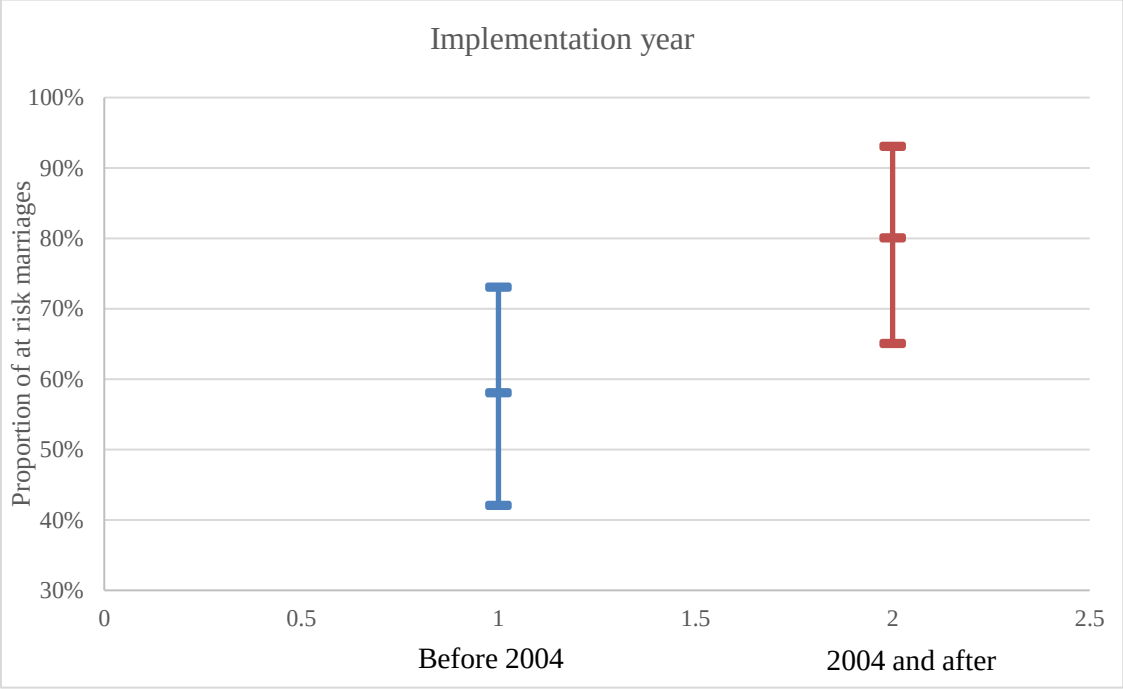

Figure 17: Confidence Interval Plot for Implementation Year Subgroup

4.5.2 Random Effect Model Estimates

As noted earlier, all the estimates provided above are based on quality effects model (50) because they are suitable for adjusting bias in individual studies. Nonetheless, random effects model is commonly used model recommended by Cochrane as an analytical approach when heterogeneity is found between studies included in meta-analysis (76). It basically allows for heterogeneity by assuming that underlying effect sizes follow a normal distribution. It argues that different studies are estimating different effect sizes, but they are related. The model assumes that the observed estimates of effect size can vary across studies due to the presence of real differences in the effect sizes in each study as well as sampling variability (chance). Therefore, even if all the included studies have large sample

sizes, the observed effect sizes would still vary across studies due to presence of the real differences in study population in terms of factors like age, length of follow-up and interventions received (76).

For the purpose of this study, we used REM as a sensitivity analysis, but our main findings were based on QEM. Table 5 illustrates comparison between Quality and Random Effects models in terms of pooled prevalence and heterogeneity by using Higgin's value i.e. I^2.

Apparently, the pooled prevalence of at-risk marriages using QEM and REM was 64% (CI: 49%, 78%) and 75% (CI: 66%, 83%) respectively. It was found that the I^2 value were almost similar for both models in all subgroups. Overall, there were lack of wide differences between the prevalence values yielded by the two models and this supports the validity of our results in this meta-analysis. Examples of subgroups with the least differences in prevalence in REM and QEM models included; region, income level, ethnicity, prevalence, diseases screened, study design, sample size and implementation year.

Table 5: Sub-group Analysis of Included Studies using QEM and REM

	Quality Effects Model			Random Effects Model		
	No. of Studies (couples at risk)	Prevalence (95% CI)	I^2	No. of Studies (couples at risk)	Prevalence (95% CI)	I^2
All eligible studies	15 (33,540)	64% (CI: 49%, 78%)	100%	15 (33,540)	75% (CI:66%, 83%)	100%
Risk of Bias						
Low ROB	6 (18,871)	52% (CI: 46%, 57%)	94%	6 (18,871)	69% (CI:61%, 78%)	99%
Intermediate ROB	7 (14,336)	79% (CI: 69%, 88%)	98%	7 (14,336)	80% (CI:73%, 86%)	98%
Region/country						
Gulf countries	6 (8514)	80% (CI: 64%, 94%)	99%	6 (8514)	87% (CI: 77%, 94%)	99%
Levant countries	2 (252)	65% (CI: 27%, 97%)	97%	2 (252)	64% (CI: 26%, 97%)	97%
Iran	4 (24,538)	58% (CI: 42%, 73%)	100%	4 (24,538)	58% (CI: 43%, 72%)	100%
Turkey	2 (32)	50% (CI: 0%, 100%)	94%	2 (32)	52% (CI: 0%, 100%)	94%
India	1 (204)	99% (CI: 97%, 100%)	-	1 (204)	99% (CI: 97%, 100%)	-
Ethnicity						
Arab	8 (8,766)	79% (CI: 63%, 93%)	98%	8 (8,766)	82% (CI: 72%, 90%)	98%
Non-Arab	7 (24,774)	58% (CI: 41%, 75%)	100%	7 (24, 774)	66% (CI: 53%, 77%)	100%
Income level						
High Income	6 (8,514)	80% (CI: 64%, 94%)	99%	6 (8,514)	87% (CI:77%, 94%)	99%
Middle Income	9 (25,026)	58% (CI: 42%, 75%)	100%	9 (25,026)	65% (CI:55%, 76%)	100%

	Quality Effects Model			Random Effects Model		
	No. of Studies (couples at risk)	Prevalence (95% CI)	I^2	No. of Studies (couples at risk)	Prevalence (95% CI)	I^2
Implementation year						
Before 2004	7 (24,692)	58% (CI: 42%, 73%)	100%	7 (24,692)	55% (CI: 43%, 67%)	100%
2004 and after	7 (8,644)	80% (CI: 65%, 93%)	98%	7 (8,644)	86% (CI: 77%, 93%)	98%
Length of Outcome Assessment (years)						
≤2 years	5 (3133)	86% (CI: 70%, 98%)	96%	5 (3133)	78% (CI: 66%, 89%)	96%
3-5 years	5 (10,769)	54% (CI: 0%, 100%)	99%	5(10,769)	76% (CI: 48%, 98%)	99%
6-10 years	3 (10,580)	65% (CI: 44%, 84%)	100%	3 (10,580)	77% (CI: 61%, 91%)	100%
11-20 years	2 (9,058)	66% (CI: 29%, 97%)	100%	2 (9,058)	63% (CI: 27%, 95%)	100%
Consanguinity (among at-risk)						
<50%	2 (145)	81% (CI: 75%, 87%)	0%	2 (145)	81% (CI: 74%, 87%)	0%
≥50%	2 (610)	83% (CI: 41%, 100%)	92%	2 (610)	93% (CI: 69%,100%)	92%
Diseases screened						
β-thalassemia	6 (24,864)	58% (CI: 41%, 75%)	100%	6 (24,864)	66% (CI: 53%, 78%)	100%
β-thalassemia & SCD	9 (8,676)	79% (CI: 63%, 93%)	98%	9 (8,676)	81% (CI: 72%, 89%)	98%

	Quality Effects Model			Random Effects Model		
	No. of Studies (couples at risk)	Prevalence (95% CI)	I^2	No. of Studies (couples at risk)	Prevalence (95% CI)	I^2
Prenatal Diagnosis						
Yes	7 (24,887)	59% (CI: 42%, 75%)	100%	7 (24,887)	73% (CI: 61%, 83%)	100%
No	8 (8,653)	78% (CI: 60%, 93%)	99%	8 (8,653)	77% (CI: 66%, 86%)	99%
Study Design						
Cross sectional	4 (2,738)	90% (CI: 71%, 100%)	96%	4 (2,738)	91% (CI: 80%, 99%)	96%
Retrospective	10 (30,220)	61% (CI: 47%, 74%)	100%	10 (30,220)	65% (CI: 56%, 74%)	100%
Sample Size						
<2000	9 (1,257)	81% (CI: 59%, 98%)	97%	9 (1,257)	80% (CI: 64%, 93%)	97%
≥2000	6 (32,283)	63% (CI: 48%, 77%)	100%	6 (32,283)	67% (CI: 53%, 79%)	100%

4.6 Sensitivity Analysis

The primary analysis of overall pooled prevalence of at-risk marriages among couples receiving premarital counseling was repeated using QEM by including only low risk studies in order to estimate its prevalence. Hence, a total of 6 low-risk studies (24,27,69–72) were included. The results indicated that the pooled estimate of high-quality studies was 52% (CI: 46%, 57%). In addition, heterogeneity decreased by a considerable amount in Cochran's Q, i.e. 89.55 (*p-value*<0.001) and I^2 value of 94% which was still high. (Figure 18)

Furthermore, when 3 of the small-size studies (27,71,72) were excluded from this plot, the overall pooled estimate did not change, i.e. 52% (CI: 47%, 57%). Cochran's Q value reduced further (Q= 72.65; *p-value*<0.001) with an I^2 value of 97%. (Figure 19)

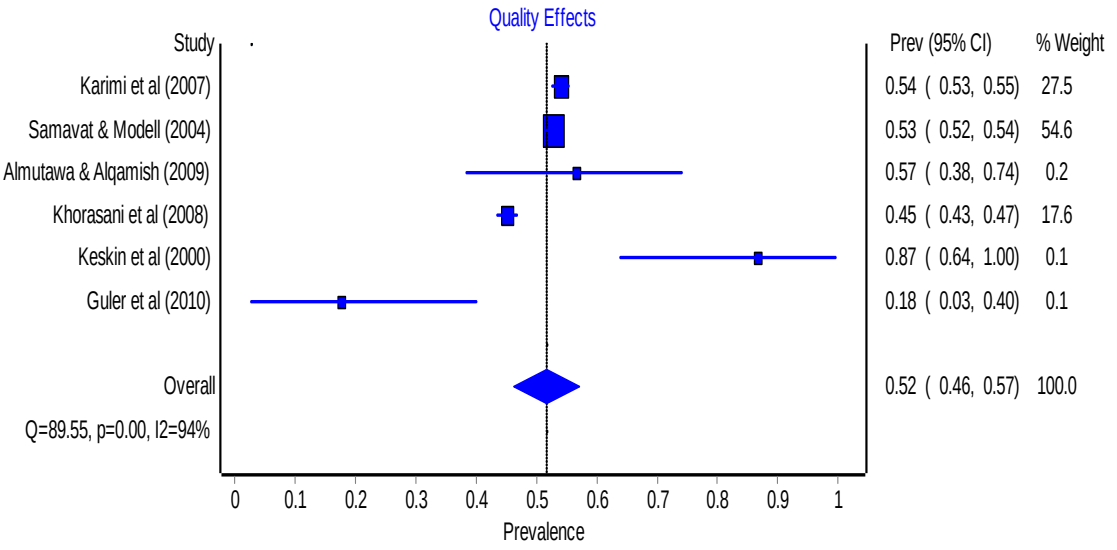

Figure 18: Forest Plot Using Sensitivity Analysis (with Low-Risk Studies)

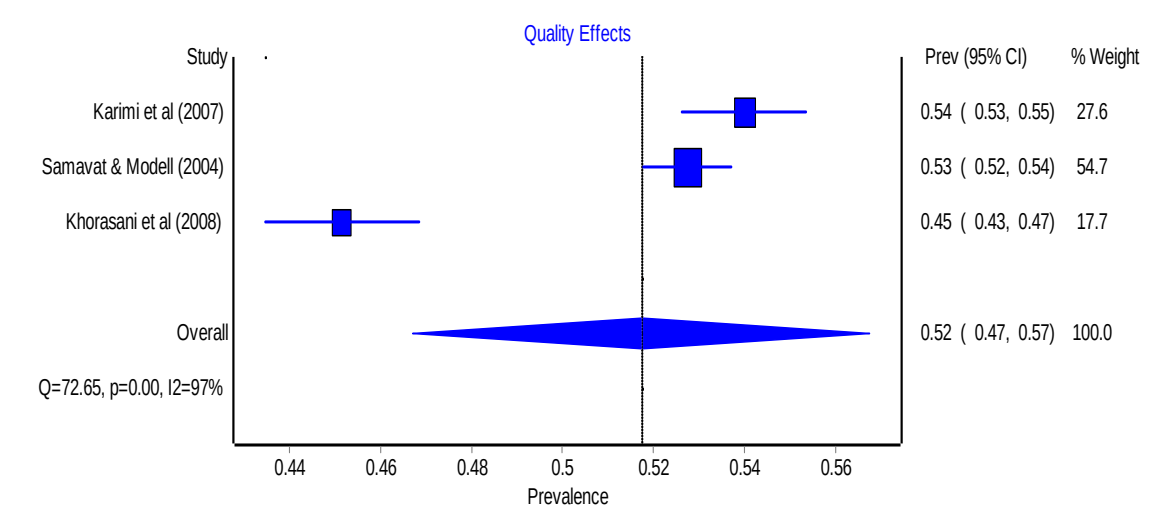

Figure 19: Forest Plot using Sensitivity Analysis (Large Studies with Low Risk)

4.7 Publication Bias

The publication bias, of the included studies in our meta-analysis, was examined using Funnel plot and Doi plot. Funnel plots show scatter plots of the study's effect estimates against sample size, could be useful in assessing the validity of meta-analyses. It is based on increase in the precision in estimating the underlying effect size with increase in the sample size of the study (77). This means that distribution of small studies scatters widely at the bottom of the graph while larger studies will be narrow at the top of the graph. When there is publication bias, the funnel plots are usually skewed and asymmetrical (77).

Hunter et al (2014) argued that classical funnel plots are not reliable methods for proportion measures (78). He, therefore, suggested to provide an alternative approach which are funnel plots including study size against log-odds, thus it could be more accurate estimate for prevalence studies. Funnel plot in Figure 20 shows that it is asymmetrical

mostly to the right side. In addition, due to subjective nature of scatter plots in funnel plots, a new method of observing publication bias was developed through a Doi plot. It replaces the conventional scatter plot with a normal quantile plot that makes up the limbs of plot through the study data (60). It provides an *LFK* index that quantifies the extent of asymmetry i.e. if value of *LFK* index is closer to zero, the Doi plot would be symmetrical. On the other hand, values of *LFK* beyond -1 and +1 are regarded as asymmetrical (60). As shown in Figure 21, Doi plot indicates asymmetry with an *LFK* value of 4.94 demonstrating publication bias.

When sensitivity analysis was done only for studies with low risk of bias (six studies), it was apparent from the plot, that there was no major publication bias. Small studies are expected to scatter more widely near the x-axis, i.e. scattered on the base of the inverted funnel (77). On the other hand, large studies are presented on the top as shown in Figure 22. The funnel plot shows that the large studies are close together towards the top of the inverted funnel thus indicating no publication bias. When Doi plot was assessed, there was no asymmetry with an *LFK* value of 0.96 thus concluding no publication bias (Figure 23).

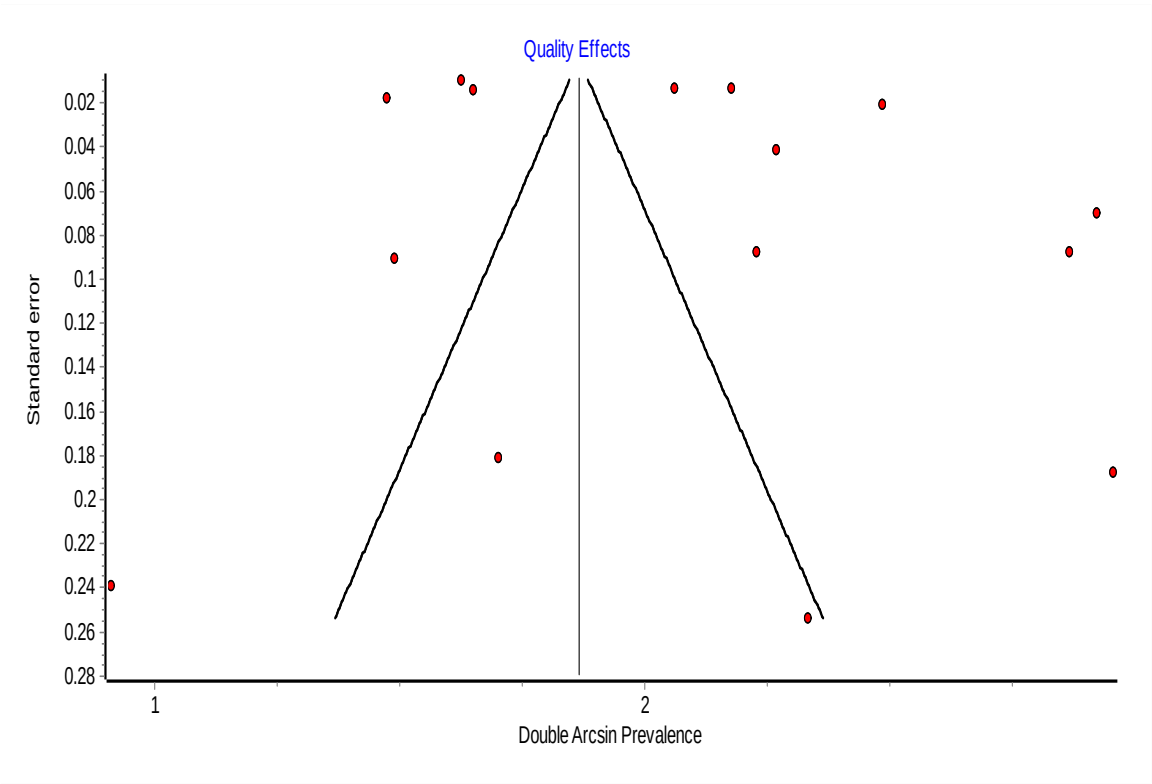

Figure 20: Funnel Plot Assessing Publication Bias (All 15 Studies)

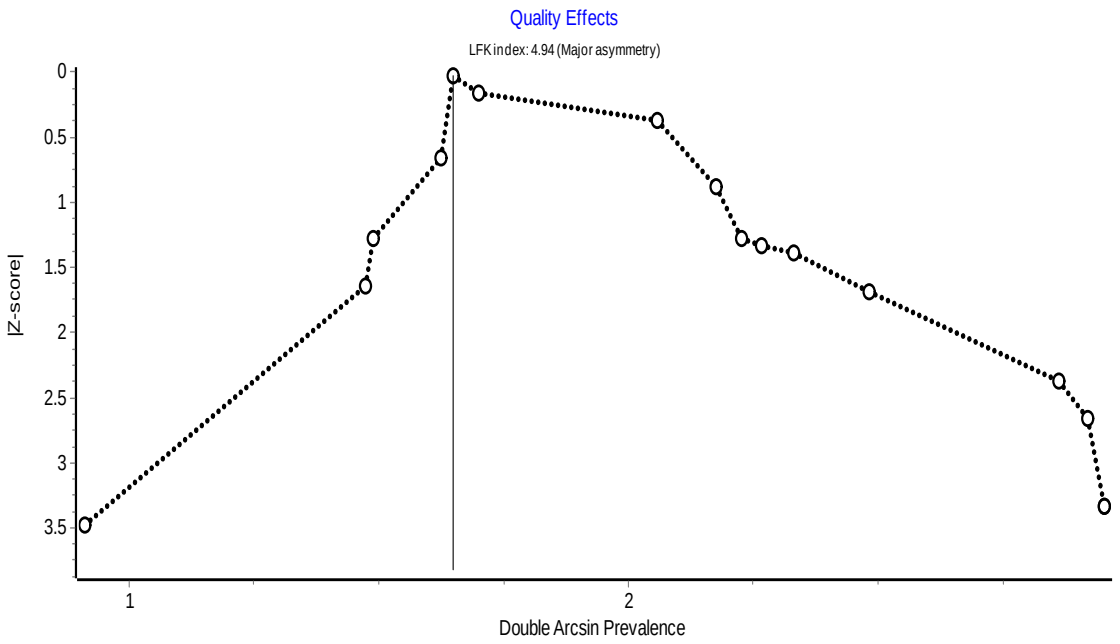

Figure 21: Doi Plot Assessing Publication Bias (All 15 Studies)

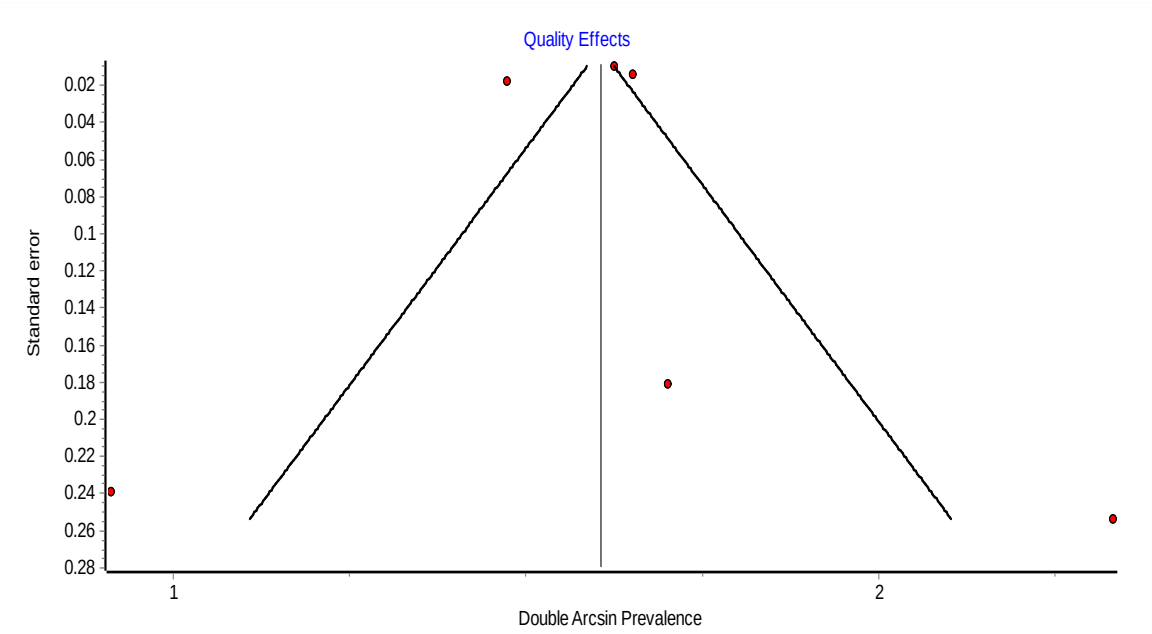

Figure 22: Funnel Plot for Sensitivity Analysis (Only Low Risk Studies)

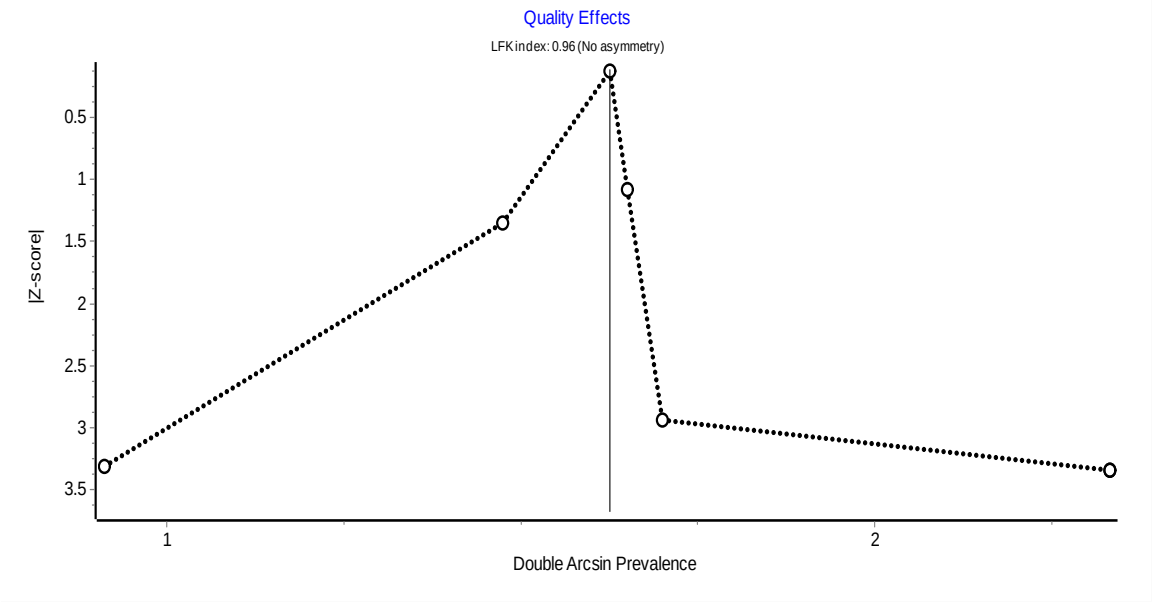

Figure 23: Doi Plot for Sensitivity Analysis (Only Low-Risk Studies)

CHAPTER 5: DISCUSSION

This is the first meta-analysis that estimated the prevalence of at high-risk marriages among high risk couples of hemoglobinopathies globally and regionally. In our study, the estimated global prevalence of at high-risk marriage of hemoglobinopathies was 64%; however, this varies widely across different regions and income level.

Our results revealed that high-income countries including Saudi Arabia and UAE had higher prevalence of at-risk marriages (pooled prevalence of 80%) compared to middle income countries (i.e. 58%) but results were not significant. In addition, based on region, estimates where the program was implemented before 2004 i.e. Iran, Turkey and Palestine had lower pooled prevalence of at-risk marriages compared to other countries of GCC and India. It could probably be explained by the fact that countries, where the PMS programs were implemented before 2004 (from 1995 to 2000), might have perceived the issue of high prevalence of at-risk marriages earlier as compared to countries where it was implemented in 2004 and after. Therefore, some amendments could have been made to the existing programs to increase the compliance rates.

The results of this meta-analysis are consistent with a scoping review (3) conducted by Saffi & Howard (2015) to evaluate the effectiveness of Premarital screening (PMS) programs in the Middle East. It concluded that PMS programs were not successful in reducing at-risk marriages by at least 65% (3). The scoping review by Saffi & Howard (2015) is based on studies in the Middle East region including narrative reviews. Furthermore, scoping review stated that the percentage of cancellation of at-risk marriages due to mandatory premarital screening was 53% in Turkey (3).

Similarly, evidence from review study in Jordan showed that PMS program discouraged 40% of at-risk marriages (16). In contrast, another review study in Jordan indicated that out of 48 at-risk couples identified in 2006, only three (6%) of them cancelled marriage plans (79). Moreover, after the implementation of PMS program in Saudi Arabia, about half of at-risk couples (51.9%) cancelled their marriage plans while the remaining half (48.1%) proceeded with their marriages (63). Similarly, results from a cost analysis study (80) in the province of West Azerbaijan in Iran show that during the period 2002-2006, the average rate of marriage cancellation among at-risk couples was 53% with a minimum rate of 38% in 2003 and a maximum rate of 69% in year 2006.

In light of the above mentioned problem, a recent study published in 2018 provided recommendations to reduce the incidence of genetic diseases on a national level (81). According to this study, health promotion and disease prevention efforts should be improved for those able to transmit genetic disorders. A follow up program after marriage is needed to ensure that couples acquire sufficient knowledge (81).

Similarly, data from the studies included in our meta-analysis indicates that there is still a wide gap to be filled to turn these programs effective. These programs are proved to be successful in screening the vast majority of eligible individuals and identifying the couples at risk while providing them education. However, it remains challenging in terms of successful reduction in the incidence of hemoglobinopathies (beta-thalassemia and sickle cell disease) due to inadequate compliance of at-risk couples with the counseling decision. These could be due to various factors such as culture, religion, preference of consanguinity among certain populations, quality of counseling sessions provided during PMS programs as well as less awareness of genetic diseases like hemoglobinopathies and

its severity among the general public.

The results of sub-group analysis in this meta-analysis showed that countries where the program was implemented before 2004 had a pooled prevalence of 58% (CI: 42%, 73%). This percentage is lower than those countries where the program was implemented in 2004 or after (pooled prevalence was 80% with CI: 65%, 93%). The examples of countries where it was implemented before 2004 are Iran, Turkey, Palestine (Gaza strip). On the other hand, it may take some time for countries in Gulf, Iraq and India to reduce the percentage of at-risk marriages.

In terms of investigating the reasons of marriage among high risk couples, a follow up study in Saudi Arabia (62) revealed that ”previous agreement and family pressures” was the most frequently reported reason, and was reported by 48% of the participants. Similarly, it was followed by “the only one in my mind” and was reported by 34% of the participants. Additionally, the majority of these participants had received counseling from general physicians, while a substantial number of couples had received counseling from other staff who did not have adequate knowledge of the diseases screened. On the other hand, some participants did not receive counseling at all (62).

Moreover, a study in Iran (68) evaluated the impact of premarital screening program over two decades. It reported the frequency of causes for the major β-thalassemia births that happened during 1997-2010. It was stated that 51.4% of the reported major beta-thalassemia cases were due to marriages that happened before starting the program. On the other hand, 14.7% of the cases were due to culture-related reasons while 9.2% of the cases were due to genetic counseling inadequacy(68).

The presence of prenatal diagnosis during pregnancy is another factor that impacts the decision of at-risk couples (82). For example, Cyprus had started the screening of beta-thalassemia and genetic counseling in 1980 but record shows that as they started prenatal diagnosis program in 1984, the number of affected births dropped dramatically from 18-20 to 6-7 per year (82).

Similarly, it has been estimated (24) that in Iran before legalizing the prenatal diagnosis and pregnancy termination, about half of the at-risk couples proceeded with the marriage while the other half separated. The separation of these high-risk couples helped in reducing the incidence of β-thalassemia (24).This indicates that the access of population to services like PND and pregnancy termination will more likely have a positive impact on reduction of hemoglobinopathies. On the other hand, this might not always be a positive factor towards reduction in the incidence of hemoglobinopathies. For example, in Saudi Arabia, there has been little change in the incidence of β-thalassemia, and the practice of prenatal diagnosis and pregnancy termination has not been widely spread. However, it is speculated that the incidence of thalassemia in Saudi Arabia may reduce, as pre-implantation genetic diagnosis is being viewed as a popular alternative (21).

The results of our meta-analysis indicated that prevalence of at high-risk marriages was lower in countries where prenatal diagnosis was provided to couples i.e. 59% (CI:42%, 75%) compared to countries where it was not provided, or no data was available i.e. 78% (CI: 60%, 93%). For example, studies from Iran, Turkey, and Iraq had reported PND data and the prevalence of at high-risk marriages were 58%, 50% and 81% respectively.

The approach of general population to actively participate in seeking services like

PND also depends on the country's prevailing healthcare systems. In a poor resource setting like Nigeria, that has a high disease burden in terms of SCD and other hemoglobinopathies, a recent study (83) reported that due to huge financial implications for raising a SCD-affected child which is even worsened by co-existence of health care financing based on out-of-pocket expenditure, SCD heterozygous women(63%) most commonly preferred the termination of pregnancy(TOP) after undergoing genetic counseling and testing. Nonetheless, in another study (84) when the attitudes of Nigerian at-risk couples for having offspring with SCD were evaluated, it was shown that two-third (66.67%) of them would call off their marriage if their blood results were not compatible. In other words, this implies that around 33.3% of couples would still proceed with the marriage decision after knowing their carrier status. This proportion is found much lower in contrast to our meta-analysis estimate in terms of middle income (lower or upper middle) countries, where the prevalence of at-risk marriages was 58% (CI: 42%, 75%) as compared to high-income countries with a much higher prevalence of 80% (CI: 64%, 94%).

On the other hand, in China where common diseases extensively screened are hemoglobinopathies, hepatitis B virus (HBV), HIV including physical examination done for all prospective couples. Interestingly, PMS programs at the national level is seen as an ideal forum for important health interventions(85). For example, it helps in increasing the uptake of folic acid by premarital women thereby decreasing the prevalence of neural tube defects. Also, PMS is seen as a good educational opportunity to reduce the exposure of future children to second-hand smoking by their fathers (85).

Nonetheless, in a region like Middle East, there could be several reasons for the poor performance of PMS programs. In this regards, the scoping review done on the Middle

East countries (3) discussed few main reasons; i.e. poor timing of the screening, combined with lack of knowledge related to inherited diseases as well as religious and sociocultural concerns that all play huge role in discouraging at-risk marriages(3).

Literature from different countries including developed world still indicate a gap in population's awareness regarding hemoglobinopathies. A survey(86) was conducted among British pregnant women who had undergone carrier testing to study their awareness of testing before the national antenatal screening program was being implemented. The study revealed that 77.4% of women had not been informed about thalassemia carrier screening, while 85.8% of women actually wanted to have been informed(86). Similar results were also shown in United States of America, as pregnant women had lack of awareness regarding genetic testing, and even if they had undergone carrier testing previously, it was ascertained that they were not able to recall undergoing the test (87).

Nonetheless, Bahrain is one of the Gulf countries where increased efforts are being diverted towards educating public about genetic disorders. A student-screening project was initiated in 1998 to screen students before they leave school. The aim of this project was mainly to raise awareness among students regarding hereditary diseases by providing students the results of their blood tests along with a booklet containing information about the disease he/she carries (88). Such project has contributed in increasing awareness among family of carrier students. In addition, the results of a study in Bahrain (88) indicated that there was a birth prevalence of 0.9% for sickle cell disease in 2002, which had decreased by almost 60% since 1984-1985 (2.1%). This was the first time in Bahrain where a prevalence of less than 1% was reported and if such trend continued, Bahrain would be the first country in the Gulf region to successfully eradicate

sickle cell disease in near future (88).

In the same way, the Cyprus Thalassemia Control Program has been successful in reducing the birth prevalence of β-thalassemia major through several important measures that were taken in the country. These include; health education, carrier screening, premarital counseling and prenatal diagnosis. The successful outcomes could be replicated in other countries following similar approach or adopting same measures (88).

To increase the success rate of PMS programs, another study (89) stressed the importance of quality of counseling sessions due to inadequate standardized guidelines. Data from this study showed that the lower compliance of at-risk couples to the counseling was associated with less awareness about these genetic diseases among public. A genetic counseling session consisting of 15-20 minutes is insufficient for couples who are informed of these diseases just before phlebotomy or upon receiving the results (89).

It is also recommended to well-establish the meaning of the "carrier status" among the public before the age of marriage(89). For example, Scriver's group achieved a 90-95% success in informing high school students through using the cooperation of organizations like governmental, non-governmental organizations, community leaders, local health personnel, as well as voluntary screening and genetic counselling over a 20-year span in Canada (90).

Additionally, in Saudi Arabia, the majority of counseling clinics are run by pediatricians due to limited number of qualified genetic counselors. Such sessions are provided in the form of one-to-one counseling after a couple receives an "incompatible status certificate". Furthermore, there are no existing standardized guidelines for the

content of such premarital counseling sessions and materials (91).

In short, high-quality genetic counseling and more effective psychological support system are needed for the at-risk couples to ensure that they have comprehensive awareness of genetic risks of future offspring, if they decide to proceed with the marriage. Such efforts should be combined with improvement in other community sectors such as education through high schools, where students can attain high knowledge regarding such genetic diseases before the age of marriage. In other words, comprehensive life cycle approaches would more likely prove to be effective in reducing and preventing at-risk marriages, which can lead to reduction the incidence of hemoglobinopathies.

CHAPTER 6: IMPLICATIONS, STRENGTHS & LIMITATIONS

6.1 Implications

Findings of this research have several important implications. It provided a better understanding of high prevalence of at-risk marriages for "carrier couples" who underwent PMS and counseling with certain populations reporting higher prevalence as compared to others that had well-established programs with specialized counselors. Some important factors that could be associated with higher prevalence include ethnicity (being Arab), awareness of PMS, implementation of PMS or whether PMS is accessible, specifically in terms of financial constraints, whether the program is voluntary or mandatory for pre-couples in the country. Understanding such factors open ways for new research that explores these factors in details. Further, investigating factors associated with high-risk marriage could lead to identification and implementation of policies to reduce this risk and its costly consequences to the family and society, in terms of genetic diseases.

6.2 Strengths

To the best of our knowledge, this is the first meta-analysis that described the prevalence of at-risk marriages for hemoglobinopathies using a sound methodological research design. It followed the recommendations of PRISMA statement as well as MOOSE guidelines.

Moreover, it is worth noting that the risk of bias assessment for included studies was done by two independent reviewers in a blinded fashion. Additionally, QEM was used which takes into consideration the risk of individual study and hence provides more accurate pooled estimates as compared to REM. Most of the studies included in this meta-analysis were of large sample sizes which increases the precision of the study estimates. Lastly, sensitivity analysis was also done to calculate the pooled estimates based on low risk studies only and it indicated no major publication bias.

6.3 Limitations

This study has several limitations, there was a lack of studies outside the Middle East region so that the prevalence of at-risk marriages could be mostly representing the Middle East region, especially, all large studies were from Saudi Arabia and Iran. However, this is in line with consanguineous marriages which are common in the region, with the highest consanguineous marriages rates in the world reported in the Arab countries.

Another limitation was that some countries offer voluntary premarital screening programs while it is mandatory in others. Useful comparisons could have been made for the prevalence of at-risk marriages of this group with the mandatory program group as part of subgroup analysis, but we could not do it due to lack of studies. Moreover, there was significant heterogeneity across studies that could pose threat or bias our pooled estimates as indicated by heterogeneity tests and Higgin's I^2 percentage.

CHAPTER 7: RECOMMENDATIONS AND CONCLUSION

7.1 Recommendations

More high-quality studies addressing the prevalence of at-risk marriages are required as well as studies on possible factors contributing to couples complying less with the counseling provided. This will help us analyze those factors by subgroups analysis and provide deeper understanding regarding this issue.

Another recommendation is increasing the awareness of public on hemoglobinopathies, especially, in the Gulf countries at the school levels and through community campaigns so that couples are well-informed of future risk of hemoglobinopathy-affected children. This could be more useful approach compared to obtaining all information right before the marriage when arrangements are already made.

Qualified genetic counselors at specialized clinics have to be available for at-risk couples to provide enough education regarding the financial and emotional burden of such genetic diseases. Finally, more epidemiological studies are needed in order to explore underlying reasons and possible interventions to reduce this high prevalence of at-risk marriages, particularly, in the Middle East region.

7.2 Conclusion

The World Health Organization (WHO) estimates that 2.9% of the world's population is affected by beta-thalassemia. This gives rise to many challenges on the health care system and consumption of resources globally. In the Middle East region, the burden of several recessive genetic disorders especially beta-thalassemia is higher compared to other regions. Additionally, most of these genetic diseases are life-threatening and are expensive to treat as well.

Therefore, several countries in the region have implemented mandatory premarital screening programs in order to reduce at-risk marriages and thus, the prevalence of such diseases.

This study was conducted in order to systematically review the global burden of at-risk marriages that is a major factor in contributing to higher prevalence of hemoglobinopathies and therefore, quantify the global prevalence using the statistical approach of meta-analysis. The results of the current study indicated that about 64% of at-risk couples proceeded with the marriage despite receiving the counseling sessions. Similarly, most prevalence estimates of the individual studies were higher than the overall pooled estimate, which indicates a serious public health problem.

However, one of the limitations of our study is that significant heterogeneity was found across studies that could pose threat to the pooled estimates meaning that there is uncertainty in the estimated prevalence and well-designed research is needed in this area for accurate estimate. Regardless, apparently, high-risk marriages is a problem that should be addressed at individual and societal/ cultural levels. Some strategies could include counseling sessions with specialized genetic counselors as well as promoting educational

campaigns to increase knowledge and awareness about the risk of hemoglobinopathies among general population before the age of marriage.

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