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Association of ABO-Rhesus Blood (RH) Groups with Type-2 Diabetes Mellitus Among Follow up Cases in Injibara General Hospital Awi Zone, Northwest Ethiopia:- A Case-Control Study

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M.Sc. Thesis

By

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SCIENCE COLLEGE

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ASSOCIATION OF ABO-RHESUS BLOOD (RH) GROUPS WITH TYPE-2 DIABETES MELLITUS AMONG FOLLOW UP CASES IN INJIBARA GENERAL HOSPITAL AWI ZONE, NORTHWEST ETHIOPIA:- A CASE-CONTROL STUDY

A THESIS SUBMITTED TO COLLEGE OF SCIENCE, DEPARTMENT OF BIOLOGY IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR MASTER OF SCIENCE IN BIOLOGY (BIOMEDICAL SCIENCE).

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Approval of thesis for defense

I hereby certify that I have supervised, read, and evaluated this thesis titled "Association of ABO-Rhesus Blood (RH) Groups with Type-2 Diabetes Mellitus among follow up cases in Injibara General Hospital Awi Zone, northwest Ethiopia" by Mr Lamesgin Sinishaw Wondim, prepared under my guidance. I recommend the thesis is submitted for oral defense.

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Approval of thesis for defense result

We hereby certify that we have examined this thesis entitled “Association of ABO-Rhesus Blood (RH) Groups with Type-2 Diabetes Mellitus among follow up cases in Injibara General Hospital Awi Zone, northwest Ethiopia” by Lamesgin Sinishaw Wondim. We recommend that the thesis is approved for the degree of “masters in biology (biomedical science).”

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DECLARATION

I undersigned, declare that the thesis comprises my own work. In compliance in intentionally accepted practice, I have duly acknowledged and referenced all materials used in this work. I understand the non-adherence to the principle of academic honesty and integrity, misrepresentation of any idea /data/source will constitute sufficient ground for disciplinary action by the university and can also take penal action from the sources which have not been properly cited or acknowledge.

……..... ……. ……

Name of candidate Signature Date

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ABBREVIATIONS/ACRONYMS

DM= Diabetes Mellitus

T2DM/T2D = Type two Diabetes Mellitus

MRC=Medical Referral Clinic

BMI= Body Mass Index

BGL= Blood Glucose Level

WHO= World Health Organization

IDF= International Diabetes Federation

ADA= American Diabetic Association

IGH=Injibara General Hospital

IR=Insulin resistance

Rh=Rhesus

Rh^{+}= Rhesus Positive

Rh^{-}=Rhesus Negative

ITAFAEDO =Injibara Town Administration, Finance and Economic Development Office

GDM=gestational Diabetes Mellitus

ABSTRACT

Diabetes is a complex multi-factorial, chronic metabolic illness characterized by hyperglycaemia, due to lack of insulin secretion, insulin action or both. Type 2 diabetes mellitus (T2DM) remains a public health problem in low and middle-income countries, including Ethiopia. Association of ABO-Rh blood group system and diverse diseases including diabetes has been examined in several susceptibility and population genetics studies with inconclusive results. Thus, a study was designed to investigate the phenotypic and allelic distribution of ABO and Rh groups and its possible association with T2DM among follow up cases and health controls attending at Injibara General Hospital, Awi Zone, Northwest Ethiopia. Data on socio-demographic, family history of diabetes BMI, alcohol drinking habit and complications related to T2DM were gathered from a total of 362 individuals, 181 patients and 181 were health controls, via interview using a structured questionnaire and patients' records. The ABO- Rh blood groups and glycemic levels of both cases and controls were also determined. The data was entered and analyzed using SPSS version 20.0. ABO allelic frequencies were determined using the Bernstein method. Differences in phenotypic distribution of blood groups were assessed using the chi-square test and P values less than 0.05 for all analyses were considered statistically significant. Most of T2DM cases were 45 years old or more with mean age of 50.82 (+ 12.40) and minimum and the maximum of 18 and 80 years old. Overall, blood group phenotypes O, Rh (+), and allele O were the most frequent while blood group AB, Rh (-) and allele B were the least represented in the studied population. Blood group A was the predominant in T2DM cases while blood group O the most frequent in health controls ($p<0.05$). Significantly higher percentages of the cases were overweight, former alcoholic and had family history of diabetes than healthy individuals. Age, ABO blood groups, family history, BMI, and alcohol drinking habits were found to be independent determinants of T2DM. However, the variables, sex, education, monthly income, occupation, marital status, glycemic level, and Rh factor were not associated with diabetes. This study suggests that people with the A and B blood groups have a higher risk of developingT2DM, but blood type O have a lower risk of T2DM. Therefore, blood group should be investigated in future clinical and epidemiological studies on diabetes and further pathophysiological research is needed to determine why individuals A and B blood groups have a higher risk of developing T2DM and blood type O have a lower risk of T2DM.

Key words/Phrases; ABO-Rh Blood group, Socio-demographic factors, Type-2 diabetes.

1. INTRODUCTION

1.1. Back ground of the study

The word diabetes describes a group of metabolic disorders expressed and recognized by the presence of an excess glucose in the bloodstreams without treatment (WHO, 2019). Diabetes mellitus (DM) is a clinically and genetically combined metabolic condition expressed by incorrect hyperglycaemia and dysregulation of nutrition (Singh *et al*., 2019). Hyperglycaemia, if left unchecked over the long term, can cause damage to various body organs, leading to the development of disabling and life-threatening health complications such as cardiovascular disease, neuropathy, nephropathy and eye disease, leading to retinopathy and blindness. On the other hand, if appropriate management of diabetes is achieved, these serious complications can be delayed or prevented (Leu and Zoknszein, 2010; IDF, 2017).

Diabetes mellitus is a group of chronic metabolic illness including: type-1 diabetes (T1DM), gestational diabetes, type-2 diabetes (T2DM) and other types of diabetes cases (Deshpande *et al*., 2008). Type 1 diabetes is caused by an autoimmune reaction where the body's immune system attacks the insulin-producing beta cells in the islets of the pancreas gland. As a result, the body produces none to very little insulin with a relative or absolute deficiency of insulin. T1DM usually affects children or young adults and accounts about 10% diabetes cases worldwide. While, T2DM, formerly known as non-insulin-dependent diabetes, is the most common type of diabetes accounting for around 90% of all cases of diabetes (Holman *et al*., 2015). In T2DM, hyperglycaemia is the result of an inadequate production of insulin and inability of the body to respond fully to insulin, defined as insulin resistance. T2DM is most commonly seen in older adults, but it is increasingly seen in children, adolescents and younger adults due to rising levels of obesity, physical inactivity and poor diet. T2DM may remain undiagnosed until complications become evident since the disease develops gradually. T2DM is one of the most popular chronic diseases and is linked with co-morbidities, such as obesity, hypertension, hyperlipidemia (increased LDL and decreased HDL cholesterol) (Jain and Saraf, 2010).

There are a number of risk factors that increase a person's risk for developing prediabetes and, ultimately, T2DM. Some of these are beyond a person's control or non-modifiable, such as: family history, race or ethnic background, age and history of gestational diabetes. Multitude of studies revealed that T2DM strongly is linked with overweight and obesity and with increasing age as well as with ethnicity and family history (Abdullah *et al*., 2010). Some important modifiable risk factors of T2DM include: obesity, poor diet and nutrition, physical inactivity, prediabetes or impaired glucose tolerance (IGT), smoking and past history of gestational diabetes mellitus (GDM) with exposure of the unborn child to high blood glucose during pregnancy (Ding *et al*., 2014; TDF, 2017). Insulin resistance is also a risk factor for type 2 diabetes and has a close association with obesity. Both obesity and IR are also strongly associated with fatty liver via an effect on the secretion of hepatokines; increased gluconeogenesis, decreased glycogen synthesis, and inhibition of insulin signaling operate by which insulin resistance, overweight/obesity, and fatty liver contribute to type-2 diabetes (Sung *et al*., 2012). Recent evidence has also suggested an association between high consumption of sugar-sweetened beverages and risk of T2DM (Malik *et al*., 2010). Other factors include inadequate intake of fruit and vegetables, whole grains and dietary fibre and high intake of energy as saturated fat (Mozaffarian, 2016).

ABO blood system is based upon the presence or absence of the two agglutinogens, the A and B agglutinogens on the surface of RBCs. When only type A agglutinogen is present, the blood is type A. When only type B agglutinogen is present, the blood is type B. When both A and B agglutinogens are present, the blood is type AB. When neither A nor B agglutinogen is present, the blood is type O. Thus, individuals are divided into four major ABO blood groups A, B, AB, and O as to the serological reactions with which their RBCs exhibit with normal human sera (Yamamoto *et al.,* 2012). The ABO and Rh genes and phenotypes exist in all human populations, but vary in their distribution across ethnic groups, races and geographical boundaries in populations throughout the world (Chandra and Gupta, 2012;Sarkar, 2018). Worldwide, group O is the most common, followed by group A, then group B with group AB as the least common (Cavalli-Sforza *et al*., 1994).

Prevalence of the blood groups is determined by the frequency of the three alleles at the ABO locus in different populations. ABO blood group antigens are polymorphic and inherited structures are presented on the surface of red blood cells. ABO antigens remain the most well-

known and medically important. They are carbohydrate structures relating to the cell-surface glycolipids and/or glycoproteins that are expressed normally on the extracellular surface of the RBC membranes and in most epithelial and endothelial cells, body fluids and organs (Yamamoto *et al.*, 2014).

Differences in blood group antigen expression can increase or decrease host susceptibility to many infections. Blood groups can play a direct role in infection by serving as receptors and /or coreceptors for pathogenic microorganisms, parasites, and viruses. In addition, many blood group antigens facilitate intracellular uptake, signal transduction, or adhesion through the organization of membrane microdomains. Several blood groups can modify the innate immune response to infection (Cooling, 2015). Several studies were conducted to examine the possible association between ABO blood group system and infectious and non-infectious diseases and both the ABO and Rh blood groups systems have been associated with a number of diseases. Some of these studies reported significant associations, suggesting that ABO blood groups could increase or decrease the likelihood of susceptibility to a disease in individuals possessing a particular ABO blood group as in Schistosomiasis (Ndamba *et al*., 1997), *Escherichia coli* O157 (Blackwell *et al*., 2002), Onchocerciasis (Opera, 2001), and HIV (Abdulazeez *et al*., 2008).

Besides, Aljooni and his colleagues observed a significant association between ABO blood type and hepatitis infection. They indicated that HBV and HCV infections were high in O blood type but low in AB (Aljooani *et al*., 2012). In relation to cancer, individuals with type A blood group were found to be susceptible cancers of Cervix (Segi *et al*., 1957), Lung (Roots *et al*., 1988), Hepatocellular (Li *et al*., 2012), Skin (Cihan *et al*., 2013), and Breast (Aly *et al*., 2014).

The number of people with T2DM worldwide has more than doubled during the past 20 years. According to the International Diabetes Federation, 415 million people are living with T2DM in 2015, and by 2040 the number will be almost 642 million (Bellou *et al*., 2018). The incidence of diabetes mellitus has been linked with many factors, but, still limited studies are done regarding the association of ABO- Rhesus blood groups with type 2 DM. Therefore, this study aimed to find out the possible linkage between ABO-Rh blood groups with type 2 diabetes mellitus.

1.2. Statement of the problem

Diabetes is a chronic condition that leads to huge morbidity and mortality resulting from the difficulties that develop during its clinical course (Alonso-Morán *et al*, 2014). The needs of diabetic patients are not only limited to sufficient glycemic control but also match with preventing a difficulty; disability limitation and rehabilitation (Shrivastava *et al.,* 2013). Type 2 diabetes mellitus is the main health problem that is increasing worldwide (Albache *et al*., 2010). T2DM has reached epidemic levels worldwide, affecting 425 million people and accounting for 4 million deaths in 2017 (Galaviz *et al*., 2018).

International Diabetes Federation predicted that the number of people with diabetes in Africa will rise from 14.2 million in 2015 to 34.2 million in 2040. More than half of the adults with diabetes in Africa live in some of the regions are having a large population such as South Africa, the Democratic Republic of Congo, Nigeria and Ethiopia (Mutyambizi *et al*., 2018). Ethiopia is one of the four most having large population countries which have the highest numbers of people with diabetes in Africa (Worede Abebaw *et al.,* 2017).

According to Ethiopian Diabetic Patients Association Report (2011), currently 2.6 million individuals are Diabetic in Ethiopia, of which more than 90% individuals are type 2 Diabetes mellitus patients. T2DM is a multifactorial disease and its appearance and development associated with genetic and environmental risk factors. The absence or presence of blood groups ABO antigens expression has been associated with increased or decreased susceptibility to various types of diseases including T2DM in diverse population. Individuals belonging to certain blood groups have shown predisposition for certain diseases. Blood group of an individual is genetically predetermined and therefore may have an association with genetically predisposed disease like diabetes mellitus (Reetu *et al*., 2018). The presence and lack of blood antigens in some blood groups induce blood membrane changes, morphologically and functionally. The structure- dependent functions of blood types can link the blood groups to health and diseases (Amjadi *et al*., 2015). However, there is limited data in this regard in Ethiopia. Thus, this study sought to determine the possible association T2DM with ABO-RH blood groups among following up cases of type 2 diabetes in Injibara General Hospital Awi Zone, northwest Ethiopia.

1.3. Objective of the study

1.3.1. General objective

The objective of the study was to assess the risk of type-2 diabetes mellitus (T2DM) in relation to ABO and Rh blood groups among diabetic patients in Injibara General Hospital.

1.3.2. Specific objective:-

1. To assess the distribution of ABO and Rh blood group phenotypes among T2DM patients and healthy controls.
2. To determine the allelic and genotypic frequency of ABO blood group in T2DM patients.
3. To determine strength of association between ABO-Rh blood groups-socio-demographic, BMI, glycemic level, alcohol and consumption habits with T2DM

1.4. Significance of the study

T2DM is a multifactorial disease, thus an association study of this type would help in identifying susceptible/ resistance ABO-RH blood types. Early identification individuals blood group would help us prevention, delay the progression of disease or its complications. This study may also contribute in identifying factors (if any) associated with glycemic controls among type-2 diabetic patients in the study area Injibara, and recommend and direction for further planning and management of this ailment .

2. LITERATURE REVIEW

2.1. History of type-2 diabetes mellitus

Diabetes mellitus (DM) was first reported in Egyptian handwritten book about 3000 years ago (Olokoba *et al*., 2012). Type 2 DM was first drawn as a parts of metabolic syndrome in 1988. Type- 2 diabetes existed to be one of the largest epidemics diseases in human history and, exactly, it is one of the main damages to human health in the 21st century (Zimmet *et al*., 2003). T2DM was identified as a disease of the pediatric age groups by late the 1970s. It changes to the significant public health problem which is not limited to North America; it also has been announced in children from Europe, Asia, Africa and Australia (Gungor *et al*., 2005).

People living with type 2 DM are more exposable to short- and long-term complications, which lead to early death. This increased morbidity and mortality specifically in developing countries like Africa (Olokoba *et al*., 2012). Women with a history of GDM have high blood pressure, blood glucose level and promote lipid profile and have a great risk of developing type-2 diabetes mellitus after childbirth (Lee *et al*., 2008).

2.2. Type-2 diabetes mellitus

T2DM is a more sophisticated metabolic conditions described by excess glucose in the blood vessels due to abnormal insulin action and/or insulin secretion (Lin and Sun, 2010). Type 2 diabetes mellitus (T2DM), also known as non–insulin-dependent diabetes mellitus or adult-onset diabetes (Lopez *et al.*, 2014). T2DM covers for between 90% and 95% of diabetes, with highest proportions in low- and middle income countries (WHO, 2019). It is a chronic disease that exists when the pancreatic β-cell function imbalance, or when the body cannot exactly use the insulin it secrets (Prabhakar *et al*., 2014). Type 2 diabetes mellitus is expressed by four main metabolic abnormalities: of which obesity, abnormal insulin action, insulin secretory disfunctions, and increased metabolic glucose amount (Weyer *et al*., 1999). If left untreated, then hyperglycemia may cause long-term microvascular and macrovascular complications. The incidence of T2DM worldwide is now increasing at more rapid rates in Africa, Asia and South America than in Europe or the U.S (Jain and Saraf, 2010).

2.3. Insulin and its roles in metabolism

Type-2 diabetes (T2D) is a complex metabolic disorder characterized by hyperglycemia in the context of insulin resistance, which precedes insulin deficiency as a result of β-cell failure (Montane *et al*., 2014). Insulin is synthesized and produced by β- cells in the pancreas. It is a peptide hormone regulates the metabolism of fat and carbohydrate in the body. It helps glucose absorption from the circulation by fat tissue and skeletal muscles (ASaraj, 2015). Genes and the environment together are important determinants of insulin resistance and β-cell dysfunction (Bellou *et al*., 2018). Insulin resistance increases a person's risk for developing impaired glucose tolerance and type-2 diabetes (Fletcher *et al*., 2002).

2.4. Risk factors for type-2diabetes mellitus

The World Health Organization has announced that unhealthy diets, sedentary lifestyles, tobacco and excessive alcohol consumption are the main risk factors for type 2 diabetes mellitus (Bertoglia *et al*., 2017). Physical inactivity increases the risk of obesity, coronary heart disease, stroke and type-2 diabetes, as well as colon and breast cancer (Guthold *et al.*, 2008). Factors responsible for the increasing epidemic of T2DM in the developing countries include urbanization, ageing population, physical inactivity and increasing obesity rates (Lopez *et al*., 2014, Gezawa *et al*., 2015). In recent years, type 2 diabetes has started to exist in children in association with increasing rates of obesity (Fonseca and John-Kalarickal, 2010). Most patients with type-2 diabetes are overweight, and obesity itself causes some degree of insulin resistance (American Diabetes Association, 2013). Obesity has been found to contribute to approximately 55% of cases of type 2 DM (Olokoba *et al.*, 2012). Chronic health threats relation with obesity are many and includes type-2 diabetes, heart disease, hypertension, and certain types of cancer (Gómez *et al*., 2011, Kennedy *et al*., 2009).

The diabetes risk assessment tool of the ADA guidelines includes sex-specific items and male sex is contributed to diabetes risk factor (Harreiter and Kautzky-Willer, 2018). A report from the United States of America (US) focused on diabetes as a major health issue for women, using mortality, morbidity and survey data to highlight that women may face different challenges across their life span than men (Rubin and Peyrot, 1998). Type 2 diabetes can occur at any age, but it is most often diagnosed in a person after the age of 40 years (Azimi *et al.*, 2008).

Alcohol influences glucose metabolism in several ways in diabetic patients as well as in non-diabetic patients. Alcohol consumption might be related to Type 2 diabetes through its effect on insulin secretion and sensitivity and has been investigated as a possible modifiable risk factor (Cullmann *et al.*, 2012). The risk factors associated with type-2 diabetes include diets rich in saturated fats, simple carbohydrates, high blood pressure, elevated plasma triglycerides (Badran and Laher, 2012).

Recently, evidence suggested a link between the intake of soft drinks with obesity and diabetes, resulting from large amounts of high fructose corn syrup used in the manufacturing of soft drinks, which raises blood glucose levels. High intake of red meat, sweets and fried foods, contribute to the increased the risk of insulin resistance and T2DM. Females with higher BMIs go to be at greater risk for T2DM than males with higher BMIs (Almubarak Faten Ahmed, 2016). Diet, acute or chronic stress, and obesity are major determinants of peripheral insulin sensitivity, and ages make worse these environmental effects (Hennige *et al*., 2003).

People with a parent with type-2 diabetes have an increased risk of the disease (Pierce *et al.*, 2001). Family history of diabetes (FHD) and lifestyle are associated with type 2 diabetes (T2DM), but little is known about the FHD diet interactions (Yanyan *et al.*, 2017). Basically family history of specific diseases reflects the consequences of genetic susceptibility, shared environment and common behaviors (Das and Ghosh, 2012). For the persons who had a family history of diabetes mellitus, the risk for developing this disease was statistically significant (OR=0.51, 95%CI=0.36-0.74, P=0.0001). According to the study revealed that low educational level, low economic level, number of children and number of people living at same house, obesity, physical inactivity, irregular food intake and hypertension were the most important environmental risk factors associated with type 2 diabetes (Belmokhtar *et al*., 2011)..

2.4. ABO/Rh blood group Risk factors for type-2 diabetes mellitus

According to Ebeye *et al.*, (2018), among Urhobos blood group O (54.63%) as most prevalent, followed by groups B (23.17%), A (19.51%), and lastly, AB (2.68%). According to Golassa Lemu *et al*., (2017), majority of Ethiopian the blood type (41.20%) had blood type 'O' followed by types 'A' (34.96%), 'B' (20.48%) and 'AB' (3.34%). However, blood type 'A' was the most frequent (44.07%) blood group among the 'highlanders and 50.42% of Nilotic natives had type 'O'.

According to Nazli et *al*., (2015), the conclusion showed that the frequency of "Rh-positive blood group" is B, A, O and AB, whereas the frequency of the most common Rh-negative blood group are A, O, B and AB respectively. According to Bener and Yousafzai (2014), the study showed that the blood group B men, the frequency of only blood group B was significantly higher, while on the contrary among diabetic women the frequency of both A and B (29.7% vs. 24.8% and 25.5% vs. 20% respectively) were significantly higher as compared with no diabetic healthy population. According to Hegde, 2015 blood groups AB & B showed less common association, whereas patients with blood group A were associated more with diabetes mellitus (DM), as compared to controls. Blood group O showed similar distribution among both groups. Higher percentage of diabetics than controls had Rh positive blood group (97.3% vs. 93.9%), whereas' less percentage of diabetics showed Rh negative blood group (6.1% vs. 2.7%). Higher percentage of diabetics with blood group B, AB and O were positive, whereas it was same in blood group A

According to Kamil *et al.,* (2010), the result showed that there A and O blood groups showed negative association with DM, which implied that A and O blood group patients have less chances of DM type 2. However, no significant association was found between DM type 2 and blood groups AB and significant association was found between DM type 2 and blood groups B.

According to Meo *et al*., (2016)**,** the study showed that blood group "B" was associated with high incidence of type-2 diabetes and blood group "O" has minimum association with type-2 diabetes. Blood group "A" and "AB" were almost equally distributed in both diabetic and non-diabetic population. According to Öner *et al*., (2016), the result showed that A blood group was the most frequent allele in the three groups (57.6%, type-2 DM; and 41.7%; controls). It was followed by O blood group (22.5%, type-2 DM; and 38.2%; controls), B blood group (15.4%, type-2 DM; and 16.0%, controls) and AB blood group (5.6%, type-2 DM and 4.2%; controls), respectively. In case of blood group, type A was more susceptible and blood type O and AB were lower risk rate of diabetes mellitus (Hailu Berhanie *et al.,* 2020).'

2.5. Cause of type-2 diabetes mellitus

Causing factors for its existence include family history of diabetes, history of gestational diabetes, abnormal metabolic glucose and ethnicity (Badran and Laher, 2012, Lopez *et al*., 2014).

Diabetes mellitus is a metabolic disorder of multiple causes (Palsamy and Subramanian, 2009). Type 2 diabetes results from interactions between genetic susceptibility, environmental factors and lifestyle choices (Hectors *et al*., 2011).

Genetic factors influencing the probable of developing T2DM include family history, history of pre-diabetes and history of gestational diabetes mellitus (Almubarak Faten Ahmed, 2016). The empirical risk of having type 2 Diabetes mellitus is increased 2 to 6-fold if a parent or other relatives have the disease (Everhart *et al.,* 2005). Many genes are taught to be involved as a cause for type two diabetes mellitus particularly mutation of genes lead the disease rarely (Goksel *et al*., 2003). A number of lifestyle factors are known to be important to the development of type 2 DM, of which inactive lifestyle, cigarette smoking, environmental toxins and continuous alcohol consumption (Alinia *et al*., 2009).

2.6. Epidemiology of type-2 diabetes mellitus

The International Diabetes Federation (IDF) estimated the global prevalence to be 151 million in 2000, 194 million in 2003, 246 million in 2006, 285 million in 2009, 366 million in 2011, 382 million in 2013 and 415 million in 2015 (Cho *et al.,* 2018). Type 2 DM is estimated that 439 million people would have by the year 2030. Diabetes is found in every population in the world and in all regions (WHO, 2019).

The incidence of type 2 DM varies substantially from one geographical region to the other as a result of environmental and lifestyle risk factor (Olokoba *et al.,* 2012). In 2011, about 14 million individuals were estimated to have diabetes in Africa, and this is expected to rise to 28 million by 2030 (Bos and Agyemang, 2013). In Ethiopia, the prevalence of diabetes was 3.5 % in 2011 (Kassahun Tesfaye *et al*., 2016). T2MD now represents 8-45% of all new cases of diabetes reported among children and escent (Baynes Habtamu, 2015). A community based study on bank workers and teachers and a hospital based study reported diabetes prevalence of 6.5% in Addis Ababa. Another community based study in northwest Ethiopia indicated that the prevalence of diabetes in the population aged 35 years and above is 5.1% in urban setting and 2.1% in rural setting (Zekewos Alemayehu *et al*., 2018). Globally, it is estimated that every six seconds, someone dies from diabetes related complications. In 2013, diabetes caused 5.1 million deaths and 548 billion dollars (USD) in healthcare expenditures alone (Bird *et al.,* 2015).

2.7. Complications of type-2 diabetes mellitus

The risk of complication in type 2 DM is directly related to prior glucose control level (Zoungas *et al*., 2012). Glycemic control is the common factor that determines death and complication from diabetes (Hardy *et al*., 2012). The complications include macrovascular diseases (hypertension, hyperlipidemia, heart attacks, coronary artery disease, strokes, cerebral vascular disease, and peripheral vascular disease), microvascular diseases (retinopathy, nephropathy, and neuropathy) and cancers (Wu *et al*., 2014**).** The morbidity and mortality associated with diabetes mellitus results from either its acute or chronic complications. The acute metabolic (hyperglycaemic) complications include: Diabetic Ketoacidosis and lactic acidosis. The chronic complications of DM affect many organ systems and are responsible for the majority of morbidity and mortality associated with the disease (Abubakar, 2014) Awareness of risk factors for developing type 2 diabetes will promote screening, early detection, and treatment in high-risk populations with the goal of decreasing both microvascular and macrovascular complications (Fletcher *et al*., 2002). .

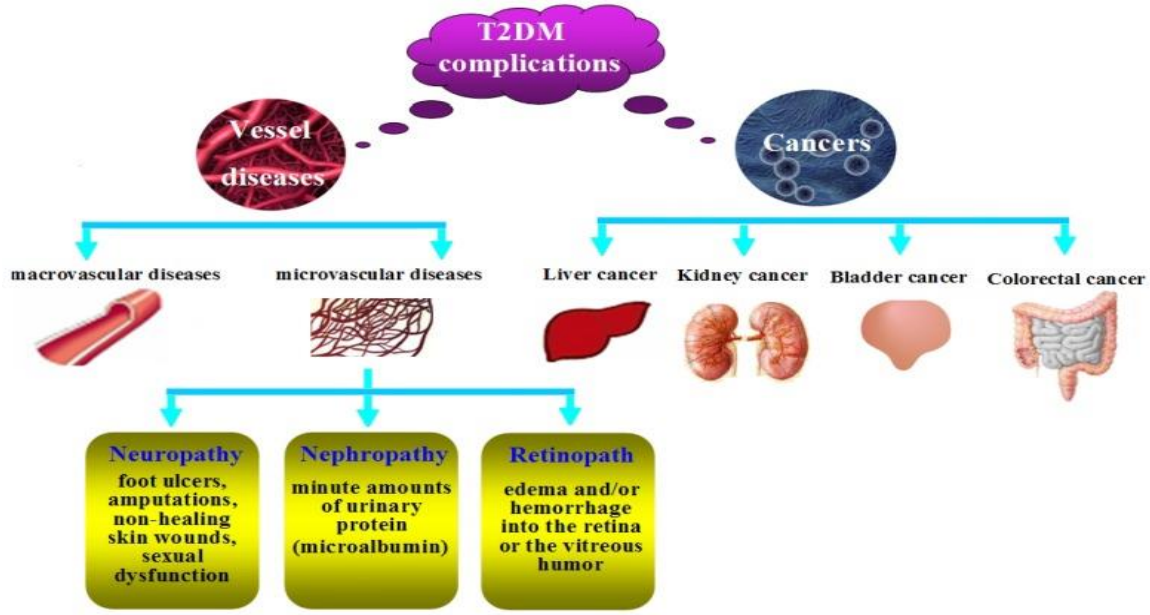

Figure 1: Classification of type-2 diabetes mellitus complication (Wu *et al*., 2014)

2.7.1. Cardiovascular disease

Cardiovascular disease is a primary cause of mortality and morbidity in both prediabetes and T2DM, the potential mechanism for which is oxidative stress that has important effects on atherogenesis and may contribute to low-density lipoprotein (LDL) oxidation **(**Wu *et al*., 2014**).**

2.7.2. Diabetic neuropathy

Diabetic neuropathy may be associated with foot ulcers, amputations, non-healing skin wounds, and sexual dysfunction. Sexual dysfunction usually occurs in young-aged diabetic patients because of oxidative stress in cavernous tissues **(**Wu *et al*., 2014). It is a life-threatening complication involves both peripheral and autonomic nerves, affecting almost half of the diabetic population (Chawla *et al*., 2016).

2.7.3. Diabetic nephropathy

Is the kidney disease that occurs as a result of diabetes (Abubakar, 2014). It is one of the most important microvascular complications, whose earliest manifestation is the presence of minute amounts of urinary protein which cannot be detected in routine urinalysis (Wu *et al*., 2014).

2.7.4. Diabetic retinopathy

Chronic hyperglycemia may cause microvascular damage to the retinal vessels, resulting in edema and/or hemorrhage into the retina or the vitreous humor because of vascular permeability (Wu *et al*., 2014). The risk of development of diabetic retinopathy (DR) in patients with (T2DM) has been found to be related to both severities of hyperglycemia and presence of hypertension (Chawla *et al*., 2016).

2.7.5. Cancers

Epidemiologic evidence has demonstrated that diabetes may elevate the risk of cancer such as colorectal cancer, liver cancer, bladder cancer, breast cancer, kidney cancer, which varies depending on the subsites of specific cancers. T2DM and cancers usually share many risk factors such as age, obesity, sedentary lifestyle (Wu *et al*., 2014).

2.8. Symptom of type-2 diabetes mellitus.

Type-2 diabetes mellitus frequently goes undiagnosed for many years because the hyperglycemia develops gradually and at earlier stages is often not severe enough for the patient to notice any of the classic symptoms of diabetes. Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the non-ketotic hyperosmolar syndrome (American Diabetes Association, 2013).

The chronic complications of untreated or inadequately treated diabetes include retinopathy, neuropathy, nephropathy, and atherosclerosis, which can lead to blindness, amputations, end-stage renal disease, and cardiovascular mortality (Gadsby, 2002). Diabetes may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. The most severe clinical symptom are ketoacidosis or a non-ketotic hyperosmolar state that may lead to dehydration, coma and, in the absence of effective treatment, death (WHO, 2019).

2.9. Pathogenesis of type-2 diabetes mellitus

The pathological sequence for type 2 diabetes is complex and entails many different elements that act in concert to cause that disease (Leahy, 2005). In developing countries, people aged 40 to 60 years are affected most, compared with those older than 60 years in developed countries. The complications of diabetes account for most of its morbidity and mortality. 65% of individuals with DM have high BP, heart disease and stroke. Death rates in diabetes are 4 times more than non-diabetes (Banoo *et al*., 2015). In the developed world, and increasingly elsewhere, type 2 diabetes is the largest cause of non-traumatic blindness and renal failure (Afzal *et al*., 2018).

Many studies have shown that inflammation plays a very important role in the pathogenesis of T2D. Inflammatory mechanisms and cytokine production activated by stress via the inflammasome may further alter the normal structure of β-cells by inducing pancreatic islet cell apoptosis. Thus, the combination of oxidative and ER stress, together with autophagy insufficiency and inflammation, may contribute to β-cell death or dysfunction in T2D (Montane *et al*., 2014). In diabetic individuals with overt fasting hyperglycemia, the triumvirate impaired insulin secretion by the pancreatic β-cells, muscle insulin resistance, and hepatic insulin resistance all play central roles in the development and progression of glucose intolerance (Akash *et al*., 2013).

2.10. Diagnosis of type-2 diabetes mellitus

In some, diabetes is diagnosed when they present with infections, especially urinary tract and skin infections and genital candidiasis (particularly, in women) (Fonseca and John-Kalarickal, 2010). A diagnosis of diabetes has important implications for individuals, not only for their health, but also because of the potential stigma that a diabetes diagnosis can bring may affect their employment, health and life insurance, driving status, social opportunities, and carry other cultural, ethical and human rights consequences (WHO, 2019).

Blood sugar level was measured at fasting using a biosensor glucometer. Glucometer was switch on, and then insert strip in glucometer and a drop of blood was placed on strip (Shams *et al.*, 2017). Well- known for the diagnosis of T2DM are based on values of fasting blood glucose, random blood glucose, and the oral glucose-tolerance test and are identical for adults and children. A random or "casual" plasma glucose value 200 mg/dL is diagnostic of diabetes if the patient has additional symptoms such as polyuria (Hannon *et al*., 2005).

The diagnosis is based on measurement of A1C level, fasting or random blood glucose level, or oral glucose tolerance testing (Patel and Macerollo, 2010). In the type 2 diabetes mellitus mildest form, the diagnosis is made in an asymptomatic child during a routine medical check-up by detection of hyperglycaemia or glycosuria. One third of patients are diagnosed by urinalysis during routine physical examination. In its severest form, the child presents with polyuria, polydipsia, and weight loss (Reinehr, 2013).

2.11. Treatment of type-2 diabetes mellitus

Type 2 DM is usually first treated by increasing physical activity, and eliminating saturated fat and reducing sugar and carbohydrate intake with a goal of losing weight (Megahed *et al.*, 2018). Lifestyle factors related to obesity, eating behavior, and physical activity play a major role in the prevention and treatment of type 2 diabetes (Wing *et al*., 2001). Moderate weight loss (5% of body weight) can improve insulin action, decrease fasting blood glucose concentrations, and reduce the need for diabetes medications (Klein *et al*., 2004).

Regular Physical activities may contribute to improvements in body composition, blood lipid profiles, hypertension, and glycemic control and reduce the risk of coronary heart disease by as much as 55% (Tudor *et al*., 2004). Epidemiologic evidence suggests that regular physical activity is a key factor in the prevention or treatment of metabolic diseases (Kavouras *et al.*, 2007). In individuals with type 2 diabetes, regular training reducesA1C, triglycerides, blood pressure, and insulin resistance (Colberg *et al*., 2016).

Worldwide, the care of T2DM patients consumes between 5 and 10% of the budget allocated to the health system due to the higher frequency of consultations and hospitalizations, and longer rehospitalizations and more complex treatments. Different phenolic compounds such as flavonoids and antocyanins have positive effects on diabetes (Ríos *et al.*, 2016).

Biguanides are one of the major classes of antidiabetic drugs, among which metformin is the most common drug used in the first line therapy for diabetes mellitus.

Metformin has been proven to be efficacious in lowering blood glucose, increasing insulin sensitivity, reducing cardiovascular and hypoglycemia risk, and is the only hypoglycemic agent to improve macrovascular outcomes and to reduce mortality rates in T2DM patients.

Sulfonylureas: are second line agents widely used in the treatment of T2DM patients who are not severely obese, which act directly on the islet β cells to close ATP-sensitive K^+ channels and stimulate insulin secretion.

Thiazolidinedione: (TZDs) are a class of insulin sensitizers, including troglitazone, rosiglitazone, and pioglitazone. They are peroxisome proliferator-activated receptor γ (PPAR-γ) ligands which control normal skeletal muscle and hepatic insulin sensitivity. The TZDs have more durable action to regulate hyperglycemia than sulfonylureas and metformin.

A*-Glucosidase inhibitors *(AGIs): including acarbose, voglibose and miglitol, are markedly effective for postprandial hyperglycemia. They inhibit intestinal mucosal enzyme (α-glucosidase) which converts complex polysaccharides into monosaccharides, thus decreasing carbohydrate absorption

Incretin-based therapies: Incretins are hormones that stimulate insulin secretion and suppress postprandial glucagon secretion in a glucose-dependent manner.

GLP-1 receptor agonists: GLP-1 receptor agonists are effective in the regulation of glucose metabolism, such as stimulating insulin production, inhibiting glucagon release, slowing nutrient absorption, and increasing feelings of satiety (Olokoba *et al*., 2012). The treatment involves diet modification, weight reduction, exercise, oral medications, and insulin (Bailes, 2002).

2.12. Control and prevention of type-2 diabetes mellitus

Diabetes patient education is considered one of the cornerstones of effective diabetes care, and is intended to improve patients' health status and quality of life (Rise *et al*., 2013). To prevent T2DM, the U.S. government and the World Health Organization (WHO) highly recommended that individuals get at least 150 min per week of modest physical activity, 75 min per week of strong physical activity, or a comparable mixture of the two intensities (Almubarak Faten Ahmed, 2016). Many cases of T2DM could be prevented with lifestyle changes, including maintaining a healthy body weight, consuming a healthy diet, staying physically active, not smoking and drinking alcohol in moderation (Zheng *et al.*, 2018).

Consumption of fruits and vegetables may protect the development of T2DM, as they are rich in nutrients, fiber and antioxidants which are considered as protective barrier against the diseases (Ha and Phuong, 2019)

2.13. Hardy- Weinberg Equilibrium

Hardy–Weinberg equilibrium (HWE) is the state of the genotypic frequency of two alleles of one autosomal gene locus after one discrete generation of random mating in an indefinitely large population (Mayo, 2008). In genetic association studies, HWE principles have been applied to detect genotyping error and disease susceptibility loci (Ryckman and Williams, 2008). The Hardy-Weinberg law in the elementary kind of two alleles of a gene establishes, that relative frequencies of genotypes in generations at autosoming inheritance correspond to term of binomial expansion $(p+q)^2$ under condition of $p+q=1$, where p and q is the frequencies of alleles in a population (Volobuev *et al*., 2013). To explore the Hardy-Weinberg equation, we can examine a simple genetic locus at which there are two alleles, A and a. The Hardy-Weinberg equation is expressed as: $p2 + 2pq + q2 = 1$, where, p is the frequency of the "A" allele q is the frequency of the "a" allele in the population. In the equation, p2 represents the frequency of the homozygous genotype AA, q2 represents the frequency of the homozygous genotype aa, and 2pq represents the frequency of the heterozygous genotype Aa. In addition, the sum of the allele frequencies for all the alleles at the locus must be 1, so $p + q = 1$. If the p and q allele frequencies are known, then the frequencies of the three genotypes may be calculated using the Hardy-Weinberg equation (Kumari, 2016).

3. MATERIALS AND METHODS

3.1. Description of the study area

This study was conducted in Injibara General Hospital, Injibara, Awi zone, Amhara National regional state. Injibara is a town and administrative center of Awi zone, northwest Ethiopia. It is located at 10°57′ latitude N and 36°56′ longitude W in the northwest part of the country at an elevation of 2560 meter above sea level. It is 435 km far from the capital city of Ethiopia, Addis Ababa and 118 km far from the administrative center of the Amhara region, Bahir Dar. The town has one general hospital and two governmental health centers and six private health care centers. According to Injibara Town Administration Finance and Economic Development Office 2015 report, the total population of the town was 35,846 of which 18,540 were males and 17,306 were females (Injibara Town Administration, Finance and Economic Development Office, 2015).

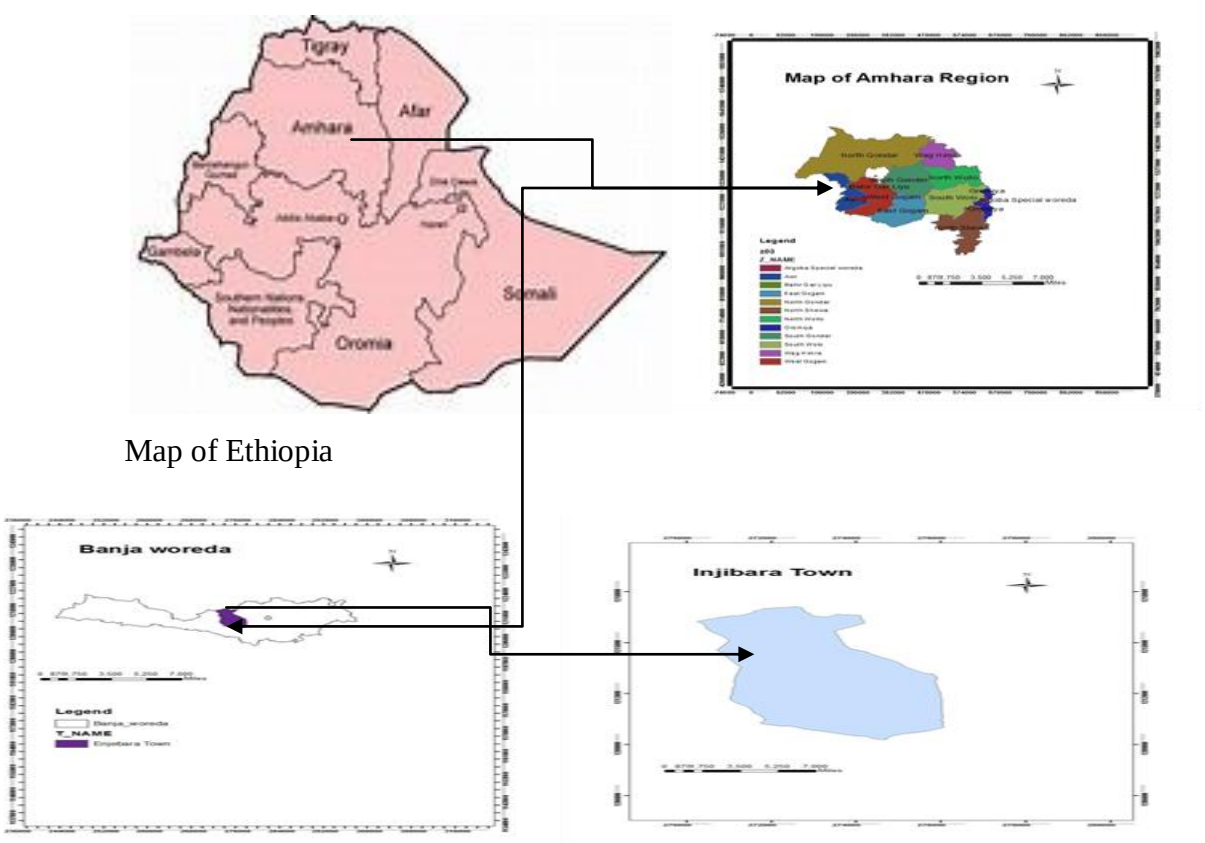

Figure 2 : Map of the study area**.**

3.2. Study populations

The source population of this study was all diabetic patients and those healthy controls who visited the Injibara General Hospital between the period of January 01/ 01/2020 and February 30/03/2020 while the study population was diabetic patients and healthy controls who visited the hospital during data collection. The controls were taken from healthy people who visited for the hospital for different health related issues and other than T2DM during data collection period.

3.3. Study design

A Case-Control study design was implemented to determine the possible association between ABO and Rh blood groups and type-2 diabetes among follow up case in Injibara General Hospital.

3.4. Sample size determination and Sampling technique

The formula for sample size calculation for case-control study depends on how many cases and controls do you need assuming…80% power, you want to detect, an odds ratio of 2.0 or greater, an equal number of cases and controls. The proportion exposed in the control group is 20%. A researcher was seeing the link between type-2 diabetic patients (case) and control group. Here number of case is qualitative variable hence this formula was used for such type of design in order to detect sample size (Charan and Biswas, 2013).

$$n = \left(\frac{r+1}{r}\right)\frac{(\bar{p})(1-\bar{p})(Z_{\beta}+Z_{\alpha/2})^2}{(p_1-p_2)^2} \qquad = 181$$

Where,

n = sample size in case group

β= desired power (typically 80%)

r = ratio of controls to cases

P* = Variability (Average proportion)

$P_1 - p_2$=Effect Size

P2=the proportion exposed in the control group

Za/2 = Level of significance

P1=Proportion in cases. Based on the above formula 181 cases and 181 controls, in total 362 subjects were included in this study. The study participants were selected using systematic sampling technique based their list for three consecutive months and took blood sugar level, but

the ABO-Rh blood groups and other data were collected once till the require sample size attained.

3.5. Inclusive and Exclusive criteria

Patients who were diagnosed with type-2 diabetes mellitus at Injibara General Hospital as per American Diabetes Association (2016) criteria were included in this study. T2DM patients who were diagnosed with other medical conditions such as mental health problems and hearing impairments and those patients who were unable to provide the appropriate information or not willing to sign the consent form were excluded. Other types of diabetes mellitus including type 1 diabetes mellitus, gestational DM and diabetes insidious patients were excluded.

3.6. Data collection

The following research tools were used to gather data: questionnaire and blood groups and venous blood or capillary blood was used to determine the glycemic level from laboratory tests.

3.6.1. Questionnaire survey

A structured questionnaire consisting of a total of 30 questions in three sections was prepared first in English language and later translated in to Amharic version. The questionnaire contains information on socio – demographic variables, BMI, blood sugar levels, family history of T2DM, alcohol drinking habits and Knowledge of the study subjects about T2DM. The purpose of the study was explained to the follow up cases of T2DM at the time of their hospital visit and invited to participate in this study. Individuals who provided consent were interviewed.

3.6.2. ABO and Rh blood group Testing

After completion of interview, about 3ml of venous blood was taken as part of the usual diagnosis at the clinic by laboratory technicians to determine glycemic labels. Then ABO and Rh blood group phenotype determination was done for both diabetes patients and healthy subjects by finger-prick method from their third fingers.

- Third fingers was pricked with fresh sterile blood lancet and wiped vigorously with cotton wool which was soaked in methylated spirit to sterilized and stimulate blood flow of the subject.
- About 0.05ml (three drops) blood was taken tested immediately following blood collection.

- The blood sample was placed at three places over a glass slide.
- To each blood drop on that was placed at separate places on the slide, a drop of anti-sera (anti-A, anti-B and anti-D) monoclonal blood grouping reagents was added and mixed by wooden stick properly.
- The slide was incubated for 3-4 min at room temperature and observed for the agglutination.

Table 1: Interpretation of ABO blood groups

Anti- A	Anti- B	Interpretation of blood groups
(+)	(-)	A
(-)	(+)	B
(+)	(+)	AB
(-)	(-)	O

Human RBCs are classified as Rhesus (+) or Rhesus (-) depending upon the presence or absence of D antigen on them. If RBCs contain D antigen, they will agglutinate in the presence of reagent containing corresponding antibody termed as RH (+). If there is no agglutination, it is considered as RH (-). The principle is haemagglutination (which is similar to ABO system). Body mass index determined as a ratio of weight in kilograms to the square of height in meters. Weight status was classified according to the WHO definitions (WHO, 2000): underweight (UW): BMI <18.5, normal weight (NW): BMI 18.5 - 24.9, overweight (OW): BMI 25 - 29.9, and obese: BMI ≥30.

3.7. Data Reliability and Validity

In order to make the study more reliable and valid, the questionnaire used for data collection in this study was adapted from questionnaires which were used in similar previous studies after making minor modifications. The participants was expressed the problems in the understanding of any of the items in the questionnaire, their comments and feedback was also noted and considered in the refinement of the final questionnaire before starting data collection.

3.8. Variables

This study consisted of dependent and independent variables. The blood group (A, B, AB and O), Rh factors (Rh positive and Rh negative) and socio-demographic and clinical variables were independent variables. The status of T2DM (being case or healthy) was considered as dependent variable.

3.9. Data analysis

All the data collected from the hospital were checked first for its completeness before entering for analysis. Then the data was entered into SPSS Version 20 for Statistical analysis and descriptive statistics like, frequencies, association of variable with diabetes mellitus (tested by Chi-square value) were estimated. Univariate and multivariate logistic regression analyses were done to identify independent factors associated with the knowledge, attitude and practices. Variables which were significant in univariate analysis were selected as a candidate for the multivariate analysis. In the Wald test from logistic regression and a p-value cut-off point of 0.25 were used. Variables which have a p-value of below 0.25 in the univariate analysis were selected to be used in the multivariate analysis.

3.10. Ethical clearance

The study proposal was reviewed and accepted and Ethical Clearance was obtained from research community service office of college of science Bahir Dar University. Permission to carry out the research was also obtained from the hospital. Informed oral consent was obtained from every study participant. All data obtained were kept strictly confidential and each sample was identified by its code number only (No names was recorded on the questionnaires and all questionnaires were kept safe).

4. RESULTS

4.1 Socio – demographic variables of the study subjects

Socio-demographic variables such as sex, age, marital status, education, income and occupation with different categories were presented (Table 2). In this study, a total 362 subjects, 181 T2DM cases and equal number of healthy controls were participated. The frequency of male and female cases was 103(56.9%) Vs. 78(43.1%) while it was 108 (59.7%) vs. 73(40.3%) in controls. The overall mean age of the study participants was 49.06 (SD =± 13.96) with minimum and the maximum of 18 and 88 years old. The mean age of the T2DM cases was 50.82 (SD =± 12.40) with minimum and the maximum of 18 and 80 years old.

Overall proportion of study participants in age categories of 18-29, 30-44 and ≥45 years old was 6.35%, 32.04%, and 61.60 %, respectively. Most (72.93%) of T2DM cases were in the age category of 45 or more years old followed by 24.86 and 2.21% cases in the age categories of 30-44 and 18-29 years of age, respectively. Likewise, the percentage of healthy control subjects in age groups of 18-29, 30-44 and ≥45 years old was 19 (10.89%), 71(39.22%) and 91 (50.27%), respectively. The frequency of single, married and divorced participants in both cases and controls were relatively comparable. Moreover, the distribution of subjects both in cases and controls in the categories of the variables, educational status, income and occupation was relatively similar and no statistically significant differences were observed.

According to their levels of education, 48 (26.52 %) of T2DM cases were illiterate, 54(29.83%) able to read and write, 16(8.84%) completed primary school, 20(11.05%) completed secondary school and 43(23.76%) diploma and above. With regard to monthly income, 21 (11.60%) and 36 (19.89%) of the T2DM cases were the lowest (500-1000 ETB) and the highest (>5000 ETB) paid, respectively, while majority 124 (68.51%) of the cases were earners of an average of monthly income between 1001-5000 Ethiopian Birr (ETB).

Table 2: Socio demographic characteristics of the study participants, at Injibara General Hospital, 2020

Variables	Categories	Cases N(%)	Controls N(%)	Total N (%)	χ^2 (df)	P-value
Sex	Male	103 (56.9%)	108 (59.7%)	211(58.3%)	0.284	0.594
	Female	78 (43.1%)	73 (40.3%)	151 (41.7%)		
Age(Year)	18-29 yrs.	4 (2.21%)	19 (10.5%)	23 (6.4%)	23.148	0.000
	30-44 yrs.	45 (24.86%)	71 (39.2%)	116 (32.0%)		
	≥45 yrs.	132 (72.9%)	91 (50.3%)	223 (61.6%)		
Marital status	Single	24 (13.25%)	25 (13.81%)	49 (13.53%)	0.563	0.755
	Married	147(81.21%)	149(82.32)	296(81.76)		
	Divorced	10 (5.52%)	7 (3.87%)	17 (4.7%)		
Education	Illiterate	48 (26.52%)	56 (30.93%)	104(57.46)	4.433	0.351
	Can read &write	54 (29.83%)	40 (22.09%)	94(51.93%)		
	Prim.school	16 (8.84%)	24 (13.25%)	40 (11.05%)		
	Seco.school	20 (11.05%)	21 (11.60%)	41 (11.33%)		
	Diploma & above	43(23.76%)	40 (22.09%)	83 (22.93%)		
Monthly income(Eth Birr)	500-1000	21 (11.60%)	18 (9.94%)	39 (10.8%)	0.658	0.720
	1001-5000	124(68.51%)	131(72.38)	255 (70.4%)		
	>5000	36 (19.89%)	32 (17.68%)	68 (18.8%)		
Occupation	Student	5 (2.76%)	11 (6.08%)	16 (4.41%)	6.067	0.300
	House wife	33 (18.23%)	29 (16.02%)	62 (17.13%)		
	Merchant	49 (27.07%)	36 (19.89%)	85 (23.48%)		
	Farmer	46 (25.41%)	57 (31.49%)	103(28.45)		
	Pr.employee	17 (9.39%)	20 (11.04%)	37 (10.22%)		
	Gov.employee	31 (17.12%)	28 (15.47%)	59(32.59%)		

4.2 Association of ABO-Rh blood groups, family history, body mass index, alcohol drinking habit, blood sugar levels with Type-2 Diabetes mellitus

The overall distribution of ABO blood phenotypes of the studied population in descending order was AB 59 (16.3%) < B 68 (18.8%) < A 117(32.3%) <O 118 (32.6%). The frequencies of A, B, O and AB blood types in T2DM patients were 44.8%, 22.1%,19.3% and 13.8%; (A>B>O>AB), respectively. Type A was the most common while type AB was the least common blood group in cases. Likewise, the frequencies of ABO blood types in healthy controls in a descending order of occurrence was O>A>AB>B, in this case blood type O predominated (45.9%) while blood type B was the least abundant (15.5%).

In Chi-square analysis, the distribution of the ABO blood phenotypes between T2DM patients and healthy controls varied significantly (p=0.000). The frequency of blood group A (81 (44.8%) was higher in cases, but O was the most abundant (45.9%) in controls. Blood group AB type, (18.8 % vs. 13.8 %;) was in healthy control and cases subjects respectively. There was no statistically significant difference in the frequency of Rh (+) and Rh (-) blood types between cases and healthy controls. The frequency of Rh (+) blood type was 93.9% in cases and it was 88.4% in controls while the Rh (-) type was 6.1% vs11.6% in cases and controls, respectively.

With regard to family history of diabetes, the frequency of T2DM patients who reported family history of diabetes was higher than (65.7%) those who had no (34.3%) family the history. More than nine out of ten (92.8%) the healthy individuals had no family history of diabetes but less one in ten reported they had family history of diabetes. In this study, there was a statistically significant association between family history and diabetes (p=0.000). Almost relatively comparable proportion of cases and controls had normal body mass index. The frequency of overweight cases was slightly higher in T2DM cases (12.71%) than the healthy controls (10.49%) whereas underweight individuals were more frequent in healthy controls (18.23%) than diabetic patients (5.52%). BMI was significantly associated with the status of diabetes (p=0.001).

In Chi-square analysis, alcohol drinking habit was significantly associated with type-2 diabetic mellitus (p=0.000). The frequency of non-alcoholic, past-alcoholic and presently alcoholic diabetic individuals were 50.82, 48.61 and 0.57% respectively, while it was 79.00, 17.12 and 3.86% respectively in controls. There were almost three fold higher (48.61%) past-alcoholic individuals among diabetic cases than the health controls (17.12%).

However, the frequency of currently alcoholic subjects was substantially higher in healthy controls than type-2 diabetic individuals. The mean glycemic level in healthy controls was 107 (SD= 11.31) with minimum and maximum of 83 and 130 ml/dL. While it was 151.19 (SD= 69.66) in diabetic cases with minimum and maximum of 83 and 470 ml/dL. In my study, more than half 100 (55.25%) of T2DM subjects had good glycemic level control while 81(44.75%) bad glycemic control. The association between glycemic level and diabetes was statistically significant (0.000).

Table 3: Association of T2DM with ABO-Rh blood groups, family history, BMI, alcohol drinking habits, glycemic levels and diabetes related complications.

Variables	Cases N (%)	Controls N(%)	Total N (%)	χ^2 (df)	P-value
Blood and Rh blood groups					
A	81 (44.8%)	36 (19.9%)	117 (32.3%)	40.324(3)	0.000
B	40 (22.1%)	28 (15.5%)	68 (18.8%)		
AB	25 (13.8%)	34 (18.8%)	59 (16.3%)		
O	35 (19.3%)	83 (45.9%)	118 (32.6%)		
Rh (+)	170(93.9%)	160 (88.4%)	330 (91.2%)	3.428(1)	0.064
Rh (-)	11(6.1%)	21 (11.6%)	32(8.8%)		
Family history of T2DM					
Yes	119 (65.7%)	13 (7.2%)	132 (36.5%)	133.973(1)	0.000
No	62 (34.3%)	168 (92.8%)	230 (63.5%)		
Body mass index in kg m^{-2}					
U(<18.5)	10(5.52%)	33(18.23%)	43(11.88%)	13.961(2)	0.001
N(18.5-24.99)	148(81.77%)	128(70.72%)	287(79.28%)		
OW(≥25-29.9)	23(12.71%)	20(10.49%)	41(11.22%)		
Alcohol Intake habits					
Non-alcoho	92(50.82%)	143(79.00%)	235 (64.90%	42.871(2)	0.000
Past-alcoho	88(48.61%)	31(17.12%)	119(32.90)		
Alcoholic	1(0.57%)	7(3.86%)	8(2.20%)		

Glycemic level					
80-130ml/dL	100(55.25%)	181(100%)	281(77.62%)	104.349 (1)	0.000
>130ml/dL	81(44.75%)	0(0%)	81(22.38%)		

BMI***
U=underweight
N=normal
Ow=overweight

4.3 Association of glycemic control with ABO-Rh blood groups and educational statuses of cases

The evaluation of the Association of glycemic control with ABO-Rh blood groups and educational statuses of patients is presented in Table 4 below. In this study, ABO blood type was marginally (p=0.055) and Rh type significantly (p=0.012) associated with glycemic control. However, level of education of T2DM cases was not significantly associated with glycemic level control (p=0.986).

Table 4: Association of glycemic control with ABO-Rh blood groups and educational statuses of cases

ABO-Rh Blood groups	**Glycemic levels of T2DM**			**χ^2 (df)**	**P value**
	80-130 ml/dL	>130 ml/dL	Total		
A	49 (60.5%)	32 (39.5%)	81	7.612(3)	0.055
B	18 (45.0%)	22 (55.0%)	40		
AB	18 (72.0%)	7 (28.0%)	25		
O	15 (42.9%)	20 (57.1%)	35		
Rh type of T2DM cases					
Rh(+)	90 (52.9%)	80 (47.1%)	170	6.024	0.012
Rh(-)	10 (90.9%)	1 (9.1%)	11		
Levels of education of T2DM cases					
Illiterate	26 (54.2%)	22 (45.8%)	48	0.359(4)	0.986
Can read and write	31 (57.4%)	23 (42.6%)	54		
Primary school completed	9 (56.2%)	7 (43.8%)	16		
Secondary school completed	10 (50.0%)	10 (50.0%)	20		
Diploma and above	24 (55.8%)	19 (44.2%)	43		

4.4. Univariate and multivariate analysis of factors associated with age, ABO blood groups, family history, BMI and alcohol drinking habits towards T2DM.

In multivariate analysis, age, ABO blood groups, family history, BMI, and alcohol drinking habits were significantly associated with T2DM ($p<0.05$). The odds of T2DM was about seven and nine times (AOR: 8.9 [95%CI, (1.8-43.9)], p=0.007 and AOR:6.5 [95%CI, (1.3-33.6)], p=0.025) higher in study subjects whose ages ranges between 30-44 years and those who are 45 or more years old than those in the younger age category, 18-29 years old , respectively. The risk of T2DM was about 4.5 and 6 times more higher in individuals with blood group B (AOR: 4.5 [95%CI, 4.5(1.8-11.8)], p=0.002*.) and blood group A AOR: 6.3[95%CI, 2.7-15.1], p=0.000), respectively compared to individuals with blood group O. The association of the blood groups A and B with T2DM was statistically significant.

Subjects who had family history of T2DM were thirty nine times more likely to be diabetic than those who had no familial history of T2DM (AOR: 38.7[95%CI, (16.7-89.8)], p=0.000**). The association was statistically significant. In this study, body mass index was significantly associated with the risk of T2DM. Overweight individuals were almost at six folds more likely (AOR: 5.6 [95%CI, (1.8-16.9) (25-29.9) to contract diabetes compared to those who were normal weight (18.5-24.9) BMI. In case of alcohol drinking habit, past-alcohol drinkers were five times more likely to develop diabetes than those who were non-alcoholic (AOR: 4.9[95%CI, 2.4- 10.4], p=0.000) (Table 5)

Table 5: Univariate and multivariate analysis of the potential risk factors associated with studied population.

Variables	Categories	COR (95%Cl)	P-value	AOR (95%Cl)	P-value
Age	18-29 yrs.	1		1	
category	30-44 yrs.	3.0(0.96-9.42)	0.049	6.5 (1.3-33.6)	0. 025**
	≥45 yrs.	6.9(2.3-20.9)		8.9(1.8-43.9)	0. 007**
Blood	A	5.3(3.1-9.3)		6.3(2.7-15.1)	0.000**
group	B	3.4(1.8-6.3)		4.5(1.8-11.8)	0.002**
	AB	1.7(0. .9-3.3)	0.000	2.1(0.8-5.6)	0. 125
	O	1		1	

Family history of T2DM	No	1		1	
	Yes	24.8(13.0-47.2)	0.000	38.7 (16.7-89.8)	0. 000**
Body mass index	N (18.5-24.99)	1		1	
	Ow ((25-29.9)	3.8(1.8-8.1)	0.000	5.6 (1.8-16.9)	0.000**
	UW<18.5)	3.88(1.5-9.6)		4.7(1.2-18.5)	0.025**
Alcohol drinking habits	Non-alco	1		1	
	Past-alco	4.4(2.7-7.2)	0.000	4.9(2.4- 10.4)	0.000**
	Alcoholic	0. 2(0. 02-1.8)		0.05(0.003-1.0)	0.051

4.5. Allelic frequency of ABO blood groups

The overall allelic frequencies of p (A), q (B) and r (O) were 0.276, 0.189 and 0.534, respectively. The frequencies of ABO blood group alleles in the population were in the order of r (O) (0.534) > p (A) (0.276) > q (B) (0.189) (Table 6).

Table 6: Allelic frequency of A, B and O in the overall study populations, case and control subjects.

Group	p (A)	q (B)	r (O)	Hardy-Weinberg Log likeli1hood	21A	*P* value
Overall	0.276 (0.018)	0.189(0.015)	0.534 (0.020)	-494.45	19.9751	0.0000
Case	0.357(0.028)	0.199(0.022)	0.443(0.030)	-232.54	0.0534	0.8173
Control	0.207(0.022)	0.181(0.021)	0.610(0.027)	-251.745	64.8811	0.0000

While the genotypic frequency were AA (p2=0.0762), AO (2pr=0.2948), BB (q2=0.0357), BO (2qr=0.2019), AB (2pq=0.1043) and OO (r2=0.2852). The frequency of p (A), q (B) and r (O) alleles among T2DM patients were 0.357, 0.199 and 0.443, respectively while the distribution was 0.207, 0.181 and 0.610 in controls, respectively.

The frequency of p (A) allele in the type-2 diabetes mellitus patients was higher and that of r (O) allele was lower relative to the control population (Table 7).

Table 7: The frequency of AA, AO, BB, BO, AB and OO blood genotypes in total, case and control population (Gene (allele); A (P); B (q); O (r)).

Study groups	Gene (allele)	Frequency	Genotype	Frequency	Phenotype	Frequency (%)
Overall						
			AA (p^2)	0.0762	A	7.62%
			AO(2pr)	0.2948	A	29.48%
			BB(q^2)	0.0357	B	3.57%
			BO(2qr)	0.2019	B	20.19%
			AB(2pq)	0.1043	AB	10.43%
			OO(r^2)	0.2852	O	28.52%
Cases						
			AA (p^2)	0.1275	A	12.75%
			AO(2pr)	0.3163	A	31.63%
			BB(q2)	0.0396	B	3.96%
			BO(2qr)	0.1763	B	17.63%
			AB(2pq)	0.1421	AB	14.21%
			OO(r^2)	0.1962	O	19.62%
Controls						
			AA (p^2)	0.0428	A	4.28%
			AO(2pr)	0.2525	A	25.25%
			BB(q2)	0.0328	B	3.28%
			BO(2qr)	0.2208	B	22.08%
			AB(2pq)	0.0749	AB	7.49%
			OO(r^2)	0.3721	O	37.21%

5. Discussion

Diabetes is a complex, chronic metabolic illness characterized by hyperglycemia, due to inadequate production of insulin or insufficient sensitivity of the cells to its action. It is a multi-factorial in nature and develops by interactions of genetic, epigenetic, immunological, life style and environmental related factors (Prasad and Groop, 2015). Type 2 diabetes mellitus (T2DM) remains a public health problem in low and middle-income countries, including Ethiopia. ABO-Rh blood group phenotypes are important for disease association and blood transfusion programs, population genetics studies. The association between ABO-Rh blood group systems has been examined in several with inconclusive findings. This study is designed to determine the phenotypic and allelic distribution of ABO and Rhesus (Rh) groups and its possible association with type 2-diabetes among follow up cases in Injibara General Hospital, Awi Zone, northwest Ethiopia.

In this study the mean age of the T2DM cases was 50.82 (SD $\pm$ 12.40) with minimum and the maximum of 18 and 80 years old. Most (72.93%) of T2DM cases were in the age category of 45 or more years old while the least (2.21%) were in the age category of 18-29 years of age. This finding agrees with the results of (Stankov *et al*.,2013) and WHO(2014) in which T2DM is more often associated with an increase in age and its onset is usually over the ages 35 years (Stankov *et al*., 2013).

Analysis of the overall distribution of ABO blood phenotypes of the studied population, revealed that group O was the most frequent phenotype while blood group AB was the least frequent; the phenotype frequencies with respect to ABO in this study can be represented as O>A>B>AB. Rhesus positive blood group was predominant, representing more than nine in ten (93.9%) of the entire population, while Rhesus-negative was less frequent. Likewise, the frequencies of ABO blood group alleles in the present population were in the order of r (O) (0.534) > p (A) (0.276) > q (B) (0.189) while the genotypic frequency were AA (p^2=0.0762), AO (2pr=0.2948), BB (q^2=0.0357), BO (2qr=0.2019), AB (2pq=0.1043) and OO (r^2=0.2852).

The distribution of ABO and Rhesus (Rh) groups varies with geographical settings and populations ethnicity worldwide. The predominance and less frequent occurrence of the O and AB phenotypes respectively is common in the majority of African populations (Hamed *et al*., 2012; Iyiola *et al*., 2012; Ndoula *et al*., 2014). The finding agrees with the reports of three

studies conducted in a southern (Tesfaye Kassahun *et al*., 2015) (Silte Zone), northwest (Mandafro Ayele and Musin Kelel, 2016) (Yilmana Densa) and western (Zerihun Teklu and Shiferaw Bekele, 2016) (Jimma) of Ethiopia. The present results were also consistent with the results reported from other different African countries Hamed *et al*. (2012) (Mauritania), Iyiola *et al*. (2012) (Cameroon) and Ndoula *et al*. (2014)) (Nigeria). Except in case of Nigerian population, where blood group B was the second most frequent. The result is inconsistent with ABO phenotype frequencies reported from the following studies. In studies from two Asian countries, India and Pakistan, the predominant occurrence of B followed by O, A and AB phenotypes was reported (Chandra and Gupta, 2012; Rehma *et al*. 2015). On the other hand, this distribution ABO phenotypes in Turkey population has A > O > B > AB pattern (Demir *et al*., 2006).

The distribution of the ABO blood phenotypes between type-2 diabetes patents and non-diabetic healthy controls ($P<0.05$) varied significantly ($p=0.000$), blood group A was predominant in cases while blood group O the most frequent in health controls. However, there was no statistically significant difference in the occurrence of Rh (+) and Rh (-) blood types between cases and healthy controls. The observed difference in the frequency of ABO blood phenotypes between patients and healthy controls might be due to small sample size, evolutionary selective pressure or has happened more likely by chance during sample collection.

In this study, the frequency of T2DM patients who reported family history of diabetes was significantly higher than those who had no family of history diabetes. On the contrary, more than ninety percent healthy individuals had no family history of diabetes. Individuals with a family history of diabetes had about thirty nine fold (AOR: 38.7[95%CI, (16.7-89.8)], $p=0.000$) increase in the prevalence of type 2 diabetes compared with individuals without a family history of the disease. This is in concordance with the reports of Cuasay *et al*. (2001) and Cederberg *et al*. (2015). An individual with a family history of type 2 diabetes with one or more first-degree relatives has a 30–70% increased risk of developing disease (InterAct, 2013; Wagner *et al*., 2013). This can be explained by the fact that a family history of diabetes is associated with a range of metabolic abnormalities and is a strong risk factor for the development of type 2 diabetes.

Likelihood of elevated risk of type 2 diabetes is mediated, in part, by both genetic and shared environmental components among family members (Groop *et al.,* 1996; Meigs *et al.,* 2000; van's Riet *et al*., 2010).

People who are overweight or obese are at increased risk for many serious diseases and health conditions, including type-2 diabetes. BMI has a strong relationship to diabetes and insulin resistance. In relation to this, the current study identified fairly higher percentage of overweight subjects among type 2 diabetic cases than healthy controls. Besides, an approximately six fold elevated likelihood of the disease was found among overweight individuals (AOR=5.6, CI: 1.8-16.9) (p=0.000).This agrees with the findings of Cuasay *et al*. (2001) and Tsirona *et al.* (2016).

The association between alcohol consumption and type 2 diabetes risk remains inconsistent in previous cross-sectional epidemiologic studies. Some studies showed that high alcohol intake increases diabetes risk among middle-aged men. However, more moderate levels of alcohol consumption do not increase risk of type-2 diabetes (Kao *et al*., 2001). Evidence from observational studies suggested a 30% reduction in risk of type 2 diabetes among moderate alcohol consumers, whereas no risk reduction was observed in heavy drinkers (Koppes *et al*., 2005). On the other hand, the findings of a cohort study conducted in general Danish population suggested that alcohol drinking frequency is associated with risk of diabetes and that consumption of alcohol over 3–4 days per week is associated with the lowest risk of diabetes, even after taking average weekly alcohol consumption into account (Holst *et al*., 2017). In case of alcohol consumption habit, the present study noted an approximately three fold higher (48.61%) past-alcoholic individuals among diabetic cases than the health controls (17.12%). However, the frequency of currently alcoholic subjects was substantially higher in healthy controls than type-2 diabetic individuals. Furthermore, past-alcohol drinking habit was associated with a five-fold increased risk of developing diabetes than non-alcoholic habit (AOR: 4.9[95%CI, 2.4- 10.4], p=0.000). This is in agreement with the findings of Tsirona and co-investigators (2016) that identified alcohol consumption as one of significant predictors of diabetes.

In accordance with other studies elsewhere, age was found to be an independent risk factor for T2DM in this study. The odds of the disease increases as age increases and the risk was about seven and nine higher in study subjects with age range between 30-44 years and among those who are 45 or more years old than those in the younger age category, 18-29 years old , respectively. This is in agreement with reports of studies conducted among Filipino-Americans in the Houston, Texas Metropolitan Statistical Area U.S.A(Cuasay *et al*., 2001) and among type 2 diabetes mellitus cases in a Greek adult population (Tsirona *et al*., 2016). In addition to well established risk factors, age-associated deteriorations in β cell function may contribute to type 2 diabetes risk. Type-2 diabetes develops only when β-cells fail to compensate for increased demand from insulin resistance. While, Type 2 diabetes (T2D) increases with age with the majority of patients being above the fifth decade of life (Koopman *et al*., 2005; Gong and Muzumdar, 2012).

The present did not find any association between Rh blood group systems and types 2 diabetes risk. This agrees with the results of several studies done in different countries of the world (Shrestha *et al*., 2014, Fagherazzi *et al*., 2015 ; Meo *et al*.,2016, Aggarwal *et al*. 2018, Khudhair and Al-Ganimi, 2018; Kehailou *et al*., 2019). Contrary to this, two studies conducted in Saudi Arabia (Alanazi *et al*., 2018) and Malaysia (Albaroodi *et al*., 2019) reported significant association of Rh group with diabetes.

In agreement with many published data, the present study found that blood groups B and A were significantly linked to five and six fold increased risk of developing diabetes, respectively compared to blood group O (Fagherazzi *et al*., 2015 ; Kehailou *et al*., 2019). Dodiya *et al*. (2016) and Mandal *et al*. (2018) documented the association of type 2 diabetes with particular blood phenotype, B and AB, respectively. However, many more studies reported no association ABO with the disease (Kamil *et al*., 2010, Khudhair and Al-Ganimi, 2018; Albaroodi *et al*., 2019). The possible explanation for these inconsistencies of results could be due to variations in the distribution of ABO blood groups among populations, ethnic groups, and sample size and study designs.

The mechanisms underlying the association between ABO blood phenotypes and type-2 diabetes are not clearly elucidated. It has been suggested that the human ABO locus may influence endothelial or inflammation markers, such as the von Willebrand factor (vWF) and the factor VIII(8) complex, present at higher levels in non- O individuals (Gill *et al.*, 1987).

In addition, ABO blood groups were associated with the plasma-soluble 1-cell adhesion molecule (ICAM-1) and TNF receptor (TNF-2 R2) (Barbalic *et al*., 2010). These markers were all associated with an increased risk of type 2 diabetes, providing a potential explanation for the observed relationships (Meigs et al., 2004; Thorand *et al*, 2006). In this study, the variable, sex, education, monthly income, occupation, marital status, glycemic level, and Rh factor were not associated with diabetes.

The major link between obesity and T2DM is insulin resistance. In the natural history of diabetes, obesity and insulin resistance precede abnormal glucose. Insulin resistance in both of these conditions is manifested by decreased insulin-stimulated glucose transport and metabolism in adipocytes and skeletal muscle. The genetic factors implicated in T2DM pathogenesis relate to a family history of the disease. Genetic polymorphisms involving insulin receptor genes expressed in the pancreatic β cells, e.g., PPRAG and IRS receptors, have been associated with T2DM pathogenesis. The mutations cause pancreatic β cell dysfunction, resulting in impaired insulin secretion or resistance. Mutations in other genes involved in the insulin transduction pathway, i.e., glucokinase and mitochondrial genes, also lead to the pathophysiological T2DM progression (Wu *et al*., 2014). Due to repressed postprandial insulin secretion reduced insulin sensitivity the pancreatic β cells are overworked, resulting in their apoptosis.

6. Conclusions and recommendations

6.1. Conclusion

Most of T2DM cases were in the age category of 45 or more years old while the least were in the age category of 18-29 years of age. Blood group O was the most frequent phenotype while blood group AB was the least frequent in the studies population and ABO blood group followed O>A>B>AB pattern . Rhesus positive blood group was predominant, while Rhesus-negative was the less frequent. In agreement with many published data, the present study found that blood groups B and A were significantly linked to five and six fold increased risk of developing diabetes, respectively compared to blood group O (Fagherazzi *et al*., 2015 ; Kehailou *et al*., 2019), Dodiya *et al*. (2016) and Mandal *et al*. (2018). The distribution of the ABO blood phenotypes varied significantly between cases and healthy controls; blood group A was predominant in cases while blood group O the most frequent in health controls.

The overall frequencies of ABO alleles in the present population were in the order of O (0.534) > A (0.276) > B (0.189) while the genotypic frequency were AA (0.0762), AO (0.2948), BB (0.0357), BO (0.2019), AB (0.1043) and OO (0.2852).

Significantly higher percentages of T2DM cases were overweight, former alcoholic and had family history of diabetes than healthy individuals. In the current study, age, ABO blood groups, family history, BMI, and alcohol drinking habits were found to be independent determinants of T2DM. However, the variables, sex, education, monthly income, occupation, marital status, glycemic level, and Rh factor were not associated with diabetes.

6.2. Recommendations

Based on the results of the present study, the following recommendations are forwarded:-

Raising the awareness of ageing, overweight and obese individuals toward the risk factors of the disease and its outcome are necessary in the diabetic patients and general population. Early precautions and consultancy service should be granted to individuals having family history of diabetes and aging segment of the population. Further molecular and pathophysiological studies are needed to elucidate plausible biological association particular blood type with the risk of type 2 diabetes.

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APPENDICES

Appendix A English version of the questionnaire

Bahir Dar University

College Of Science

Department Of Biology

Questionnaires to be fill by T2 DM patients Respondent's code----------

Dear respondents:

The main objective of this questionnaire is to collect information about risk of type-2 diabetes mellitus in relation to ABO blood group and Rh factor from diabetic patients attending in Injibara General Hospital towards the type-2 diabetic disease. Your responses are confidential and are not used for other purposes rather than this study. Therefore in order to obtain relevant and reliable information that would contribute to the success of this study your participation is vital and I kindly request your cooperation to answer all the questions frankly.

Thank you!!!

Don't write your Name

Part A. **Socio-demographic factors**

1. Sex A, Male B , Female
2. How old are you? ----------
3. Blood group type A. A B. B C. AB D. O
4. Rh factor A. Positive B. Negative
5. Your marital status A. single B, married C, divorce D, widow
6. Your level of education A. Illiterate B. can read and write C. primary school completed D. secondary school completed E. Diploma and above
7. Your family average monthly income in birr ----------
8. Your occupational status A. Student B. House wife C. Merchant D. Farmer E. Private employee F. Gov. Employee
9. Do you have a familial history of DM2? A. yes B. no
10. Anthropometric measurement a. Height in meter ---------- b. Weight in kg------ c. Body mass index (BMI) ----------
11. Cigarette Smoking habit A. current Smoker B. Past smoker C. None smoker

12. Alcoholic drinking habit A. Alcoholic B. Past alcoholic C. None alcoholic

13. Do you walk (Exercise) regularly? A. Yes B. No

14. If your response is yes for question 13, how often?

A. 30 minutes per day or 150 minutes per week

B. Less than 30 minutes per day or less than 150 minutes per week

C. More than 30 minutes per day or more than 150 minutes per week

D. I had never the habit of physical Exercise

15. Your current blood sugar level? A. 80-130mg/dl B. >130mg/dl

Part B: Questions on Knowledge of DM2 patients about the disease and potential risk factors so please circle the letter corresponding to your response.

1. Is T2 DM communicable disease? A. Yes B. No
2. What is T2 Diabetes mellitus?

A. It is an unusually elevated and uncontrolled blood Sugar level.

B. It is a hereditary disease of obese individuals.

C. Disease in which the body's cells fail to respond appropriately to insulin.

D. All. E. I do not know

3. What are the major risk factors associated to increase the severity of T2 DM?

A. Age B. Obesity C. Unhealthy life style D. Physical inactivity E. all F. I don't know

4. What are the symptoms of type 2 Diabetes mellitus? A. very thirst B. frequent urination C. deep hungry D. unusually increased levels of glucose in the blood E. all G. I don't know
5. What should be done to manage T2 DM? A. monitoring blood sugar level B. Strict control of HBP C. Control of lipids D. Diet modification E. Physical exercise F. all G. I don't know
6. The regular intake of which of the following food staff is advisable for DM2 patients?

A. Vegetables B. high energy density foods C. foods rich in fat D. I don' know

7. The regular and unusually much intake of which of the following food staff should be avoided by T2DM patients?

A. vegetables B. high energy density foods C. foods rich in fat D. B and C E.I don' know

8. What are the complications of T2DM?

Part C: Questions related to the Attitude of T2 DM patients about the Disease, so Answer the following questions by marking (√) on 1= strongly Disagree, 2= Disagree, 3= undecided, 4= agree and 5= strongly agree.

No	Items	Alternatives				
		1	2	3	4	5
1	T2 DM is curable disease.					
2	T2 DM is more fatal disease.					
3	Proper taking T2 DM medication in prescribed by Physician helps to treat the disease.					
4	T2 DM is non -communicable disease.					
5	Eye check -up and urine test helps to the diagnosis of T2 DM.					
6	Blood glucose level will fall below normal when one takes drugs.					
7	Following controlled and planned diet is used to manage T2 DM.					
8	Obesity and high blood pressure are the risk factors to worsen severity of T2 DM**.**					

Appendix B consent form

Dear participants: Code----------

My name is Lamesgin Sinishaw Wondim. I am Master of Science student at Bahir Dar University, College of science. I conducted academic research in partial fulfillment of the requirements for the degree of Master of Science to determine the Risk of Type-2 Diabetes Mellitus in Relation to ABO Blood Group and Rh factor in Injibara General Hospital, Awi Zone. Therefore, you are invited to be a participant of this study by giving response to questioners relating this issue. If you agree to participate in this study please put your answers for questioners given to you.

Participation in this study is completely voluntary and you have the right to refuse from participating in this study at any time and refusal to participate will not result loss of any benefits you receive.. If you have under stood the explanation, I kindly request you to participate in the study.

Thank you in advance for your cooperation!!!

Put your signature as illustrated below

Participant's response:

"I am clear about the study and agree to participate"

Participant 'signature---------- date----------

Appendix C: - Amharic version of the questionnaire

በሁለተኛው አይነት የስኳር በሽታ ህመምተኞች አማካኝነት ለመሞላት የቀረበ መጠይቅ

ባህርዳር ዩኒቨርስቲ

ሳይንስ ኮሌጅ

ስነ-ህይወት ትምህርት ክፍል

ውድ የጥናቱ ተሳታፊወች:

የዚህ መጠይቅ ዋና ዓላማ በእንጅባራ አጠቃላይ ሆስፒታል የሚጠቀሙ የሁለተኛው አይነት የስኳር በሽታ ህመምተኞች ስለበሽው ያላቸውን ግንዛቤ እና በሽታውን የሚያበብሱ ሁኔታዎችን የሚለይ ትግበራ ማሰስ ነው።ለዚህ መጠይቅ የምትሰጧቸው መልሶች ከጥናቱ አላማ ውጭ ለየትኛውም ተግባር የማይውሉ መሆኑን እርግጠኛ መሆን ይኖርባችኋል።በመሆኑም ለጥናቱ መሳካት የእናንተ ተሳትፎ ወሳኝ መሆኑን አውቃችሁ ከዚህበታች ለተዘረዘሩት ጥያቄዎች ግልጥመልስ በመስጠት እንድተባበሩኝ ስንል በአክብሮት እንጠይቅወታለን።

ስለ ትብብርዎ እናመሰግናለን!!!

1ኛ. የአጠቃላይ ሁኔታ ጥያቄዎች

1. **ፆታ ሀ ወንድ ለ ሴ ት**
2. **እድሜ** .....
3. **የጋብቻ ሁኔታ? ሀ ያላገባ (ች) ለ ያገባ (ች) ሐ አገብቶ የፈታ መ አግብታ የፈታች**
4. **የደም አይነት ሀ, A ለ, B ሐ, AB መ, o**
5. **አር ኤች አይነት ሀ. አዎንታዊ ለ አሉታዊ**
6. **የትምህርት ደረጃ ? ሀ.ማንበብ እና መጻፍ የማይችል ለ ማንበብ እና መጻፍ የሚችል**

   ሐ አንደኛ ደረጃ ትምህርት የአጠናቀቀ መ ሁለተኛ ደረጃ ትምህርት የአጠናቀቀ

   ሠ ዲፕሎማ እና ከዚያ በላይ
7. **የቤተሰብ የገቢ መጠን በኢትዮጵያ ብር**.....?
8. **የስራ ሁኔታ? ሀ ተማረ ለ የቤት እመቤት m ሐ ንግድ መ አርሶ አደር**

   ሠ በግል የተቀጠረ ረ በመንግስት የተቀጠረ

9. በቤተሰብ ውስጥ የሁለተኛው አይነት የስኳር በሽታ የለበት ዘመድ አለዎትን ?
ሀ. አዎ ለ. የለኝም

10. የሰውነት መለኪያዎች

ሀ. ቁመት በሜትር .....

ለ. ክብደት በኪሎግራም.....

ሐ. ክብደት በካሬ ቁመት ንጥጥር (ኪ.ግ/ሜ.....

11. ሲጋራ የማጨስ ልምድ? ሀ. አጨሳለሁ ለ. በፊት አጨስ ነበር ሐ. አላጨስም

አልኮል የመጠጥ ልምድ ሀ. እጠጣለሁ ለ. በፊት እጠጣ ነበር ሐ. አልጠጣም

12. በተከታታይ የአካል ብቃት ያደርጋሉ? ሀ. አዎ ለ. አላደርግም

13. ለጥያቄ ቁጥር አስራ ሁለት መልስዎ አዎ ከሆነ ለምን ያህል ጊዜ እና ደቂቃ እንቅስቃሴ ያደርጋሉ?

ሀ በቀን 30 ደቂቃዎች ወይም በሳምንት 150 ደቂቃወች

ለ በቀን ከ30 ደቂቃዎች በላይ ወይም በሳምንት ከ150 ደቂቃወች በላይ

ሐ በቀን ከ30 ደቂቃወች በታች ወይም በሳምንት ከ150 ደቂቃወች በታች

መ እንቅስቃሴ የማድረግ ልምዱ የለም

14. የደምህ የስኳር መጠን በሚሊ ግራም ስንት ነው? ሀ, 80-130mg/dl ለ, >130mg/dl

2ኛ. የእውቀት መጠይቅ

15. ሁለተኛው አይነት የስኳር በሽታ ተላላፊ በሽታ ነው ትላለህ (ሽ)?
ሀ. አዎ ለ. አይደለም

16. ሁለተኛው አይነት የስኳር በሽታ ምንድን ነው?

ሀ. በደም ውስጥ የስኳር መጠን መጨመር የሚስከትለው በሽታ ነው

ለ. ከፍተኛ የሰውነት ክብደት ያላቸውን ሰዎች የሚጠቃ በሽታ ነው

ሐ. የሰውነት ህዋሳት እንሱሊን ቅመም ማምረት ሲቆሙ፣በአነስተኛ መጠን ሲመርቱ እና አጠቃቀማቸው ላይ ችግር ሲፈጠር ሊመጣ የሚችል በሽታ ነው

መ. ሁሉም ሠ. አላውቀውም

17. ይህን በሽታ ወደ ከፋ ሁኔታ ሊያደረሱ የሚችሉ ሁኔታወች የትኞቹ ናቸው?

ሀ. የእድሜ መጨመር ለ. ከመጠን ያለፈውፍረት ሐ. ጤናማ ያልሆነ የአኗኗር ሁኔታ

መ. የአካል ብቃት እንቅስቃሴ አለማድረግ ሠ. ሁሉም ረ. አላውቀውም

18. ከሚከተሉት ውስጥ የሁለተኛው አይነት የስኳር በሽታ ምልክት የቱ ነው?

ሀ. ፈጣን ውሀ ጥም ለ. ቶሎ ቶሎ መሽናት መ. የደም ስኳር መጠን መጨመር

ሐ. ከባድ ርሀብ ሠ. የክብደት መቀነስ ረ. ሁሉም ሰ. አላውቀውም

19. የዚህን በሽታ ጉዳት ለመቀነስ እና እንደ ጤናማ ሰው ለመኖር መደረግ ከሚገባቸው ነገሮች ውስጥ የትኞውይመከራሉ ?

ሀ. የደም የስኳር መጠንን ማስተካከል

ለ. የደም ግፊትን መቆጣጠር

ሐ. ከፍተኛ የሆነ ቅባት አለመመገብ መ. አመጋገብን ማሻሻል

ሠ.የአካል ብቃት እንቅስቃሴማድረግ ረ. ሁሉም ሰ. አላውቀውም

20. በዚህ በሽታ የተያዘ ሰው በተከታተይ እንዲመገበው የሚመከረው የምግብ አይነት የቱ ነው?

ሀ. አትክልት ለ. ከፍተኛ ሀይል ሰጭ ምግቦች ሐ. ቅባት የበዛባቸው ምግቦች

መ. አላውቀውም

21. በዚህ በሽታ የተያዘ ሰው በተከታተይ እንዳይመገባቸው የሚመከሩ የምግብ አይነቶች የትኞቹ ናቸው? ሀ. አትክልት ለ. ከፍተኛ ሀይል ሰጭ ምግቦች

ሐ. ቅባት የበዛባቸው ምግቦች መ.ለእናሐ ሠ.አላውቀውም

22. የሁለተኛው አይነት የስኳር በሽታ ተጽናዎች ምንድን ናቸው.....?

3ኛ. ከሚከተሉት የሁለተኛው አይነት የስኳር በሽታ የአመለካከት መጠይቅ መልስ ይሆናል በሚሉት ቦታ ላ ይ የራይት ምልክት (√) ያስቀምጡ፡፡ 1= በጣም አልስማማም ፣ 2=አልስማማም ፣ 3= አልወሰንሁም ፣ 4= እስማማለሁ እና 5= በጣም እስማማለሁ፡፡

ተ.ቁ	ዝርዝር ተግባራት	አማራጮች				
		1	2	3	4	5
23	ሁለተኛው የስኳር ህመም መዳንየሚችል በሽታ ነው፡፡					
24	ይህ የስኳር ህመም በጣም ገዳይ በሽታ ነው፡፡					
25	በሀኪምዎ ትዛዝ መሰረት መድሀኒት መውሰድ ለማገገም ይረዳል፡፡					
26	ሁለተኛው የስኳር ህመም ተላላፊ አይደለም፡፡					
27	. የአይን እና የሽንት ምርመራ በማድረግ በሽታውን መለየት ይቻላል፡፡					
28	መድሃኒት በምንወስድበት ጊዜ በደማችን ውስጥ ያለው የስኩር መጠን ከትክክለኛው መጠን ይቀንሳል ፡፡					
29	የአመጋገብ ሁኔታን ማስተካከል በሽታውን ለመቆጣጠር ይረዳል፡፡					
30	በሽታውን ክብደት እና ከፍተኛ የደም ግፊት የሚያበብሱ ሁኔታዎች ናቸው፡፡					

Appendix D: - Amharic version of consent form

የስምምነት ማረጋገጫ

ውድ ተሳታፊዎች

መለያ ቁጥር.....

እኔ በባህርዳር ዩኒቨርስቲ በሳይንስ ኮሌጅ ትምህርት ክፍል የሁለተኛ ዲግሪ ተማሪ ስሆን የመመረቂያ ጽሁፍ በመስራት ላይ አገኛለሁ፡፡ የጥናቱ ዋና ዓላማ በእንጅባራ አጠቃላይ ሆስፒታል የሁለተኛው አይነት የስኳር በሽታን የሚያበብሱ ሁኔታዎች መለየት ነው፡፡ስለዚህ ለዚህ ለጥናቱ መሳካት በፈቃደኝነት ላይ የተመሰረተ ተሳትፎ ከፍተኛ አስተዋጽኦ ይኖረዋል፡፡አንተ(ች) የምትሰጠው(ጭው) ማነኛውም ዓይነት መረጃ ለጥናታዋ ፅሁፍ ካልሆነ በስተቀር ለሌላ አገልግሎት የማይውል መሆኑን ሙሉ እግጠኛ መሆን ይኖርባችኋል፡፡በመሆኑም ለጥናቱ መሳካት የእናንተ ተሳትፎ ወሳኝ መሆኑን አውቃችሁ ግልጥ መረጃ በመስጠት እንድተባበሩኝ ስንል በአክብሮት እንጠይቅወታለን፡፡

የተሳታፊ ፊርማ..... ቀን .....

ስለ ትብብርዎ እናመሰግናለን!!!

Appendix E: - Ethical clearance

ሳይንስ ኮሌጅ
የድህረ ምረቃ፣ምርምርና ማህበረሰብ አገልግሎት ም/ዲን
ባሕር ዳር ዩኒቨርሲቲ
ባሕር ዳር - ኢትዮጵያ

Wisdom at the source of the Blue Nile

Science College
The Graduate, Research & Community Services V/Dean
Bahir Dar University
Bahir Dar – Ethiopia

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website: www.bdu.edu.et

ቁጥር:PGRCSVD/116/2012 ዓ.ም
ቀን : 17/09/2012 ዓ.ም

Ethical Clearance Approval Form

Applicant's Name: Lamesgin Sinishaw

Research Title	Risk of type-2 diabetes mellitus in relation to ABO blood group and Rh factor: a case-control study
Researcher (s) Name (s)	Lamesgin Sinishaw

Thank you for submitting your application for ethical clearance, which was considered at the College of Science Research Ethics Committee meeting on 25 May 2020. The committee has reviewed your ethical application, issues pertaining to participants, consent form, debriefing, and relevant questionnaires.

The researcher should keep the confidentiality of the identity of research participants and data that will be obtained from them. Any serious adverse events or significant changes which occur in connection with this study and /or which may alter its ethical consideration must be reported immediately to the committee for a possible ethical amendment.

We are therefore pleased to inform you that the College's Ethical Clearance Committee has approved your study from an ethical point of view.

With kind regards

Dr. Tsegaye Kassa Gogie
P.G.R.C.S. Vice Dean

Office of Research & Community Services
Bahir Dar University College of Science

CC//

- Dean office
- The Graduate, Research and Community Services V/Dean
- Department of Biology

College of Science

Appendix F**. ABO blood group**s **and Rh** factors **identification.**

Injibara General Hospital

Observing blood sample in test tube **using centrifuge**

Blood groups shaking using shaker **observing the result after shaking**