



University of Babylon
College of Medicine

Study the Role of Cytomegalovirus Infection and Autoimmunity in Patients with Type 1 Diabetes Mellitus

A thesis

Submitted to the council of the college of medicine.
University of Babylon in partial fulfillment of the
Requirements for the degree of Master of science in
Medical Microbiology

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1430 A.H

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

((قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا

عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ))

صَدَقَ اللَّهُ الْعَلِيُّ الْعَظِيمُ

سُورَةُ الْبَقَرَةِ

آيَةُ (32)

Dedication

To soul of my mother and father;

To my brother

ACKNOWLEDGMENTS

I would like to express my thanks to "ALLah" the most Gracious and most merciful.

It is a pleasure to express my deep appreciation to my supervisors **Dr. Mohammed Aboud** and to **Dr. Mohammed Sabri** for their valuable guidance, assistance cooperation, throughout the course of preparing my thesis .

I am thankful to **Dr. Ali Khair-Allah**, Dean of college of medicine and **Dr. Hussien Naji** assistant of Dean of College of Medicine ,Babylon university for providing all the needed facilities which are essential for successful completion of the present work.

I would like to thank **Dr. Habeeb S. Naher** and all Teachers and staff of Department of Microbiology in the Medical College in Babylon university.

Sadeq



كلية الطب

دراسة دور الإصابة بفايروس انتفاخ الخلية والمناعة الذاتية في مرضى السكري النوع الأول

رسالة

مقدمة الى مجلس كلية الطب/ جامعة بابل
كجزء من متطلبات نيل درجة الماجستير
في علم الاحياء المجهرية الطبية

تقدم بها

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ABBREVIATION

Symbol	DESCRIPTION
DM	Diabetes mellitus
ADA	American diabetes association
NDDG	National diabetic data group
ECDCM	Expert committee on the diagnosis and classification of diabetic mellitus
UKPDS	United kingdom prospective diabetes study world health organization
WHO	World health organization
USA	United State of America
EMR	Eastern Mediterranean region
OGTT	Oral glucose tolerance test
IDDM	Insulin dependent diabetes mellitus

NDDM	Non insulin dependent diabetes mellitus
HLA	Human Leukocyte antigen
MHC	Major histocompatibility complex
BMI	Body mass index
TNF	Tumor necrosis factor
UK	United kingdom
GAD	Glutamic acid decarboxylase
CMV	Cytomegalovirus
T1D	Type 1 diabetes
CRS	Congenital rubella syndrome
EBV	Epstein –Barr virus

IFA	Immune fluorescent antibody
ELISA	Enzyme linked immune sorbent assay
AICA	Anti islet cell antibody
Ab	Antibody
IGT	Impaired glucose tolerance
TMB	Tetra Methel Benzoat
HRP	Horse Reddish Peroxidase

Summary

Chronic Cytomegalovirus (CMV) infection were reported in several epidemiological studies as one cause of type 1 diabetes mellitus (T1DM) by number of clinical studies.

The aims of this study were to investigate the role of CMV as one of the causative factors for type 1 DM in different age groups compared with healthy control.

This study was conducted in the center of Endocrinology and Diabetes and National Diabetes Center in AL-Karkh Health directorate during the period from August 1st 2008 through March 2009.

The study population consisted of 120 patients (60 male, 60 female) all of them were recently diagnosed with type 1 DM Diabetic patient and healthy control were tested for anti CMV (IgM,IgG), antiGAD antibody, anti islet cell antibody. Fasting blood glucose level were also tested for healthy and patients group.

There were association between chronic CMV infection and occurrence of T1D mirrored by level of anti GAD antibody titer and anti islet cell antibody in different age group. There is increase

in titre of anti GAD and anti Islet cell antibody in correlation with increased in titre of anti CMV IgG antibody

Age group (6months-10ys),(11-20ys) show highly association between chronic CMV infection and occurrence of type 1 autoimmune diabetes when compared to other age group.

Anti GAD antibody titre had show high association to anti CMV IgG antibody titer more than anti islet cell antibody except in old age group anti islet cell antibody explained association with anti CMV IgG antibody more than anti GAD antibody , and the level of GAD antibody begin to decreased with increase in age.

Anti CMV IgM antibody not effect on auto immunity in T1D only indicate reactivation of infection or acute infection.

Difference in sex has no effect on CMV infection.

This illustrate the role of chronic CMV infection as one of the environmental factor for type 1 DM in different age groups.

Further studies are needed to determine the role of other virus as environmental factor for type 1 DM.

1-1 INTRODUCTION

Type one auto immune diabetes was previously encompassed by the terms insulin – depending diabetes, type 1 diabetes, or juvenile – onset diabetes result from autoimmune mediated destruction of the beta cell of the pancreas. The rate of destruction is quit variable, being rapid in some individuals and slow in other (Gulay et al ,2007).

The rapidly progressive form is commonly observed in children, but also may occur in adult (Gilliam et al ,2005)

The slowly progressive form generally occurs in adults and is sometimes referred to as latent auto immune diabetes in adult (LADA) some patients particularly children and adolescents, may present with ketoacidosis as the first manifestation of the disease. Others have modest fasting hyperglycemia and or ketoacidosis in the presence of infection or stress. Still other, particularly adults may retain residual. Beta cell function, sufficient to prevent ketoacidosis, for many years (Humphray et al ,1998),Individuals with this form of type 1 diabetes often become depending insulin for survival eventually and are at risk for ketoacidosis (Willis et.al 1996) at this stage there is little or no insulin secretion as manifested by low or undetectable levels of plasma C-peptide (Hathout et al ,2003).

Marker of immune destruction including islet cell auto antibodies and/or auto antibodies to glutamic acid decarboxylase(GAD) are present in 85-90% of individuals with type 1 diabetes mellitus when fasting diabetic hyperglycemia is initially detected (verge et al , 1996).

There is a genetic predisposition to autoimmune destruction of beta cell and it is also related to environmental factors, One of these environmental factors is infection with some type of virus. Certain viruses have been associated with beta cell destruction. Diabetes occur in some patient with congenital rubella, coxsackie B virus, cytomegalovirus(CMV),and other viruses(e.g adenovirus and mumps) have been implicated in inducing the diseases (Akerblom et al ,2002). CMV infection can cause B-cell damage including degranulation and the characteristic inclusion bodies have been found in the B-cell of children who died with disseminated CMV (Jensen ,1990). Moreover, case report have described IDDM developing in CMV-infected individual .Antibodies against CMV have been shown to cross react with islets cells(Pak et.al,1990).which probably shares antigenic determinants with CMV protein.nigro et al found that 35%of newly diagnosis IDDM patient showed serological evidence of recent CMV infection (Zimmet ,1995)).and that the percentage of these patients who had islet cell and GAD antibodies was similar to that reported by pak et al.nicoletti et al observed statistically significant association between high titer of IgG antiCMV antibodies and islet-cell auto antibodies in IDDM patient (Nicoietti et al , 1990) They suggested that chronic CMV infection may be associated with the production of islet cell and GAD auto antibodies .

AIMS OF STUDY

- 1- Explaining the role of chronic CMV virus infection as an environmental factor for development of type 1 DM in different age groups.
- 2- Explaining the role of chronic CMV infection in the formation of GAD antibody and islet cell antibody as one cause of type 1 diabetes in different age groups.
- 3- Explaining the role of age in CMV infection and anti GAD and islet cell antibody formation.

1.2 Diabetes Mellitus (DM):

1.2.1 Definition:

Diabetes mellitus (DM) is heterogeneous group of metabolic disorder characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, protein, water & electrolytes metabolism resulting from defects in insulin secretion, insulin action or both (Shaheed and james,2006).

Symptoms

- Polyuria due to the osmotic diuretic
- Thirst due to loss of fluid and electrolytes
- Weight loss due to fluid depletion and accelerated breakdown of fat (Albret , 1998)

Complication

- Staphylococcal skin infection
- Retinopathy noted during visit to the optician
- Polyneuropathy causing tingling and numbness in the feet
- Impotence
- Arterial disease resulting in myocardial infarction or peripheral gangrene (Engelgau et al , 1997)

1.2.2 Classification of Diabetes mellitus:

The etiologic classification of (DM) currently recommended by WHO and the American Diabetes Association (ADA). This

classification differs considerably from the previously recommended classification which used the terms insulin-dependent diabetes (IDDM) and non insulin – dependent diabetes (NIDDM) (WHO ,1999).

The Etiologic Classification of (DM) Currently Recommended by
WHO and the American Diabetes association (ADA, 1999)

I.Type 1 diabetes (B cell destruction, usually leading to absolute insulin deficiency)

A. Immune-mediated B. Idiopathic

II.Type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance)

III. Other specific types

- A. Genetic defects of B cell function
- B. Genetic defects in insulin action
- C. Diseases of the exocrine pancreas
- D. Endocrinopathies

III. Other specific types

E. Drug- or chemical-induced

F. Infections

- 1. Congenital rubella
- 2. Cytomegalovirus
- 3. Others

G. Uncommon forms of immune-mediated diabetes

H. Other genetic syndromes sometimes associated with diabetes

- 1. Down's syndrome
- 2. Klinefelter's syndrome
- 3. Turner's syndrome
- 4. Wolfram's syndrome
- 5. Friedreich's ataxia
- 6. Huntington's chorea
- 7. Laurence-Moon-Biedl syndrome
- 8. Myotonic dystrophy
- 9. Porphyria
- 10. Prader-Willi syndrome
- 11. Others

IV. Gestational diabetes mellitus (GDM)

1.2.3 Type 1 DM:

During the past two decades, accumulating information has led to the recognition that the predominant form of IDDM is of Immune etiology, that the disease can present at any age and that individuals are not insulin dependent early in the course of disease (ECDCM ,2003).

The ADA (1997) had divided the idiopathic forms of diabetes resulting from B-cell loss and / or severe insulin secretory deficiency into Type 1 A and Type 1B.

Type 1A is the immune — mediated form and is best characterized by the presence of Islet autoantibody and insulinitis with selective destruction of Islet B cells (Foulis et al.,1991). Type 1A diabetes is strongly associated with specific human leukocyte antigen (LA) alleles and almost always progresses to severe insulin deficiency (Nepom and Kwok, 1998).

The term Type 1B diabetes is a reserved form of diabetes with severe insulin deficiency without evidenced of B cell autoimmunity (ADA, 1999).

An interesting form of potential Type 1B diabetes was reported in Japan and this type is characterized by lymphocytic infiltration of the exocrine pancreas with increased serum amylase level and an acute development of diabetes (Imaqawa et al., 2000).

1.2.4 Type 2 (DM):

Is the commonest form of diabetes accounting for 85 — 95 % of all cases worldwide National Diabetes Data Group (NDDG)

Chapter one

Introduction and literature Review

(NDDG, 1979) and its global prevalence is increasing rapidly as a consequence of the westernized life — style? It is destined to become one of the most important and costly disease Expert Committee on the Diagnosis and Classification of DM (ECDCM) (ECDCM, 2001).

Type 2 diabetes is characterized by disorders of both insulin action and insulin secretion both are usually present at time of diagnosis and either may be the prominent feature (Zalloua et al, 2002). Type 2 diabetes often remains undiagnosed for many years because hyperglycemia is often not severe enough to provoke noticeable symptoms of diabetes and many patients have only moderately raised blood glucose level (Harris, 1989). Ketoacidosis is infrequent in this type of diabetes, it occurs spontaneously only very rarely and usually arises in association with the stress of another illness (Umbierrez et al.,1995).

People with type 2 diabetes do not need insulin treatment to survive and some can be treated for many years with diet and exercise alone or in combination with antidiabetic tablets United kingdom prospective diabetes study(UKPDS)(UKPDS, 1995)

1.3 Epidemiology:

1.3.1 Epidemiology of Type I (DM):

Most studies of type 1 diabetes have considered only the disease occurrence in children typically under the age of 15 years (Mordes et al,1996).

Type 1 diabetes rarely occurs during the first of life and the incidence rises sharply until age 12–14 years and then declines (Neu et al,2007).

The magnitude of the geographic variation in the occurrence of type1 diabetes is very large according to a recent compilation of data from 100 studies conducted in 50 countries (Patrick et al., 1989).

The lowest age adjusted incidence rates for the population on aged 0-14 years are in Asia , the Caribbean and Latin America ranging from 0.1 - 3.5 per100.000 per year, and the highest are in Nordic countries. The United Kingdom,

Canada , New Zealand , Portugal and Sardinia ranging from 21.2 - 36.8 per 100.000 per year and the incidence rate in white population in United States range from 11.7-16.4 per 100.000 per year (Karvonen et al., 2000).

Diabetes epidemiology research international group (1988) reported that the incidence rate in United States for non whites is lower than that for whites in the population aged 0-14 years. The

Incidence rate in the United States during the first three decades of the 20th century in white population younger than age 15 years was fairly constant however during the next three decades the rate almost tripled (Onkamo et al,1999)

The incidence rate for children younger than age 15 years in Oslo , Norway rose from 6.2 per 100000 per year for the years 1925 to 10.8 per 100000 per year for the year 1956 and then to 1981).

In Finland the incidence rate for children aged 1-14 years sharply increased reaching a value of 44.8 per 100.000 per year in 1996 and the increase was greatest in the age group 1-4 years (tuomilehto et al., 1999).

During the period 1989 to 1994 (Uorodiab. Ace study Group 2000) reported that the average annual increase in incidence rate in European countries was 3.4 % overall, but it varied among centers and by age. As in finish population the increase was 6.3 % for age group 0-4 years and lowest for age group 10-14 years.

Seasonal variability diagnosis of type 1 diabetes attracted much attention in the 1970s (Gleason et al., 1982). In most population the peak incidence were in winter months. It was statistically significant (Levy — Marchal, 1995).

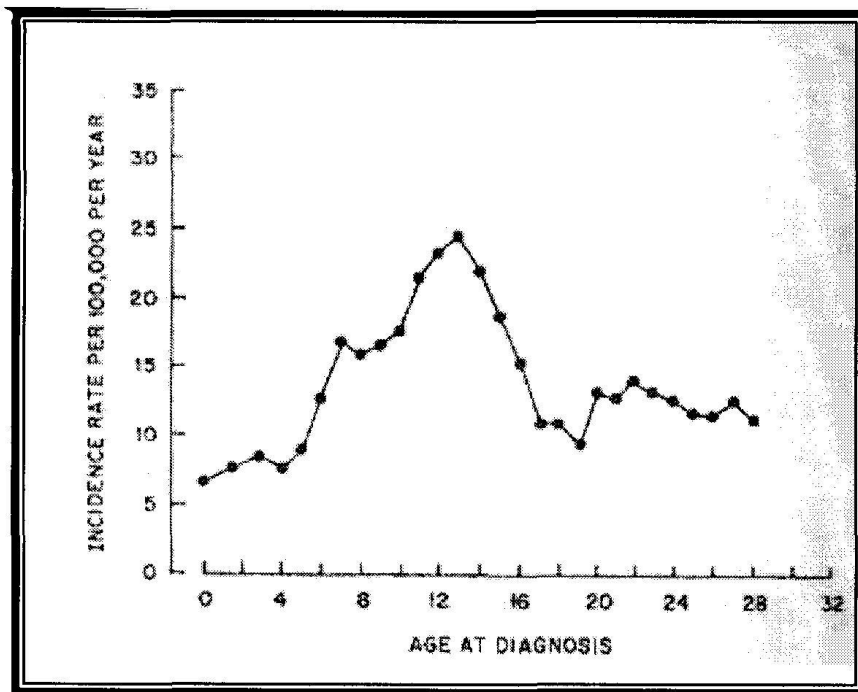


Figure (1-1): Incidence rate of Type 1 diabetes according to individual years of age (Christau et al., 1979)

1.4 Diagnostic criteria for (DM):

If a patient has symptoms such as thirst, polyuria, unexplained weight loss, drowsiness or coma and marked glucose urea. The diagnosis of diabetes can be established by demonstrating fasting hyperglycemia (Gavin et al., 1997). Oral glucose tolerance test (OGTT) is required to confirm or exclude the diagnosis of diabetes, if fasting glucose concentration is in the diagnostic range for diabetes, an OGTT is not required for diagnosis (WHO, 1999).

Table (1-1): Diagnostic criteria for diabetes mellitus and related stages of Glycemia (Genuth et al., 2003).

	Glucose concentration mg/dL (m mol/L)	
	Capillary whole blood	Venous plasma
Diabetes mellitus		
Fasting	≥ 110 (≥ 6.1)	≥ 126 (≥ 7.0)
or		
2-hour postglucose	≥ 200 (≥ 11.1)	≥ 200 (≥ 11.1)
Impaired glucose tolerance		
Fasting (if measured)	< 110 (< 6.1)	< 126 (< 7.0)

and		
2-hour postglucose	140-199(7.8-11.0)	140-199(7.8-11.0) 1

Chapter one

Introduction and literature Review

1.5 Etiology of Diabetes Mellitus:

1.5.1 Type 1 DM:

1.5.1 .1 Genetic Factor:

Studies in monozygotic (Identical) twins suggest that the genetic component accounts for 30-40 % of the total risk of developing IDDM (Atkinson and Maclaren, 1994).

Inheritance of susceptibility to IDDM does not depend on a single gene but is determined by several genes at different loci (Todd et al., 1981). The most important appears to reside in the HLA major histocompatibility complex (MHC) class II region on the short arm of chromosome 6, this locus account for the genetic susceptibility. Other susceptibility genes of lesser important include the region of the insulin gene itself on the short arm of chromosome 11 and the a region on the long arm of chromosome 2 (Davies et al., 1994). The first evidence of allelic association between the HLA complex and type 1 diabetes was reported for the HLA- B15 allele (Singal and Blajchman, and in other studies, a positive association of type 1 diabetes with HLAB8 and negative association with HLA B7 were established (Nerup and Pociot , 2001).

Higher risk were shown to be associated with HLA class II specificities namely DR3-DR4 where as DR2 (now named DRI5) was suggested to involved protection (Rewers et al ,2002), and DR3 is in linkage disequilibrium with B8 and DR4 is in

disequilibrium with B15 and more than 90% of white patients with type 1 diabetes have DR3 and / or DR4 as compared with 40 % to 50 % of control (Wassmuth and Lemmark,1989). The greatest risk of type 1 diabetes is conferred by the simultaneous presence of DR3 and DR4 which are found together in 30 % to 50 % of patients but in only 1% to 6% of controls (Deschamps et al., 1991).

1.5.1 .2 Environmental Factors:

Type 1 diabetes is the result of an unfavorable interaction between environmental factors and an inherited predisposition to the disease (Atkinson and Maclaren, 1994).

The environmental factors that might lead to type 1 diabetes had received less attention than the genetic factors even though individual susceptibility depends more on the environment than on the inherited predisposition (Fohlman and Frimon, 1993). Mordes et al(1996) suggested that the delayed exposure to a common virus may predispose to destruction of the β -cells. Epidemiologic evidence supporting this hypothesis is the low risk of type 1 diabetes associated with crowding early social mixing (McKinney et al., 2000).

Markers of recent infection have been associated in many different circumstances with type 1 diabetes such as the presence of antibodies at the time of onset of type 1 diabetes and in maternal serum obtained during the gestation of children who subsequently developed diabetes (Patterson et al,1996). Viral infection were reported to play a role as initiator of autoimmunity or as promoter

of progression of autoimmunity to type 1 diabetes or both (Graves et al, 1997).

Chapter one

Introduction and literature Review

Early exposure to cow's milk protein is a trigger of β -cell autoimmunity and subsequently development of type 1 diabetes in susceptible individual (Norris and Scott,1997).(Norris et al, 1996) suggested that the decreased frequency and duration of breast feeding could be predispose to type 1 diabetes and this effect could be to lack of protective effect of breast feeding or to exposure to an initiator of autoimmunity in cow's milk.

Atkinson et al, (1993) founded that a neonatal exposure to cow's milk which contains bovine albumin can cross the neonatal gut intact and they suggest elicit an immune response which may cross react with specific β -cell antigens.

1.5.1.3 Immunological Factors:

Several lines of evidence suggest that type 1 diabetes is a T-cell mediated autoimmune disease (Michael et al,1994) and the onset of the disease associated with an infiltration of mononuclear cells in and around the islets of langerhans and this inflammation termed insulitis (Parveen Kumar, 2002). Therefore the cellular composition of the inflammatory infiltrate and most cells are lymphocytes T-cells of these CD8 (Cytotoxic / suppressor) cells out-number CD4 cells (Bottazzo et al., 1985).

Type 1 DM is associated with other autoimmune disease and increase the risk of the development of these disease and conversely and patients with Addison's disease , hashimoto's thyroiditis and pernicious anemia have an increased risk of

developing type 1 DM (Dreu and Notkins, 1987). Two additional observations provided support for the idea that autoimmunity is

important to the pathogenesis of type 1 diabetes, the first was disease's association with HLA genes and the HLAB 15 and B8 were more frequent among diabetic patients compared with control (Signal and Blajchman, 1973) , second the presence of islet— cell specific autoantibodies (Lernmark et al., 1978).

Numerous studies have shown abnormalities in the number of T-cells during and before the clinical onset of type 1 diabetes (Peakman and Vergani, 1994).

1.6 Pathogenesis of DM:

1.6.1, Type 1 DM:

Type 1 DM is due to eradication of the pancreatic islet β -cell and the onset of the disease was associated with an infiltration of mononuclear cells in and around the islets of langerhans (Parveen kumar,2002) and numerous studies have shown abnormalities in the number of T-cells during and before the clinical onset of type 1 diabetes (Peakman and Vergani, 1994). However insulitis is associated with the clinical onset of hyperglycemia and the islet seen first infiltrated by macrophages (Bach, 1994).

Environmental factors known to be associated with type 1 diabetes may interact in the autoimmune destruction of islet β -cells in many different ways and host tissues could be destroyed by immune responses during the process of removing pathogen (Nerup et al, 1987). Other mechanisms probably underlie the autoimmune B-cell destruction that develops spontaneously as an autosomal recessive trait (Jacob et al, 1992).

Type 1 diabetes a chronic autoimmune disorder and development of disease divided into series of stages beginning with genetic susceptibility and ending with complete or nearly

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complete β cell destruction associated with a lack of C - peptide secretion (Eisenbarth, 1986). The great majority of individuals expressing islet autoantibodies that react with multiple islet antigens progress to loss of first phase insulin secretion (Bingley et al, 1994). Islet autoantibodies can appear during the first year of life but also can first develop in adults (Verge et al, 1996). Progressive loss of first phase insulin secretion characteristically precedes the development of hyperglycemia (Colman et al, 2000).

The detection of progressive loss of first - phase insulin secretion in the presence of islet autoantibodies led to hypothesis that type 1 diabetes is a chronic autoimmune disorder (Eisenbarth, 1986) and other alternative hypothesis that chronic loss of first phase insulin secretion results from functional inhibition of insulin secretion by the immune system and that the actual destruction of pancreatic β -cell is acute and follows a long latency period during which islet autoantibodies are expressed (Shimada et al, 1996).

The available histological data and the observation that the loss of B-cell mass correlates with loss of first phase insulin secretion (McCulloch et al, 1988).

Pancreas from individuals who have died at the onset of diabetes often have lost most β -cells but still have a significant mass of islet B-cells remaining (Faulis et al., 1986). Several years after the onset of diabetes as reflected by loss of C-peptide secretion, the remaining cell mass is lost and the chronic destruction that precedes type 1 diabetes that, continues after the development of hyperglycemia (McCulloch et al., 1988).

Glutamic Acid Decarboxylase (GAD)enzyme is present in pancreatic cells Antibodies to an isoform of (GAD 65) are present in up to 90% of recent – onset IDDM patients, although they are more frequent in females. GAD 65 antibodies are also associated with other diseases such as stiff man syndrome and autoimmune polyendocrine syndrome, but the epitopes may differ from IDDM.

GAD 65 is membrane – bound in the islets and is structurally different from the cytoplasmic GAD 67 isoform also found in the brain (John et al ,1997).

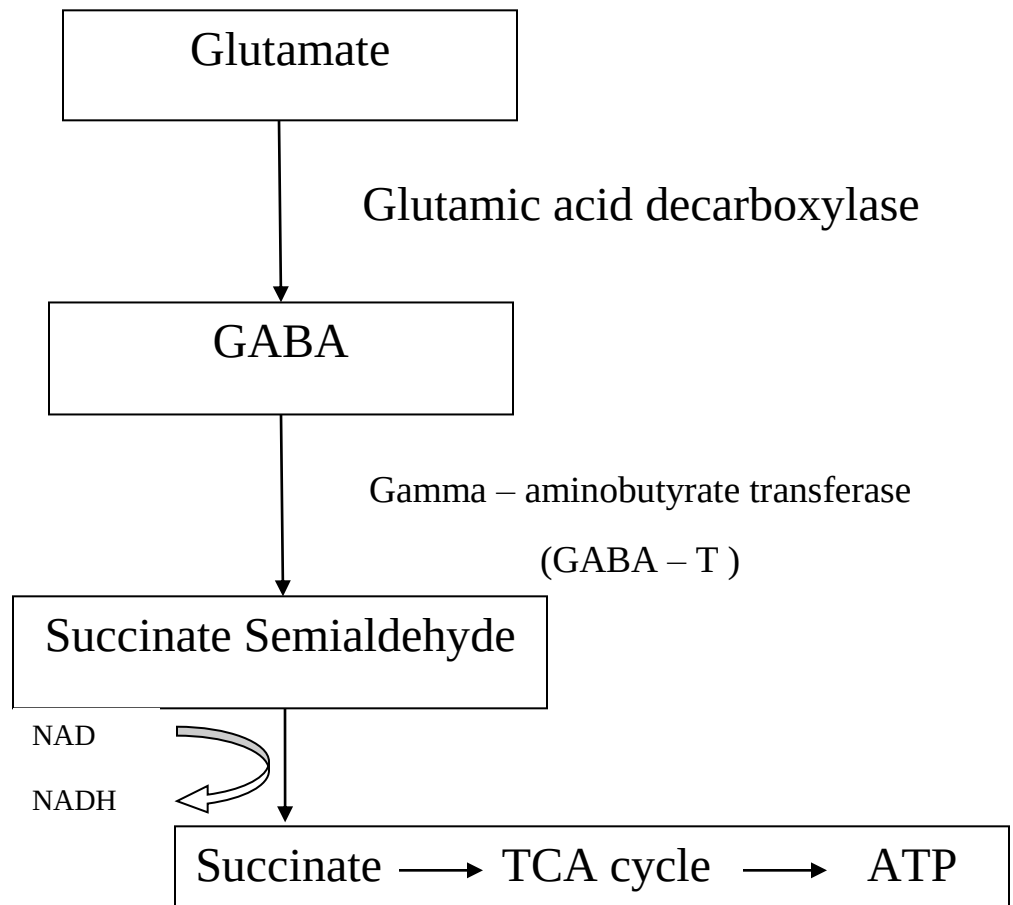


Figure (1 – 2) The GAD path way generates GABA and also succinate which can enter the tricarboxylic acid (TCA) cycle to produce ATP, the importance of these dual roles of the GAD pathway in β -cell function (Johnc et al, 1997).

1.7 Viral Infection and Its Role in the Pathogenesis of Diabetes:

A number of viruses have long been considered to be associated with the development of IDDM in humans (Barnett et al, 1981). Evidence for these associations has come primarily from many studies of seasonal and geographical variations in the onset of the disease including clustering of cases pointing to an effective agent (Yoon, 1990). Diagnosed patients with type 1 diabetes may show serological and other evidence of viral infection and there were many reports of IDDM developing soon after or during viral infection (Szopa et al, 1993).

Viruses may damage islet, β -cells either directly by the cytolytic effects of the virus or indirect by an autoimmune response that is initiated or enhanced by the virus (Yoon, 1990) and individual susceptibility depends more on the environmental agents than on the inherited predisposition which include viral infections especially those caused by Coxsackie and other enteroviruses (Fohlman and Friman, 1993). The most prominent proposed environmental triggers for type 1 diabetes are infection particularly viruses (Ginsberg – Fellner et al, 1984).

Viral infection may play a role as initiator of autoimmunity or as promoter of progression of autoimmunity to type 1 diabetes or both (Graves et al, 1997).

Viral infection may initiate autoimmunity or precipitate diabetes in subjects' with autoimmunity and cytoplasmic islet cell antibodies or insulin autoantibodies have been detected after mumps infection (Helmk et al, 1986). So that these antibodies have been detected after Rubella, Measles, Chicken pox and Coxsackie virus infection (Bodansky et al, 1986).

Congenital rubella and enteroviral infections are clearly associated with the development of type 1 diabetes (Dahlguist and Kallen, 1997) and diabetes occurs in individuals with high risk

HLA-alleles and occurs years after the rubella infection (Rubinstein et al, 1982).

Both β -cell autoimmunity and diabetes may be caused by enteroviral infection perhaps acquired in utero (Clements et al. 1995). Viruses have long been suspected to contribute to the onset of T1D and the earliest observations were the onset of T1D sometimes followed acute infections (Honeyman et al, 2000) This association occurred with greater frequency at certain times of year which often indicates a viral cause. Recently epidemiological studies have shown the presence of virus specific IgM antibodies in recent T1D patients (Szopa et al, 1993) and the most convincing evidence comes from studies in which viruses isolated from the pancreas of patients died from acute T1D caused by diabetes in animal by the destruction of beta cells (Champsaur et al, 1982).

To date over a half - dozen human viruses have been reported to be associated with human T1D and these include: coxasackie B, rubella, mumps, cytomegalovirus, Epstein Barr, Varicella zoster, retrovirus and rotavirus (Hee- Sook and Jiwan, 2003).

Table (1-2): Viruses associated with the development of type 1 diabetes mellitus in humans (Hee-sook and Jiwan, 2003)

Virus	Involvement of genetic factors	Remarks
RNA Viruses Picornaviridae		
Coxsackie B virus	Not determined	Evidence from epidemiological studies, anecdotal reports and isolated viruses causing diabetes in infected animals.
Retroviridae		
Retrovirus	Not determined	Association of beta cell-specific expression of retroviral gene with development of human autoimmune IDDM
Togaviridae		
Rubella virus	Not determined	Possible association with autoimmune IDDM, especially congenital rubella syndrome
Paramyxoviridae		
Mumps virus	Yes	Possible induction of islet - cell autoantibodies.
Reoviridae		
Rotavirus	Not determined	Association with islet autoimmunity
DNA Viruses Herpesviridae		
Cytomegalovirus	Not determined	Association with autoimmune IDDM

Epstein-Barr virus	Not determined	Possible induction of autoimmune IDDM
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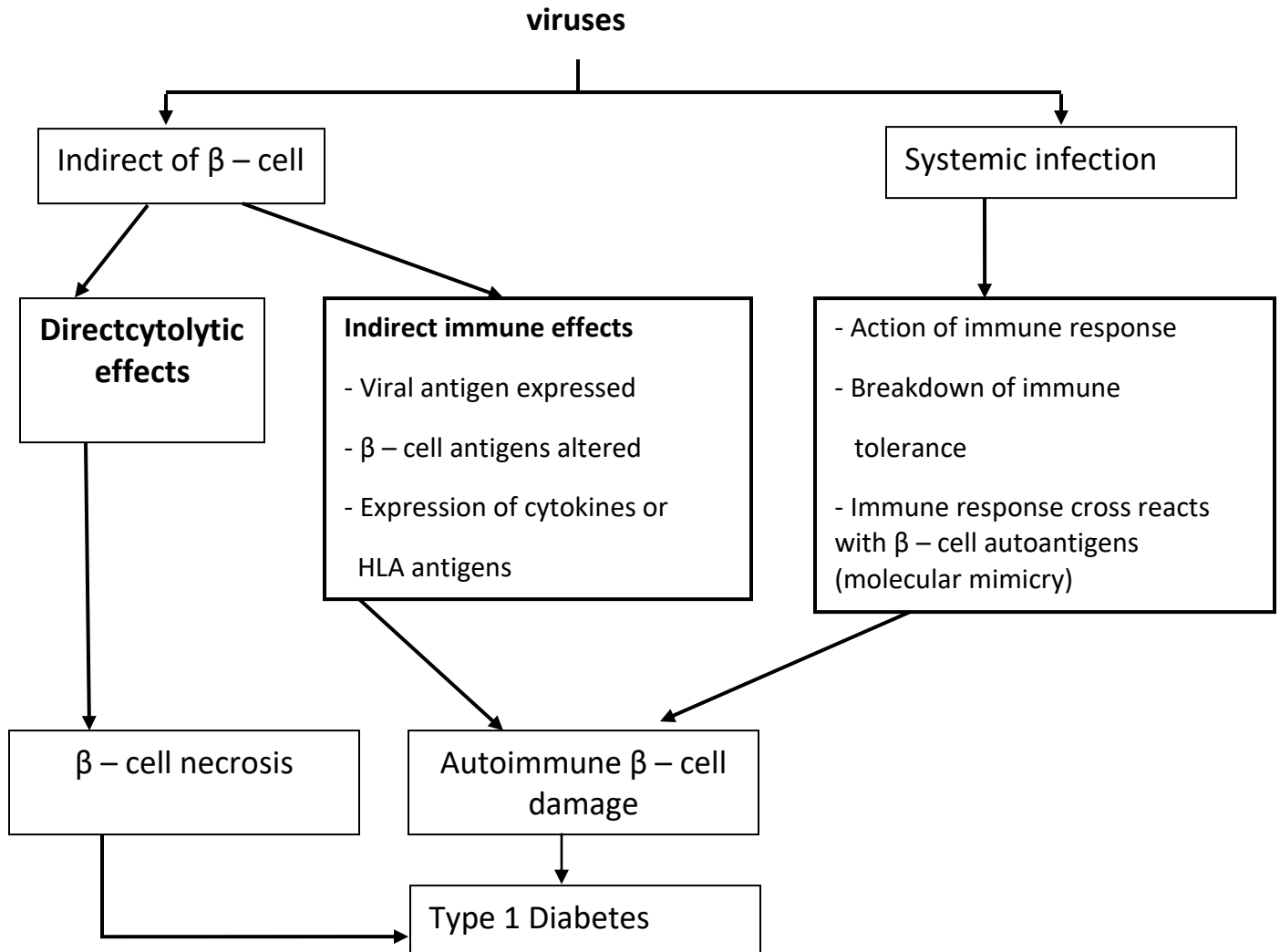


Figure (1-3) : possible mechanisms for virus induced diabetes (Hee-Sook and Jiwan, 2003)

1.8 Virus – mediated Autoimmune type 1 Diabetes:

The autoimmune T1D results from the T-cell – mediated destruction of pancreatic beta cells and the event that triggers this autoimmune is not known (Schattner and Rager – Zisman, 1990).

Several evidences from animal models suggest that viruses cause T1D in some cases and viruses have also been implicated as possible triggers of other autoimmune (Talal et al, 1992).

Virus could alter the target tissue of the host such that the tissue becomes recognized as foreign by the host's immune system, thus, triggering an autoimmune response (Champsaur et al, 1982) and these alterations could include modification of surface antigens into immunogenic forms such as, induction of new antigens, release of sequestered antigens during host cell lysis, incorporation of cellular antigens with viral envelope or up regulation of MHC class I and / or class II molecules on the target tissue of the host (Parkkonen et al, 1992).

The virus could alter the immune system of the host resulting in autoimmune attack of beta cells which lead to polyclonal B-cell activation and production of autoantibodies (Bodansky et al, 1986) so that the autoimmune attack of beta cells may lead to release of lymphokines such as interferon (IFN) α and tumor necrosis factor (TNF) which in turn recruits immunocytes to the host tissue (Vriarte et al, 1987).

The activation of immune cells that, result in a break - down of immune tolerance or disruption of the Th1\Th2 immune balance (Szopa et al, 1993). Antigenic epitopes on the virus could be similar to molecules on the host tissue (molecular mimicry), thus, causing generation of antigen - specific T effector cells and / or antibodies that recognize the host target cell leading to the development of autoimmunity (Juhela and Hyoty, 2000).

The antiviral antibodies arising as a result of viral infection could lead to the formation of an anti-idiotypic antibody and these antibodies could be autoreactive if the first antibody was made against the part of the virus that reacts with the host (Cavallo et al, 1992).

1.9, Direct Cytolytic Infection and Destruction of Beta-Cells:

As well as triggering beta cell- specific autoimmunity viruses may cause diabetes by directly infecting and destroying beta cells (Yoon, 1990). Studies on animal models have shown that three picornaviruses are associated with this mode of development of T1D encephalomyocorditis (EMC) virus, meningovirus and the Coxsackie virus (Szopa et al, 1993). Picornaviruses have a small non - enveloped icosahedral particle and single stranded positive sense RNA genome (Morishima et al, 1982).

The capsid is comprised of four polypeptides of which the major capsid protein VP1 is responsible for viral attachment to cell (Atkinson and McLaren, 1994). When susceptible cells are infected with picornaviruses, the cells are destroyed due to inhibition of DNA, RNA and protein synthesis of the infected cells (Rossini et al, 1993).

1.10, Coxsackie viruses:

Coxsackie viruses are small RNA containing viruses (picornaviruses) of the enterovirus class and there are six B serotypes (B1 - B6) (Lonnrot et al., 2000). Epidemiological studies show a strong association between the development of T1D and enterovirus infection and T cell response to enterovirus have been detected in new onset diabetes patient (Juhela et al, 2000). So that enteroviral RNA was detected with high frequency in the serum or lymphocyte of T1D patients (Yin et al, 2002).

Lonnrot et al, (1998) reported that T1D developed after enteroviral infection. Coxsackie B4 viruses have been isolated from the pancreases of patients with acute-onset of IDDM (Yoon et al, 1979). Coxsackie B viruses may induce diabetes through several mechanisms. They have very strong cytolytic activity and in animals may directly destroy enough of the B-cell mass to cause IDDM (Toniolo et al, 1982).

Autoimmune mechanism may also be involved perhaps because the immune response rise against the virus crossreacts

with specific β -cell antigens (Kaufman et al, 1982) and further possibility is that a defective coxsackie B-virus lacking the usual high lytic activity could cause persistent infection of B- cells , resulting in autoimmune B-cell destruction (Foulis et al, 1990).

1.11, Rubella virus:

Rubella virus is non-segmented single stranded RNA enveloped virus that belongs to the Togavirus family and it has implicated in T1D (Patterson et al, 1981).

The patients with congenital rubella syndrome (CRS) had a higher incidence of T1D than the general population with about 10-20 % developing diabetes between ages of 5 - 20 years (McIntoch and Menser, 1992). Ginsberg - Fellner et al (1984) showed that the islet cell and anti-insulin antibodies were found in 50 - 80 % of diabetic patients with CRS whereas these antibodies were present in about 20 % of non- diabetic patients with CRS and they suggesting an underlying autoimmune disorder. Genetic susceptibility may be also involved as patients with CRS and diabetes had a significantly increased frequency of HLA- DR3 and significantly decreased frequency of HLA-DR2 (Menser et al, 1978).

Rubella virus appears to be able to directly infect beta cells as shown by in-vivo and in vitro studies (Hee-sook and Jiwan, 2003). Human fetal islets exposed to rubella virus contained rubella viral antigens in both beta and non- beta cells and have lowered level of insulin production (Numazaki et al, 1989). Rubella viral antigens on beta cells or rubella virus - altered antigens on the surface of beta cells may be perceived as foreign by the host's immune system leading to beta cell specific autoimmunity (Patterson et al, 1981).

Ou et al, (2000) suggested that the rubella virus exposure may lead to the generation of viral antigen - specific cytotoxic T-

cells that also recognize beta cell specific antigen (s) in susceptible individuals.

1.12, Mumps virus:

Mumps virus is an enveloped single - stranded virus belonging to the paramyxovirus family and it was one of the first viruses implicated in the development of human T1D as several cases were reported in which mumps infection appeared to precede the onset of T1D (Gamble, 1980).

Infection with mumps virus may induce autoimmunity as some children I appear to develop islet cell autoantibodies during parotiditis (Helme et al, 1980).

Price et al, (1978) in their study have showed that human beta cells could be I infected with mump virus.

Mumps infection of human insulinoma cell line induced the release of IL-1 and IL-6 and up regulated the expression of HLA class I and Class II antigens (Cavallo et al, 1992) and that pancreatic beta cells infected with mump virus had increased expression of only HLA class I molecules (Parkkonen et al, 1992).

Vuorinen et al, (1992) showed that the mump virus is capable of replicating in the exocrine pancreas.

The cytokines released by mumps virus infected cells and increased expression of HLA molecules by infected beta cells may lead to an immune response against the beta cells or may increase pre-existing autoimmune processes directed against beta cells (Hee-Sook and Jiwan, 2003).

Hyoty et al, (1993) concluded that the elimination of natural mumps infections by vaccination may have been responsible for decreased risk of developing T1D and (Hummel et al, 2000) concluded that there is no association with childhood mumps vaccinations and the development of isletautoimmunity or T1D.

1.13, Epstein - Barr virus (EBV):

EBV is a double stranded DNA enveloped virus that belongs to the herpes virus family, EBV has been implicated in the etiology of autoimmune disease (Marchini et al., 1994).

A temporal link between EBV infection and the onset of T1D has been reported in rare number of cases (Chikazawa et al, 1985). Children with recent onset IDDM had lower IgG antibody titers against EBV capsid antigen than in non - diabetic controls and the specific immune responses to EBV were abnormal (Hyoty et al, 1991).

Molecular mimicry may be involved as with other virus and the BOLF1 protein of EBV has an 11-residue sequence homologous to a portion of the B- chain of the HLA-DQW8 antigen which includes the ASP-57 residue thought to be important in determining susceptibility to IDDM (Sairenji et al, 1991). However, sera from IDDM patients did not bind to either the B-chain of DQW8 or to BOLF1 and another part of the HLA-DQ, also near residue 57 has been implicated (Horn et al, 1988).

This is a pentapeptide sequence (GPPAA) which is repeated successively six times in the BERF4 encoded epitope of EBV (Parkkonen et al, 1994).

1.14, Retrovirus:

Retroviruses are RNA viruses that contain reverse transcriptase and there is increasing evidence that retroviral infections may be related to the development of human IDDM (Suenaga and Yoon, 1988). The expression of endogenous retroviruses by β -cell is associated with insulinitis and IDDM in NOD mice (Nakagawa et al., 1992). Endogenous retroviruses have also been implicated in humans. (Hao et al, 1993) showed that the anti-insulin autoantibodies from IDDM patients and their non diabetic first degree relatives cross react with retroviral P73 antigen in up 75 % of cases where as only 3 % of non-diabetic unrelated controls.

The presentation of a retroviral antigen on the beta cells by antigen presenting cells such as macrophages and dendritic cells may be the initial step in the autoimmune destruction of beta cells

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(Liang et al, 1995), In addition (Pak et al,1995), showed an immune response to specific antigen on target cells involves the activation of CD+4 T cells by antigens presented on the surface of macrophage or other antigen presenting cells and (Jun et al, 1999) support this possibility as elimination of macrophages resulted in the prevention of beta cell-specific autoimmune process in NOD mice.

1.15 Cytomegalovirus:

1.15.1, Definition:

Cytomegalovirus (CMV) is a virus related to the group of herpes viruses, infection with CMV can cause no symptoms or can be the source of serious illness in people with weak immune system. CMV infection is also an important cause of birth defects (Yow, 1989).

1.15.2, Routes and symptoms of CMV infection:

The symptoms of a CMV infection vary depending upon the age and health of the person infected and how the infection occurred (Weller, 1971).

Infants who are infected before birth usually show no symptom of a CMV infection after they are born (Fowler et al,1992). Some of these infants can develop hearing , vision , neurological and developmental problems over time (Williamson, 1982).

In few cases there are symptoms at birth which can include premature delivery , being small for gestational age , jaundice , enlarged liver and spleen , microcephaly , seizures , rash and feeding difficulties (Stagno, 1986), also some infants are at high

risk for developing hearing , vision , neurologic and developmental problems (Handshow, 1971).

Newborns can also contact CMV infection during or soon after birth by passing through birth canal of an infected mother and most of these infants show no symptoms, however, a few may develop pneumonia or other symptoms (Yow, 1989).

CMV infections that occur in children after the newborn period usually don't cause significant illness and some of them develop pneumonia, hepatitis or rash (Weller, 1971).

Older children who become infected with the virus may have mononucleosis I like symptoms including fatigue, muscle aches, headache, fever enlarged liver and spleen and these symptoms are generally mild (Boppana et al, 1997).

Cytomegnlovirus May Be transmitted person to person in several different ways, all requiring close contact with virus bearing material, in apparent infection is common during childhood and adolescence (Jawetz, 2004).

In people who have received organ transplants or in people whose immune systems are weakened, CMV can cause serious infections and may involve the lungs, nervous system and eye (Lowance et al, 1999).

1.15.3, Morphology of CMV:

CMV is a typical herpes virus, the central DNA containing core is surrounded by a capsid composed of 162 capsomeres each of which is a hollow I hexagon in cross section (Yamane et al, 1983) and the capsid is surrounded by a poorly demarcated area, the tegument which is itself surrounded by a loosely applied envelope (Plotkin et al, 1976).

When CMV is propagated in cell cultures two morphological forms are produced from the virus - specific proteins and envelope, the first termed dense body and the second has been termed non - infectious enveloped particle (Yow, 1989).

1.15.4, Epidemiology:

Infection may be acquired during delivery following ingestion of infected maternal genital secretions or soon afterwards by ingestion of breast milk containing CMV, these means of transmission combine to infect between 2 % and 10 % of infants by the age of 6 months in all parts of the world (Stagno et al,1980) Child to child transmission has been documented in play groups (Pass et al, 1986).

In populations of poor socioeconomic background the majority of children have primary CMV infection by the onset of puberty (Griffiths and Baboonian, 1984). In countries with good social circumstances roughly 40 % of adolescents have been infected and increases by approximately 1 % per annum (Pass et al, 1986).

The prevalence of CMV IgG antibodies in organ transplant recipients and individuals who acquired HIV infection via blood, heterosexually or through contaminated needles reflects their socioeconomic grouping (Cope et al, 1997).

HIV positive male homosexuals have a very high prevalence of CMV IgG I antibodies (Typically 95 %) (Bowen et al, 1997).

Congenital CMV infection has its highest incidence in the poorest communities and most women in poor societies are infected before reaching child bearing age (Fowler,et al, 1992). Antibody prevalence may be moderate (40-70 %) in adults in high socioeconomic groups in developed , countries in contrast to a prevalence of 90 % in children and adults in developing nations and in low socioeconomic groups in developed countries (Hengel et al., 1998).

1.15.5, Risk Factors for CMV disease:

A primary infection is a major risk factor during pregnancy and for recipients of solid organ transplants but not for bone marrow transplant or patients with AIDS (Meyers et al, 1990).

A high CMV load was initially shown to be important in neonates with congenital infection and in renal, liver, bone marrow transplants and AIDS patients (Cope et al, 1997).

There are different cellular mechanisms of pathogenesis may be activated at different virus loads, thus, immunopathology may be triggered by low virus loads, while high virus loads may be required to damage sufficient cells by lysis to cause clinically recognized disease (Yurochko et al, 1997).

1.15.6, Incubation period:

The date of infection and date of onset of virus excretion can be reliably predicted which gives an estimate of 4-8 weeks for the incubation period of primary infection (Fowler et al, 1992). Allograft recipients typically have recurrent CMV infections in the second or third month following transplantation where as recurrent CMV infections in adult seem to be age dependent (Glenn, 1981).

(Stagno et al, 1975) reported that the virus excretion from the cervix increased as pregnancy progressed and where as the CMV excretion was actually suppressed during early pregnancy.

1.15.7, Host Defenses:

The host defense responses in those with normal immunity keep CMV suppressed into a latent state in most individuals for most of the time (Fleisher et al, 1982). Severe CMV disease is restricted to individuals with impaired cell mediated immunity and it can be concluded that this arm of the immune response provides most protection against disease (Koszinowski et al, 1990) so that the ability of the humoral system to provide a supportive role, especially in keeping CMV viral loads below critical thresholds should not be dismissed (Landini, 1992).

1.15.8, Humoral Immunity:

Antibodies IgG class is produced promptly during primary infection and persists for life (Fowler et al, 1992). Women with primary CMV infection transmit the virus in utero have higher levels of total IgG but lower levels of neutralizing antibodies (Boppana and Britt, 1995). IgM class antibodies are produced on primary but not recurrent infection of immunocompetent individuals and persist for 3-4 months and immunocompromised patients may fail to produce IgM antibodies (Landini, 1992).

Therefore one third have IgM responses detectable with recurrent infections (Boppana and Britt, 1995). With intrauterine infection IgM antibodies are produced by the fetus also IgG class responses are present (Fowler et al, 1992).

1.15.9, Cell - Mediated Immunity:

CMV specifically suppresses the function of the cell designed to respond _ against it (Pass et al, 1983). The CMV - infected cell evade lysis by T - cytotoxic and natural killer (NK) cells (Riddell and Greenberg, 1997).

Immediately after uncoating the tegument protein PPUL83 is available in the cell to phosphorylate the IE72 isoform of the major immediate - early protein and so prevent its presentation as target epitope (Surcel et al, 1988).

At immediate - early times, PUS3 retain class 1 HLA molecules within the endoplasmic reticulum (ER) (Van Herrath et al, 1998).

Once the virus infected cell has moved into early phase of CMV gene I expression, PUS2 and PUSH act to re-export class I HLA molecules back from the lumen of (ER) into the cytoplasm (VonHerrath and Oldstone, 1996). PUS6 blocks the activity of the transporter associated with antigen presentation so that epitopes

derived from CMV proteins cannot be presented as targets (Pass et al,1983). The cell becomes a target for NK cells which recognize

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the absence of there normally ubiquitous molecules and to prevent this CMV encodes another protein , PUL18 which is homologous to class I HLA and acts as decay to prevent NK cells attacking the CMV infected cells (Riddell and Greenberg,1997).

1.15.10 Laboratory Diagnosis:

1.15.10 - A, Polymerase Chain Reaction and Antigen Detection Assays:

PCR assays have replaced virus isolation for routine detection of CMV infections (Kidd et al, 1993).

The PCR assays are designed to detect replicating virus, not latent viral genomes and blood and urine are most commonly tested (Fox et al, 1992). PCR assays can provide viral load data which appears to be important in predicating CMV disease (Darlington et al, 1991). Monoclonal antibodies against viral antigens can be used to detect virus positive leukocytes from patients (Kidd et al, 1993).

1.15.10 -B, Isolation of virus:

The virus can be recovered most readily from throat washing and urine (Hengel et al, 1998). Cultures 2-3 weeks are usually needed for the appearance of cytological changes consisting of small foci of swollen translucent cells with large intranuclear inclusions (Vanden Berg et al, 1991).

1.15.10 –C, Serology:

Many types of assays can detect CMV IgG antibodies which include neutralization , complement fixing (CF) enzyme immunoassay (EIA), Immuno-fluorescence assay for viral late antigen (IFA-LA) , latex agglutination , radioimmunoassay (RIA) (Revello and Gerna,2002). The detection of IgG antibodies against CMV is indicative of infection some time in the past and the

seropositive individual is liable to experience reactivation of latent infection (Musianietal, 1990).

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The presence of IgG antibodies in, thus, is a marker of potential infectivity (Lazzarotto, 1998).

Detection of viral IgM antibodies suggests a current infection (Hekker et al, I 1979).

1.15.11 ,Role of cytomegalovirus in Diabetes Mellitus:

Cytomegalovirus (CMV) belongs to the herpes virus family and is a double stranded DNA enveloped virus (Chee et al, 1990). Human CMV can be congenital although the disease may not appear until later in life and infection can also be transmitted perinatally or postnatally through close contact or breast milk (Stagno, 1980).

CMV has been implicated in T1D by a number of clinical studies. (Ward et al, 1979 and Yasumoto et al, 1980) reports that the child with congenital CMV infection and women with CMV infection who both developed T1D latter after extensive pancreatitis. (Jensen et al, 1980) showed that the children with fatal viral infections, viral cytopathology of the pancreas and characteristic inclusion bodies in the beta cells were found in 20/45 cases of CMV infection indicating that CMV can infect pancreatic beta cell.

Pak et al, (1988) showed that 20 % T1D patients had cytomegalovirus genomic DNA in their lymphocytes compared to only 2 % of normal control.

Nicoletti et al, (1990) founded that non-diabetic sibling of T1D patients had a significant association between high titers of anti CMV antibodies and islet cell autoantibodies but no correlation between anti- CMV antibodies with HLA I DR antigens.

Pak et al, (1988) showed that 80 % of patients who had both anti- CMV antibodies and the CMV genome also had islet cell autoantibodies.

CMV infection can cause β -cell damage including degranulation and the characteristic inclusion bodies have been found in the B cells of children who died with disseminated CMV (Visseren et al, 1997). It is possible that molecular mimicry may involve some cases of CMV induced diabetes because CMV proteins share a resemblance with protein in the islet cells of the pancreas (Ivarsson, 1993).

Pak et al, (1990) found that human CMV can induce an islet cell antibody that reacts with a 38 KD autoantigen expressed in human pancreatic islets and (Hiemstra et al, 2001) suggested that the human CMV may be involved in the induction of autoimmunity by molecular mimicry of the beta cell autoantigen GAD65. (Nigro et al, 1988) showed that a CD4⁺ T cell clone reactive to GAD65 isolated from prediabetic patient cross react with a peptide of human CMV major DNA binding protein.

2.1 Materials

2.1.1 Patients

Hundred and twenty diabetic patients and equal number of control non diabetic subjects were enrolled in this study. Diabetic patient attending center of endocrinology and diabetes and National center for the treatment and researches of diabetes (Al-Mustansiriya University) college of medicine. The blood samples collected from newly diagnosis diabetic type 1 diabetes during the period from August 1st 2008 through march 2009 from 60 male and 60 female at different age group. Study patient were interviewed directly similar questionnaire measurement and investigation were applied where as the control groups included in questions only about age and sex

2.1.2 Equipments

<u>NO.</u>	<u>NAME OF INSTRUMENT</u>	<u>NAME OF COMPANY</u>
<u>1</u>	<u>BIO KIT(ELIZA READER)</u>	<u>Bio kit(Italy)</u>
<u>2</u>	<u>INCUBATOR</u>	<u>Fisher(Germany)</u>
<u>3</u>	<u>CENTERIFUGE</u>	<u>Universal(Germany)</u>
<u>4</u>	<u>FLOURECENT MICROSCOPE</u>	<u>Nikon(Japan)</u>
<u>5</u>	<u>WATER BATH</u>	<u>Memmert(Germany)</u>
<u>6</u>	<u>SPECTROPHOTOMETER</u>	<u>Optima(Japan)</u>
<u>7</u>	<u>KAHN SHAKER</u>	<u>United kingdom</u>
<u>8</u>	<u>MICROPIPETTE(0-20µl,0-50µl,10-100µl,100-1000µl)</u>	<u>Eppendrof(Germany)</u>

<u>NO.</u>	<u>NAME OF KIT</u>	<u>COMPANY</u>
<u>1</u>	<u>Kit for detection of blood sugar</u>	<u>Randox(United kingdom)</u>
<u>2</u>	<u>Anti CMV IgM Ab enzyme immune assay test kit</u>	<u>Bio check</u>
<u>3</u>	<u>Anti CMV IgG Ab enzyme immune assay test kit</u>	<u>Bio check</u>
<u>4</u>	<u>Anti GAD Ab ELISA kit</u>	<u>DRG international,USA</u>
<u>5</u>	<u>Monkey pancreas indirect fluorescent antibody(slides for detection anti islet cell</u>	<u>The binding site(United kingdom)</u>

Chapter two

Material and method

2.1.3 Kits used for assay

2. 2 Inclusion criteria,

2. 2.1 study group

1-Newly diagnosed type 1 Diabetic patients (120 patient)

2-Patients on insulin therapy

2.2.2 Control group

1-Subjects with normal fasting blood glucose(<110 mg/dl) (120) subjects.

2-The subjects not receive oral hypoglycemic therapy or insulin previously.

2.3 Questionnaire,

The questionnaire form includes age, sex, type of treatment, time of diagnosis, familial history

2.3.1 Familial history

Assessed by ask the patient with type 1 (DM) about the presences of one or both parent, brother or sister (first degree relative)uncle, aunt, grand father, grandmother(second degree relative to excluded genetics causes)

2.3.2 Time of diagnosis

It means the time of diagnosis the sample collected from only newly diagnosis or maximum 2 month

2.3.3 Type of treatment

Only patients type 1 diabetes that received insulin

2.3.4 Age

To divide patients with CMV infection and type 1 diabetes as age group.

2.4 Investigation

The following values where measured for each patient in study groups. (IgM,IgG anti CMV antibodies) ,antiGAD antibodies, islet cell antibodies, fasting blood sugar as well as for control group. sample of venous blood was drawn from each subject in the

study groups and control groups, about three milliliter in sterile plain tube then separated by universal .

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centrifuge at 1500 r.p.m and serum sample collected in sterile plain tube and after that kept for 5 mint at -20°C.

2.4.1 Fasting blood glucose (F.B.G)

It is determined for the subjects in the control and patient groups using the glucose kit ,any subject with F.B.G greater than 110mg/dl will be taken within patient groups while F.B.G less than 110 mg/dl considered as control .

Procedure

- a. serum sample (10µl) were taken
- b. then added 1ml from Randox reagent provided with kit.
- c. test sample were kept in water bath at 37 C for 10 mints.
- d. after that read in spectrophotometer at 500nm.

2.4.2 CYTOMEGALOVIRUS (CMV) IgM ENZYME IMMUNOASSAY TEST KIT

Enzyme immunoassay for the detection of IgM antibodies to CMV in human serum.

INTENDED USE

The CMV IgM ELISA was intended for use in the detection of IgM antibodies to Cytomegalovirus (CMV) infection in human serum.

PRINCIPLE OF THE TEST

Purified CMV antigen was coated on the surface of micro wells. Diluted patient serum was added to the wells, and the CMV IgM specific antibody, if present will bind to the antigen. All unbound materials were washed away. HRP-conjugate was added, which binds to the antibody-antigen complex. Excess HRP-conjugate was washed off and a solution of TMB reagent was added, The enzyme conjugate catalytic reaction was stopped at a specific time. The intensity of the color generated was proportional to the amount of IgM specific-antibody in the sample, The results were read by a micro well reader compared in a parallel manner with calibrator and controls.

REAGENTS

Materials provided with the kit:

- Micro titer wells: purified CMV antigen coated wells (12x8 wells)
- Enzyme conjugate reagent (red color): red cap. 1 vial(12 ml)
- Sample diluent (blue color): 1 bottle (22 ml) •
- Negative control: range stated on label. natural cap. (150ml/vial).
- Cut-off calibrator: yellow cap. CMV *IgM* Index = 1 (150 ml/vial)
- Positive control: range stated on label. red cap. (150 ml/vial)
- Wash phosphate buffer concentrate (20x): 1 bottle (50 ml).
- TMB reagent (One-Step): 1 vial (11 ml)
- Stop solution (1N HCl): natural cap. 1 vial (11 ml)

SPECIMEN AND REAGENT PREPARATION

1. Serum specimens were transferred from freezer to water bath at 37°C.

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2. kit was transferred from refrigerator to kept at room temperature (25°C) before assay.
3. 50 ml of wash buffer (20x) were diluted in distilled water to prepare 1000 ml of wash buffer (1x).

ASSAY PROCEDURE

Assay procedure followed according to catalog provided with kit.

1. Ninety six of coated wells were placed into the holder.
2. Test samples were diluted with sample diluents (1:40 dilution) and negative control, positive control, and calibrator by adding 5 µl of the sample to 195 µl of Sample Diluents. then Mix well.
3. 100 µl of diluted sera, Calibrator, and Controls were dispensed into the appropriate wells. For the reagent blank, 100 µl Sample Diluent were dispensed in 1A well position. After that the holder were taped to remove air bubbles from the liquid and mix well.
4. Incubated at 37°C for 30 minutes.
5. After the end of the incubation period, liquid was removed from all wells. The microtiter wells were rinsed and flicked 5 times with diluted washing buffer.
6. Then 100 µl of enzyme conjugate were added to each well, mixed gently for 10 seconds.
7. Incubated at 37°C for 30 minutes.
8. The enzyme conjugate were removed from all wells. The plate was rinsed and flicked 5 times with diluted wash buffer.
9. 100 µl of TMB reagent were dispensed into each well, mixed gently for 10 seconds.
10. Incubated at 37°C for 15 minutes.
11. Then 100 µl of Stop Solution (1N HCl) were added to stop reaction.
12. The plate were mixed gently for 30 seconds.

11. Finally O.D. was read at 450 nm *after 10 minutes* with a micro well reader.

2.4.3 CYTOMEGALOVIRUS (CMV) IgG ENZYME IMMUNOASSAY TEST Kit

Enzyme immunoassay for the detection of IgG antibodies to cytomegalovirus (CMV) in human serum.

INTENDED USE

The CMV IgG ELISA was intended for use in evaluating a patient's serologic status to (CMV) infection.

PRINCIPLE OF THE TEST

Purified CMV antigen was coated on the surface of micro wells. Diluted patient serum was added to the wells, and the CMV IgG specific-antibody, if present will bind to the antigen. All unbound materials were washed away. HRP-conjugate was added, which binds to the antibody-antigen complex. Excess HRP-conjugate was washed off and a solution of TMB reagent was added. The enzyme conjugate catalytic reaction was stopped at a specific time. The intensity of the color generated was proportional to the amount of anti CMV IgG-specific antibody in the sample. The results were read by a micro well reader, compared in a parallel manner with calibrators and controls.

RAGENTS

Materials provided with the kit

- Microtiter wells: purified CMV antigen-coated wells (12x8 wells)
- Enzyme conjugate reagent (red color): red cap. 1 vial (12ml)
- Sample diluent (green color): 1 bottle (22 ml)
- Negative calibrator: 0 IU/ml. natural cap. (150ml/vial)
- Cut-off calibrator. 1.2 IU/ml. yellow cap. (150 ml/vial) CMV IgG index-

- Positive calibrator: 6 IU/ml. red cap. (150 ml/vial)

- Positive calibrator, 18 IU/ml. green cap. (150 ml/vial)
- Negative control: range stated on label. blue cap. (150 ml/vial)
- Positive control: range stated on label. purple cap. (150 ml/vial)
- Wash phosphate buffer concentrate: 1 bottle (50 ml, 20x)
- TMB reagent (One-Step), 1 vial (11ml)
- Stop solution: 1N HCL, natural cap. 1 vial 11ml

SPECIMEN AND REAGENT PREPARATION

1. Serum sample were placed in water bath at 37 °C after freezing
2. All reagents were allowed to reach room temperature (18-25 °C) before use.
3. 50 ml of wash buffer (20x) were diluted in distilled water to prepare 1000 ml of wash buffer (1x). Then Mix well before use.

ASSAY PROCEDURE

Assay procedure followed according to the catalog provided with kit.

1. Ninety six of coated wells were placed into the holder.
2. Test samples were diluted(1:40 dilution) using the provided sample diluents and negative control, positive control, and calibrator by adding 5 µl of the sample to 195 µl of Sample diluent.then Mix well.
3. After that, 100µl of diluted sera were dispensed as well as calibrator and control, in to the appropriate wells. For the reagent blank 100µl of Sampled were dispensed in 1A well position. Then the holder was taped to remove air bubbles from the liquid and mix well.
4. Then Placed in incubator at 37Cfor 30 minutes.
5. At the end of incubation period, liquid were removed from all wells. The micro titre wells were rinsed and flicked 5 times with diluted wash buffer .

6. Enzyme conjugate (100µl) were dispensed into each well and Mix gently for 10 seconds.
7. The micro titer wells were incubated at 37°C for 30 minutes.
8. Enzyme conjugate were removed from all wells. The micro titer wells were rinsed and flicked 5 times with diluted wash buffer (1x).
9. After this 100µl of TMB Reagent were dispensed in to each well and Mixed gently for 10 seconds.
10. Incubated at 37°C for 15 minutes.
11. Stop solution 100µl (1N HCL) were added to stop reaction.
12. Mix gently for 30 seconds.
13. The O.D was read at 450nm after 10 mints with micro well reader.

2.4.4 MONKEY PANCREAS IFA SLIDES

INTENDED USE

Monkey pancreas sections were intended for use in indirect immunofluorescence assays (IFA), screening human serum for circulating antibodies to pancreatic islet cells as an aid in the diagnosis and treatment of insulin-dependent diabetes mellitus (IDDM).

PRINCIPLE

These slides were used in an indirect immunofluorescence technique where patient samples and appropriate controls were incubated with the sections. The unreacted antibodies were washed off and then appropriate fluorescein- labelled conjugates were applied Unbound conjugate was washed off as before. Slides were Viewed with a fluorescence microscope and positive samples

produce apple-'green fluorescence that corresponds to areas of the section where autoantibody has bound.

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Material and method

Test Procedure

Procedure followed by the researcher for this test according to catalog provided with kit.

1-Mounting medium: the mounting medium was removed from the fridge and allowed it to reach room temperature (25°C) before assay.

2- Dilute patient samples

patient samples were diluted (1/5) by adding 40µl of serum to 160µl of PBS buffer (pH 7.1).

3-Substrate slides

Substrate slides were allowed to reach room temperature (25°C) prior to removal from pouches.

Then slides were labeled appropriately and placed in the humid chamber and added positive and negative controls to appropriate wells. 50µl of diluted patient samples were added to the remaining wells.

4-Slide incubation.

Slides were incubated for 30 minutes in a humid chamber at room temperature (25°C).

5- PBS WASH

Slides were removed from humid chamber and rinsed briefly with PBS squeeze bottle. Slides were placed in a rack and immerse in PBS and agitate or stir for 5-10 minutes.

6- Addition of fluorescent conjugate

excess PBS was aspirated and then blotted around wells. Slides were returned to humid chamber and immediately covered each well with a drop of appropriately diluted fluorescent conjugate.

7-Slide incubation.

Slides were incubated for 30 minutes in humid chamber at room temperature (25°C), in the dark.

8-PBS Wash. Wash again as mention previously. 2 -3 drops of 1% Evans Blue was added per 100mL of PBS prior to slide immersion.

9-Mounting with cover slip.

One slide was removed at a time from PBS wash and quickly dries around the wells and then added a drop of mounting medium to each well. After that, carefully the slide was lowered onto the cover slip, Wiped excess medium from around edge of cover slip.

10- slide was viewed under fluorescence microscope.

2.4.5 Qualitative ELISA test for the detection of circulating autoantibodies against GAD antigen.

INTENDED USE

The GAD ELISA was an in vitro qualitative ELISA test for the detection of circulating autoantibodies to glutamic acid decarboxylase (GAD) antigen in prediabetic high risk individuals as well as 1DDM diabetic patients. This kit was intended for Research Use Only in the United States.

PRINCIPLE OF THE TEST.

Purified GAD antigens immobilized onto microwells. GAD specific IgG antibodies present in the patient's serum sample were allowed to react with the antigen. The excess unbound serum proteins were washed-off from the microwells. An enzyme (alkaline phosphatase) labeled goat-antibody, specific to human IgG was added to the GAD antibody complex. After washing off

Excess un reacted enzyme conjugate from the microwells , a substrate (PNPP) was added and the color generated is measured spectrophotometrically. The intensity of the color developed was

given directly the concentration of GAD autoantibodies in the test serum sample. GAD positive and negative controls serve as an internal quality control to ensure valid result.

REAGENT PREPARATION AND STORAGE

1. GAD-Enzyme Conjugate Reconstitution

Accurately was transferred 5 ml of the conjugate diluent into the bottle containing 1.0 ml of the enzyme conjugate (concentrate). Then closed the bottle and mixed thoroughly by inversions this diluted reagent expires 30 days after reconstitution. Each bottle contains enough conjugate for 6 strips. Reconstitute as needed.

2. Sample Diluent buffer

The entire contents (25 ml) were transferred into 100 ml of distilled/deionized water in a suitable container and mixed thoroughly, the container was labeled as sample diluent and stored at 2-8°C until use. The diluted reagent was stable until the expiration shown on the vial.

3. Wash Solution

The entire contents were transferred into 480 ml of distilled/deionized water in a 500 ml container. Mixed thoroughly the container was labeled as wash solution. The diluted reagent was stable until the expiration shown on the vial.

4. Serum Sample Preparation

10 µl (0.010 ml) of serum sample were accurately pipette into 1.0 ml of the working sample diluents into an already labeled glass tube. After this, mixed thoroughly.

ASSAY PROCEDURE

Procedure followed according to instruction manual provided with kit.

1. Ninety six of micro well provided with kit were dispensed in holder provided. The microwell strips were snapped firmly in place.
2. The strips were labeled with a marking pen.

3. After that 100 μ l (0.1 ml) of calibrators, positive and negative controls, and the diluted serum samples were dispensed into the appropriate microwells. Wells A1 and B1 were reserved for blank and contain no sample,
4. The plate were covered with a parafilm/plastic wrap (to prevent contamination) and incubated for 1 hour at room temperature.
5. After a 1 hour incubation, the contents in the microwells were dumped and blotted the plate dry by tapping gently onto paper towel a few times.
6. 100 μ l (0.1 ml) of reconstituted Enzyme Conjugate reagent were added to all microwells except wells A1 and B1
7. The plates were covered with a parafilm / plastic wrap and incubated it in the dark at room temperature for one hour.
8. At the end of the incubation, the microwells were washed three times with wash buffer.
9. After this 100 μ l (0.1 ml) of substrate solution were added to all microwells including wells A1 and B1. The substrate reagent was dispensed at a rapid steady pace without any interruption.
10. The plate was covered and incubated in the dark for 30 minutes at room temperature
11. At the end of 30 minutes, after substrate, addition 50 μ l (0.05 ml) of the stopping solution was added into each well at a rapid steady pace without interruption.
12. The plate reader was blank and read the absorbance of the plate at 405 nm. A1 or B1 wells were used to blank the plate reader. They have no sample, no conjugate only substrate reagent and stopping solution.
13. The data were calculated according to O.D by ELISA reader.

	<u>Value</u>	<u>Result</u>
GAD	<1.00unit/ml	Negative
	>1.05 unit/ml	Positive
	1 – 1.05unit/ml	indeterminate (borderline)

Positive result (> 1.05) indicates the presents of anti GAD antibodies in the patient's serum sample.

Negative result (< 1) indicates the absence of GAD auto antibodies or below the limit of resolution of the test.

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2.5 Statistical analysis

T-test were depended for statistical analysis, Statistical significance was considered whenever P value was less than 0.05, while different percentages were tested using chi-square test. (SPSS computer program).

3.1 ROLE OF CMV INFECTION IN TYPE 1 AUTOIMMUNE DIABETES (T1DM) IN AGE GROUP(6M-10y)

The results were presented in the following pages in Table and figures according to age group. The specific IgM and IgG anti CMV antibody followed by anti GAD antibody and anti islet cell antibody(AICA).

The specific anti CMV IgM antibody were measured in I.U/ml.No significant difference in anti CMV IgM antibody level in sera of male and female type 1 diabetes patients group (0.181 ± 0.0905 i.u/ml), (0.3108 ± 0.1554 i.u/ml) respectively, when compared with IgM antibody level in sera of male and female for control group as seen in Table and Figure (3-1A)

Table (3-1A) Specific anti CMV IgM antibody in age group (6m to 10 y)

Groups	Sex	No.	Age(Years) mean $\pm$ SD	P	IgM(I.U/ML) mean $\pm$ SD	P
Control	male	7	6 $\pm$ 1.57		0.00871 $\pm$ 0.0004	
	female	7	5 $\pm$ 2.1		0.13342 $\pm$ 0.0667	
Patients	male	7	7 $\pm$ 1.9	P>0.05	0.181 $\pm$ 0.0905	P* >0.05
	female	7	8 $\pm$ 2.6	P>0.05	0.3108 $\pm$ 0.1554	P** >0.05

*p probability between male patient and male control

**p probability between female patient and female control

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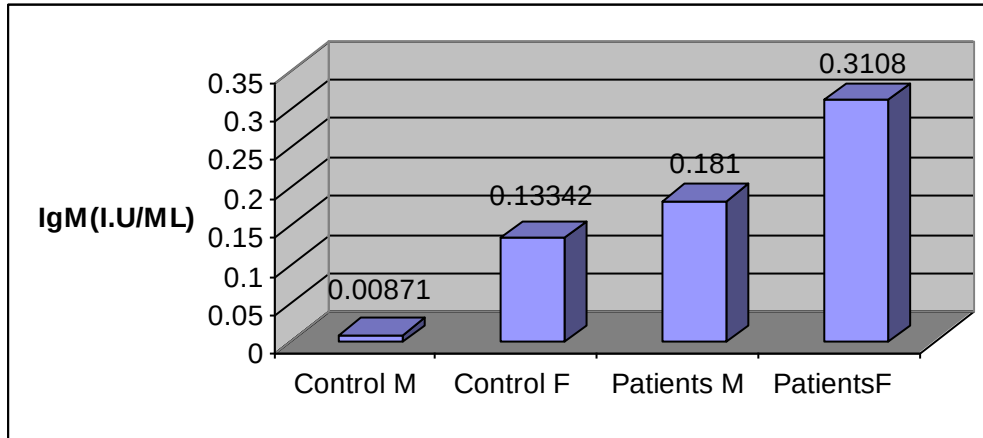


Fig. (3-1A) Level of anti CMV IgM antibody in age group (6m to 10 y)

Table and Figures(3-1B) ,(3-1C) ,A significant increase in IgG antibody titer (10.425 ± 7.077 i.u/ml), (6.703 ± 3.3515 u/ml) in male and female patient respectively, when compared with control group.

Table (3-1B) Specific anti CMV IgG antibody in age group (6m to 10y)

Groups	Sex	No	IgG(I.U/ML) mean $\pm$ SD	P
Control	male	7	0.0794 $\pm$ 0.003	
	female	7	0.083 $\pm$ 0.035	
Patients	male	7	10.425 $\pm$ 7.077	P* <0.05
	female	7	6.703 $\pm$ 3.3515	P** <0.05

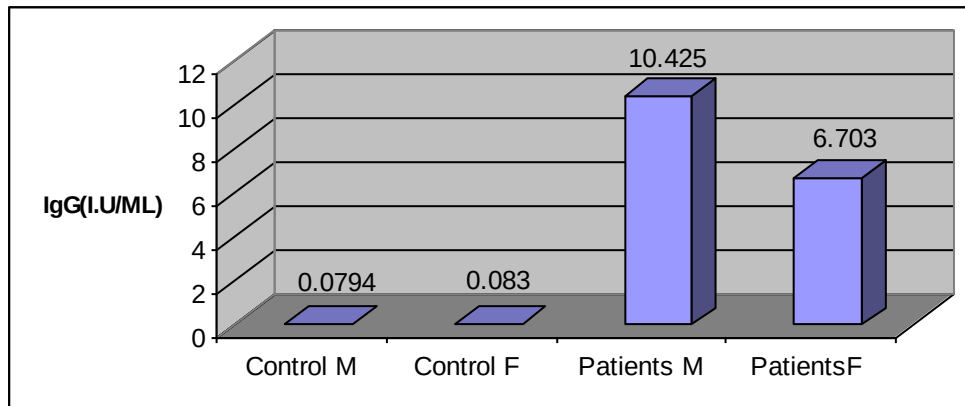


Fig. (3-1B) Level of anti CMV IgG antibody in age group (6m to 10 y)

Level of anti GAD antibody in male ($1.284 \pm 0.538 \text{ u/ml}$) and female patient were ($1.4785 \pm 0.547 \text{ u/ml}$) respectively, There is an elevation in the titer when compared with control group as appeared in the following Table and Figure (3-1C).

Table(3-1C) Specific anti GAD antibody in age group (6m to 10 y)

Groups	Sex	No	AGAD (Unit /ML) mean $\pm$ SD	P
Control	male	7	0.0148 ± 0.0003	
	female	7	0.0475 ± 0.041	
Patients	male	7	1.284 ± 0.538	$P^* < 0.05$
	female	7	1.4785 ± 0.547	$P^{**} < 0.05$

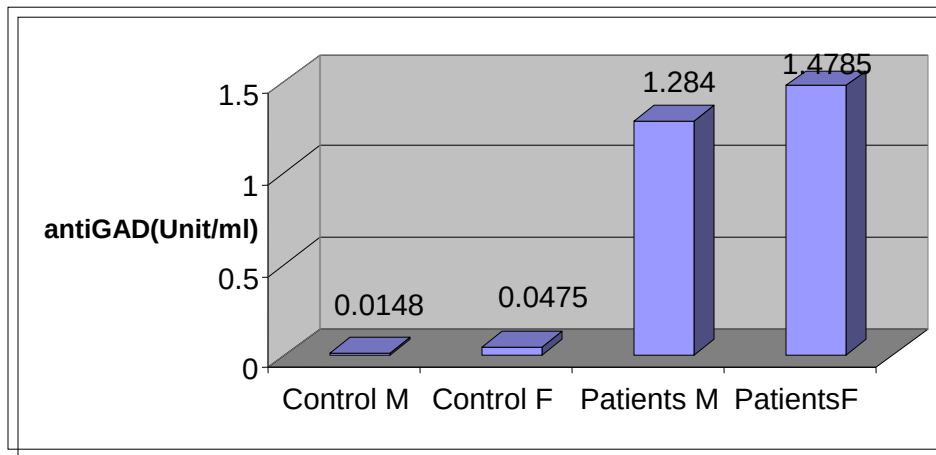
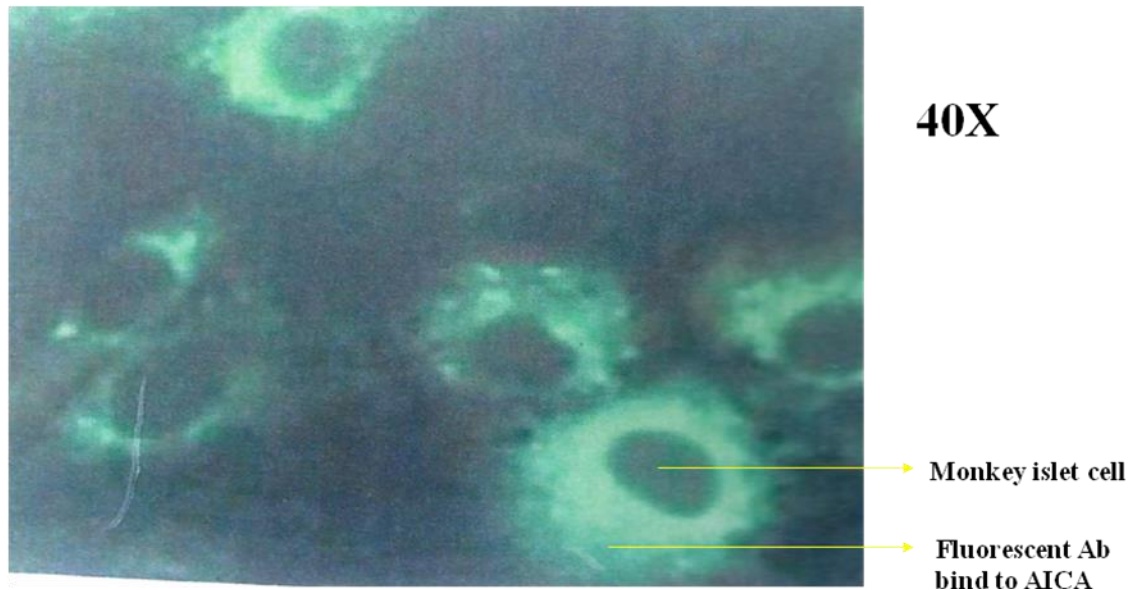


Fig. (3-1C) Level of anti GAD antibody(AGAD) in age group (6m to 10 y)

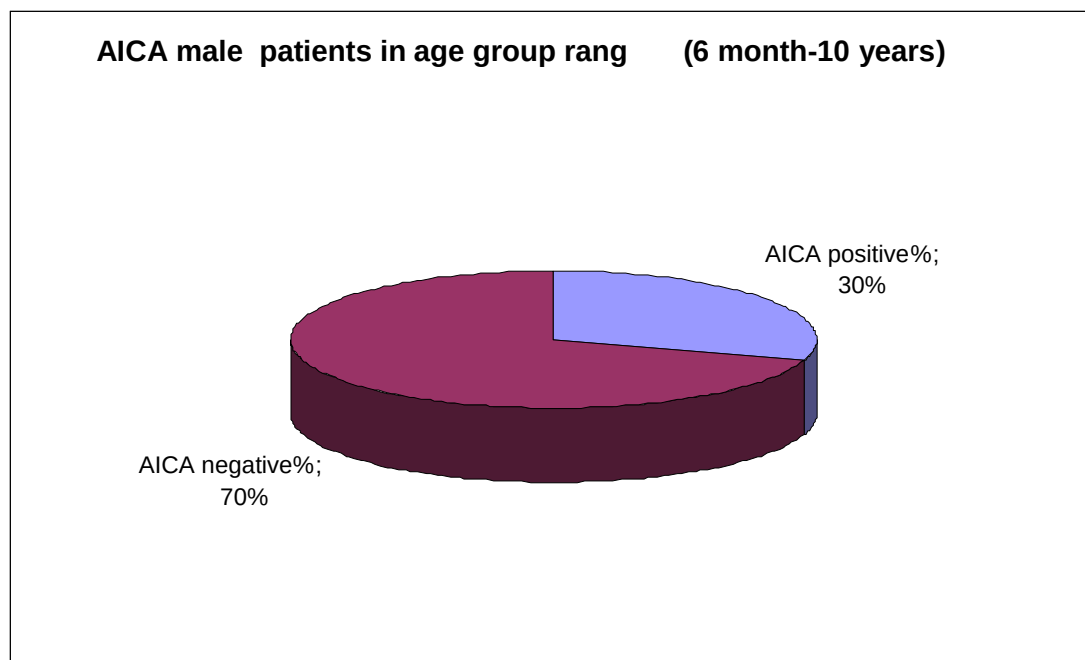
Anti islet cell antibody(AICA) in age group(6m-10y)were presented in Table (3-1D) , Figure (3-1D) ,(3-1E) , The percentage of positive AICA for male and female were 30 %,10 % respectively which were considered high when compared with healthy control for male and female and had given negative results in all age groups.

Table(3-1D) Specific anti islet cell antibody(AICA) in age group range(6m-10y)

<i>Patients groups</i>	<i>Positive AICA%</i>	<i>Negative AICA%</i>
<i>Male</i>	<i>30%</i>	<i>70%</i>
<i>Female</i>	<i>10%</i>	<i>90 %</i>



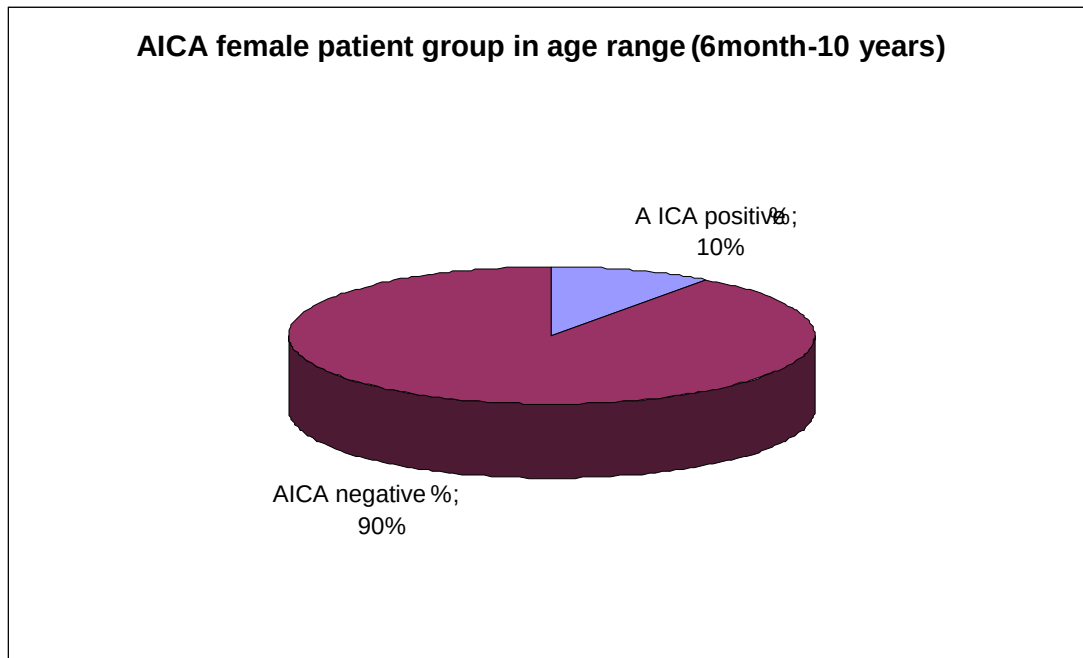
Figure(3-1) Show the specific AICA (IgG) fluorescent technique



Figure(3-1D) Positive percent of AICA for male patient in age group range(6m-10y)

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Figure(3-1E)Positive percent of AICA for female patient in age group range(6m-10y)

From above findings there is a relation between increasing of anti CMV IgG antibody titer with elevation of anti GAD antibody titer, In addition, total positive percent for anti islet cell as result of chronic CMV infection causes cross reaction between anti CMV IgG antibody and AICA add to that GAD enzyme because of molecular mimicry with CMV protein (Ivarsson,1993)

We found this association by increasing in titer of anti CMV IgG antibody in sera of newly diagnose T1D(male and female),Also there is a significant increasing in anti GAD antibody in addition to total positive percent of anti islet cell.

These results agreed with (Versallotti ,1998) who found the association between chronic CMV infection in children which was more likely to be associated with T1D.

This had given good indicator that newly diagnosed diabetic children with chronic CMV infection (high level of IgG antibody) caused induction for anti GAD antibody formation by cross reaction of Ab with GAD antigen and islet cell causes breaking for immune tolerance GAD enzyme was considered as sequestered antigen, in some few cases and may be a direct infection for islet cell causes cytolytic effect for β -cell.

3.2 ROLE OF CMV INFECTION IN TYPE 1 AUTOIMMUNE DIABETES IN AGE GROUP(11-20y).

Table and Figure(3-2A) , slight elevation in serum CMV IgM antibody for male and female with T1D(0.499 ± 0.249 i.u/ml), (0.794 ± 0.397 i.u/ml) respectively, compared with control groups for both sexes, female patient slightly have titer of anti CMV IgM antibody more than male patient.

Table(3-2A) Specific anti CMV IgM antibody in age group (11 – 20 y)

Groups	Sex	No.	Age(Years) mean $\pm$ SD	P	IgM(I.U/ML) mean $\pm$ SD	P
Control	male	18	17 $\pm$ 0.61		0.47 $\pm$ 0.39	
	female	18	17.5 $\pm$ 2.57		0.26 $\pm$ 0.253	
Patients	male	18	14 $\pm$ 2.52	P $\leq$ 0.05	0.499 $\pm$ 0.249	P* $<$ 0.05
	female	18	13 $\pm$ 2.91	P $\leq$ 0.05	0.794 $\pm$ 0.379	P** $<$ 0.05

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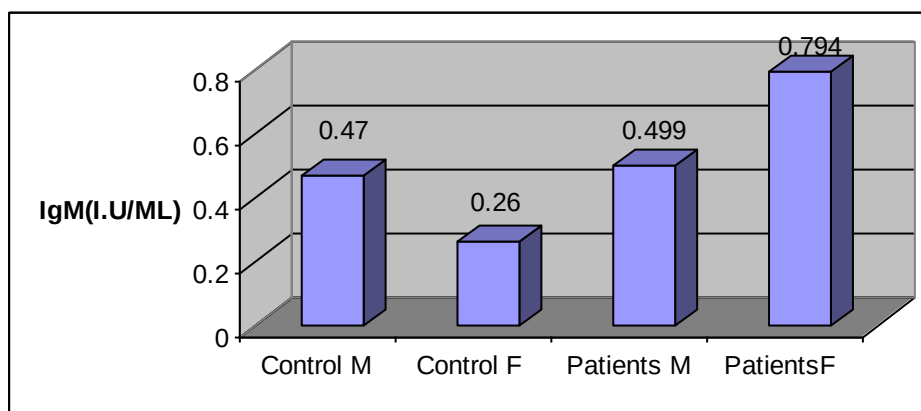


Fig. (3-2A) level of anti CMV IgM antibody in age group (11 – 20 y)

A significant increase of antiCMV IgG antibody titer in male patient (6.614 ± 3.307 .u/ml) while in female patient (6.488 ± 3.244 i.u/ml) compared with control person (male and female) without significant difference between male and female patient as noted in Table and Figure(3-2B).

Table (3-2B) Specific anti CMV IgG antibody in age group (11 – 20 y)

Groups	Sex	No	IgG(I.U/ML) mean± SD	P
Control	male	18	0.355±0.1775	
	female	18	0.046±0.023	
Patients	male	18	6.614±3.307	P* <0.05
	female	18	6.488±3.244	P** <0.05

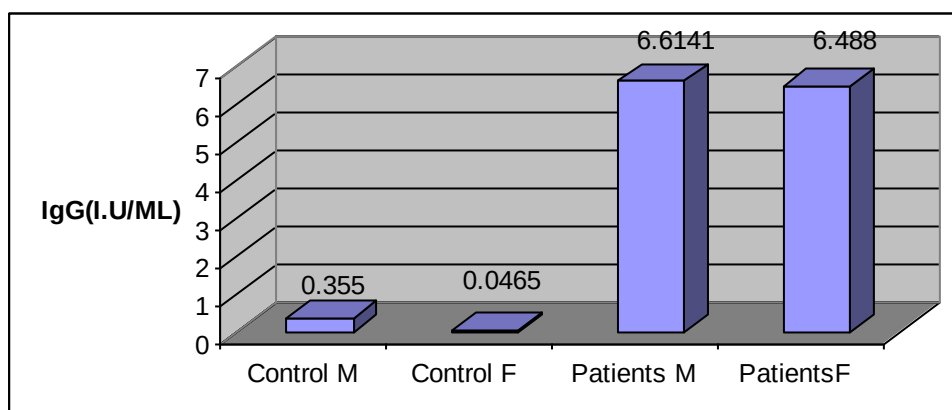


Fig. (3-2B) level of anti CMV IgG antibody in age group (11 – 20 y)

However, this increasing also we found in Table and Figure (3-2C) ,by following results (3.179 ± 2.580 i.u/ml), (2.6617 ± 2.247 u/ml) for male and female in anti GAD antibody titer when compared in titer of anti GAD antibody in sera of control group, when noted this result male patient showed more affected in anti GAD antibody than female patient when compared with result of healthy control.

Table(3-2C) Specific anti GAD antibody in age group (11 – 20 y)

Groups	Sex	No	AGAD (Unit /ML) mean $\pm$ SD	P
Control	male	18	0.029 $\pm$ 0.00411	
	female	18	0.255 $\pm$ 0.00102	
Patients	male	18	3.179 $\pm$ 2.580	P* <0.05
	female	18	2.6617 $\pm$ 2.247	P** <0.05

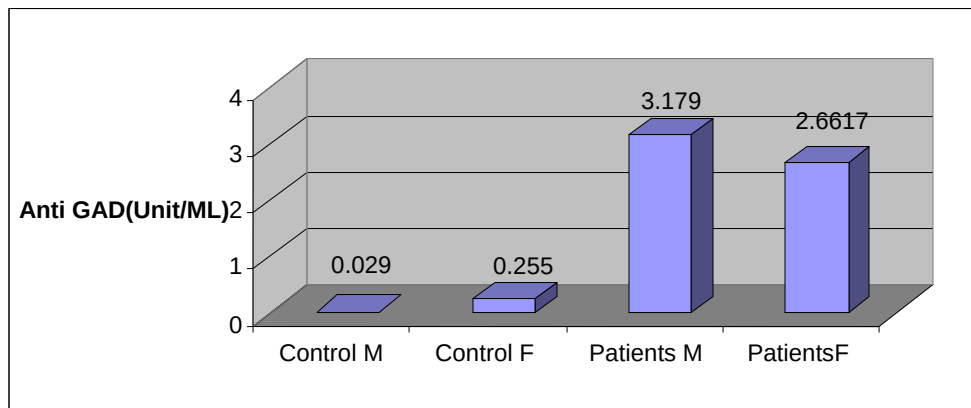


Fig. (3-2C) level of anti GAD antibody in age group (11 – 20 y)

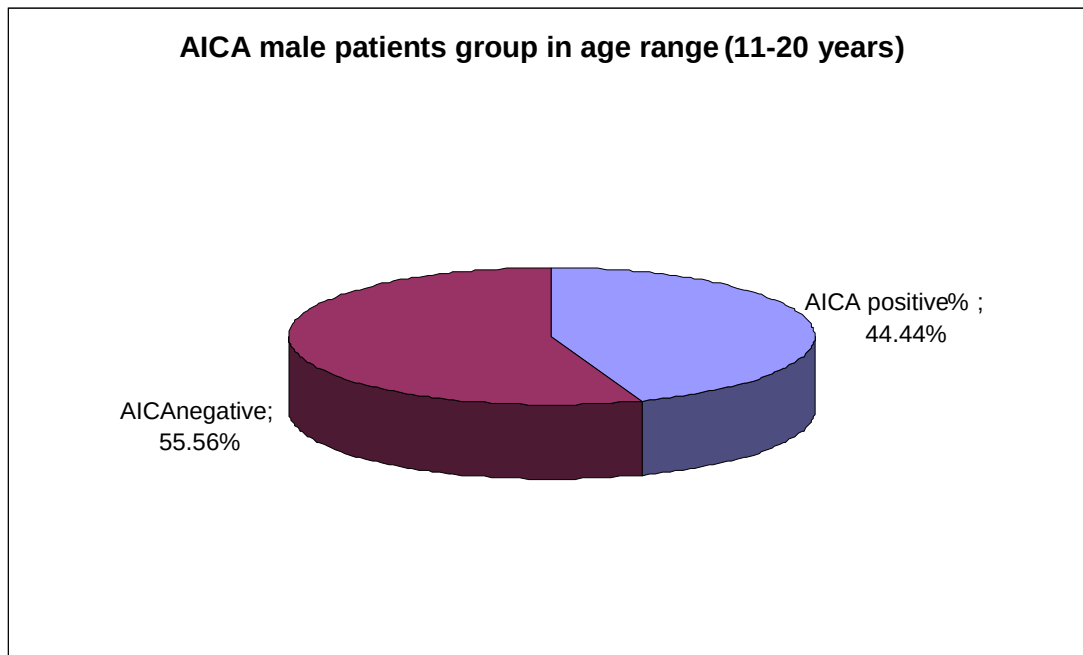
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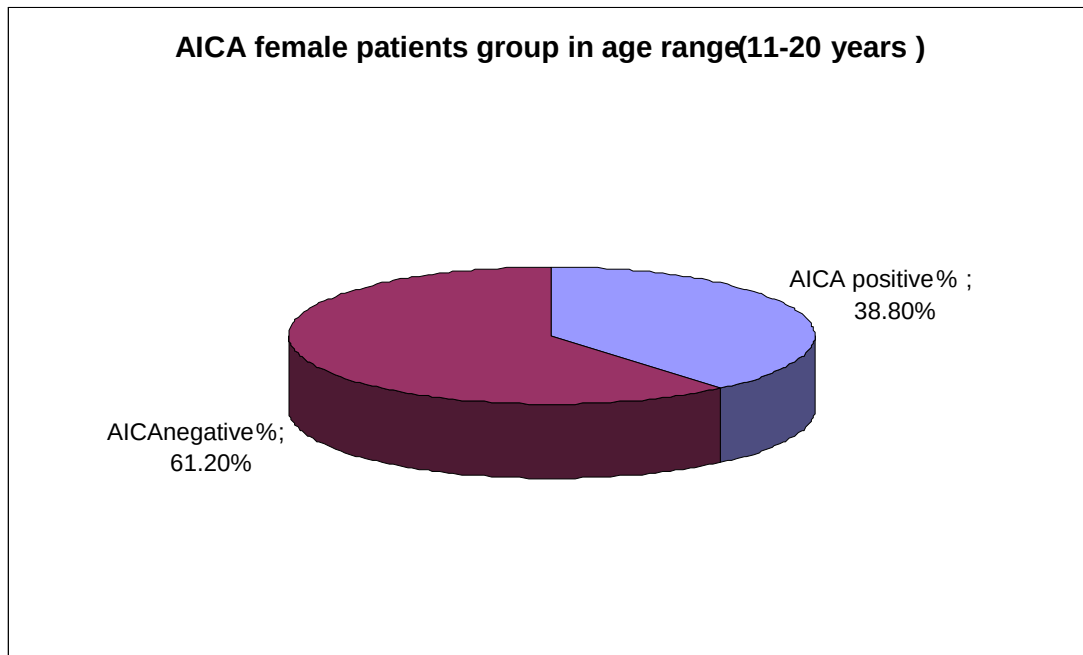
Total positive anti islet cell antibody , 44.44% for male, 38.8% where as female patient was more than first age group (6m-10y), Positive anti islet cell antibody for male more than female as seen in the following Table (3-2D), Figure(3-2D),(3-2E).

Table(3-2D) specific AICA in age group range(11-20y)

Patients groups	Positive AICA%	Negative AICA%
Male	44.44%	55.56%
Female	38.8%	61.2%



Figure(3-2D) Positive percent of AICA for male patient in age group range(11-20y)



Figure(3-2E) Percent of AICA for female patient in age group range(11-20y)

This explains that this age group shows highly association between chronic CMV infection and occurrence of T1D by increasing in anti GAD antibody. Anti islet cell antibody in this age group as a result of chronic CMV infection and these auto antibodies were considered as marker for development of autoimmunity that ended with occurrence of type 1 autoimmune diabetes.

These results are in consistent with (Joran et al, 2005) which studied association between coxsackie B virus, CMV infection and indication of T1D between adolescence by peak elevation in anti GAD antibody in this age. In those studied patients in correlation with increased titer of anti CMV IgG, as explained by our study with total positive anti islet cell antibody for both sex with increase in load of anti CMV IgG antibody. This increase chance Of

autoimmunity development in these age groups was considered the first step for T1D.

3.3 ROLE CMV INFECTION IN TYPE 1 AUTOIMMUNE DIABETES IN AGE GROUP (21-30y).

Level of anti CMV IgM antibody which indicated acute infection or reactivation of infection is related to an elevation in IgM antibody for CMV in male patient(1.73 ± 1.383 i.u/ml) in comparism with healthy control of male person, which is non significant increasing in anti CMV IgM antibody for female patient with T1D(0.44 ± 0.22 i.u/ml) while in female control(0.064 ± 0.032 i.u/ml) this explained in the following Table (3-3A), Figure(3-3A).

Table (3-3A) Specific anti CMV IgM antibody in age group (21 – 30 y)

Groups	Sex	No.	Age(Years) mean $\pm$ SD	P	IgM(I.U/ML) mean $\pm$ SD	P
Control	male	7	26 $\pm$ 2.69		0.0091 $\pm$ 0.005	
	female	7	25 $\pm$ 4.25		0.064 $\pm$ 0.032	
Patients	male	7	27 $\pm$ 3.73	P>0.05	1.73 $\pm$ 1.383	P* <0.05
	female	7	25 $\pm$ 2.81	P>0.05	0.44 $\pm$ 0.22	P** >0.05

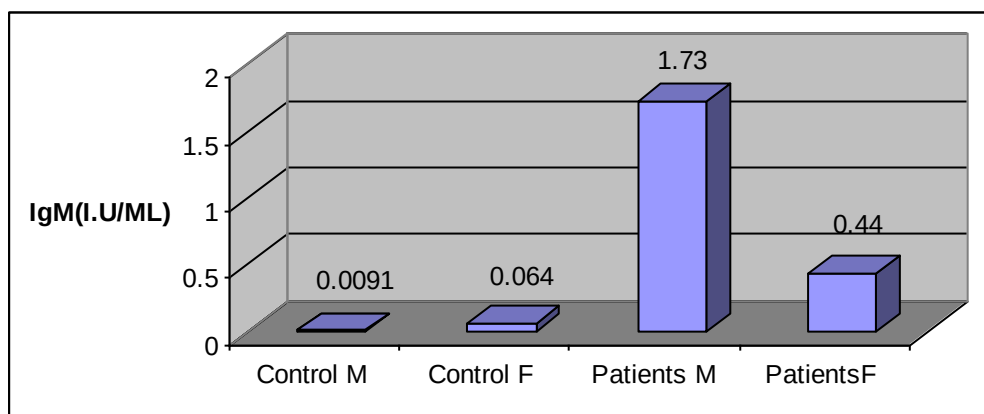


Fig. (3-3A) Level of anti CMV IgM antibody in age group (21 – 30 y)

Second table and figure (3-3B), shows a clear elevation in titre of antiCMV IgG antibody in sera of male and female patients with new diagnosis T1D (6.222 ± 5.435 i.u/ml), (7.967 ± 7.377 i.u/ml) respectively, in comparison with health control (male, female).

Table (3-3B) Specific anti CMV IgG antibody in age group (21 – 30 y)

Groups	Sex	No	IgG(I.U/ML) mean $\pm$ SD	P
Control	male	7	0.079 $\pm$ 0.037	
	female	7	0.0561 $\pm$ 0.028	
	male	7	6.222 $\pm$ 5.435	P* <0.05

Patients	female	7	7.967±7.377	P**<0.05
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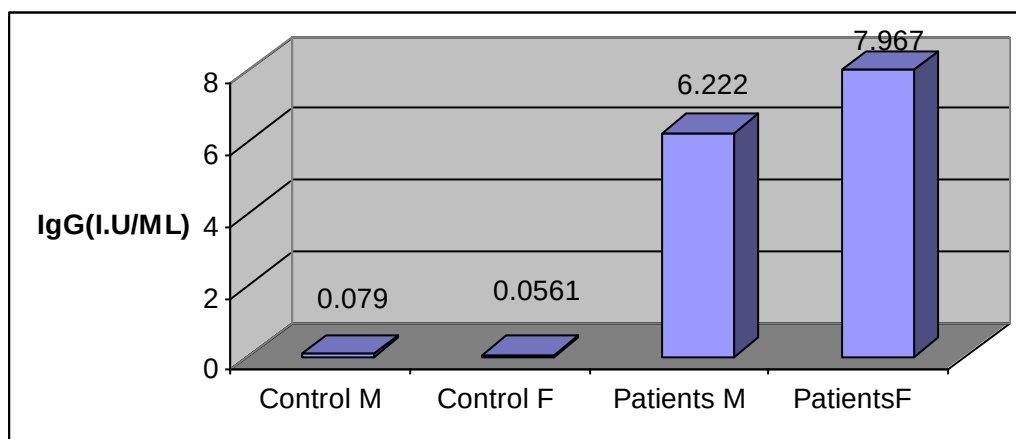


Fig. (3-3B) Level of anti CMV IgG antibody in age group (21 – 30 y)

When linked these results with Table and Figure(3-3)C, for level of anti GAD antibody in sera of newly diagnosed diabetic patient(1.981 ± 1.408 unit/ml), (2.0342 ± 1.31 unit/ml) for male and female respectively.

This result leads to clear fact (increasing and lowering of anti CMV IgG antibody effect on level of anti GAD antibody).

Table (3-3C) Specific anti GAD antibody in age group (21 – 30 y)

Groups	Sex	No	GAD (Unit /ML) mean± SD	P
Control	male	7	0.0571±0.047	
	female	7	0.151±0.096	

Patients	male	7	1.981±1.408	P* < 0.05
	female	7	2.0342±1.31	P** < 0.05

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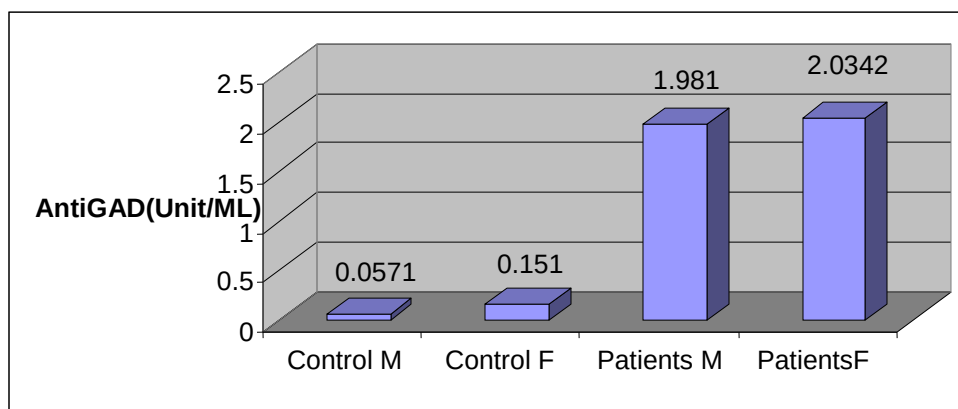


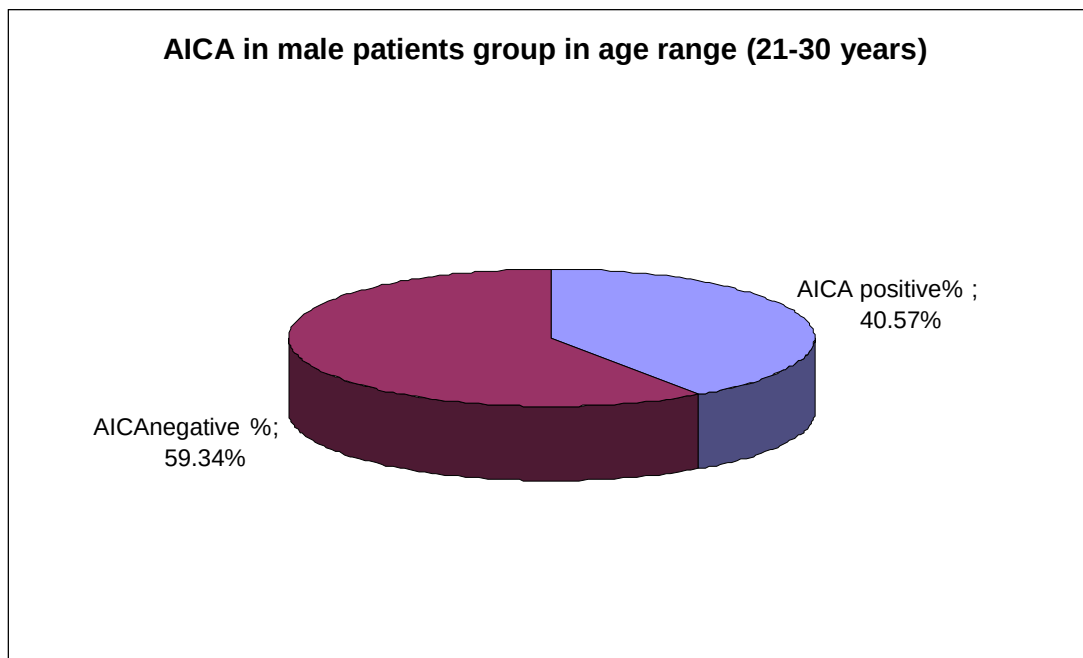
Fig. (3-3C) Level of anti GAD antibody in age group (21 – 30 y)

Male positive ICA, 40.57% while female patient 42.85 % which remains high in comparison with healthy control and is totally given negative result by immune fluorescent technique .

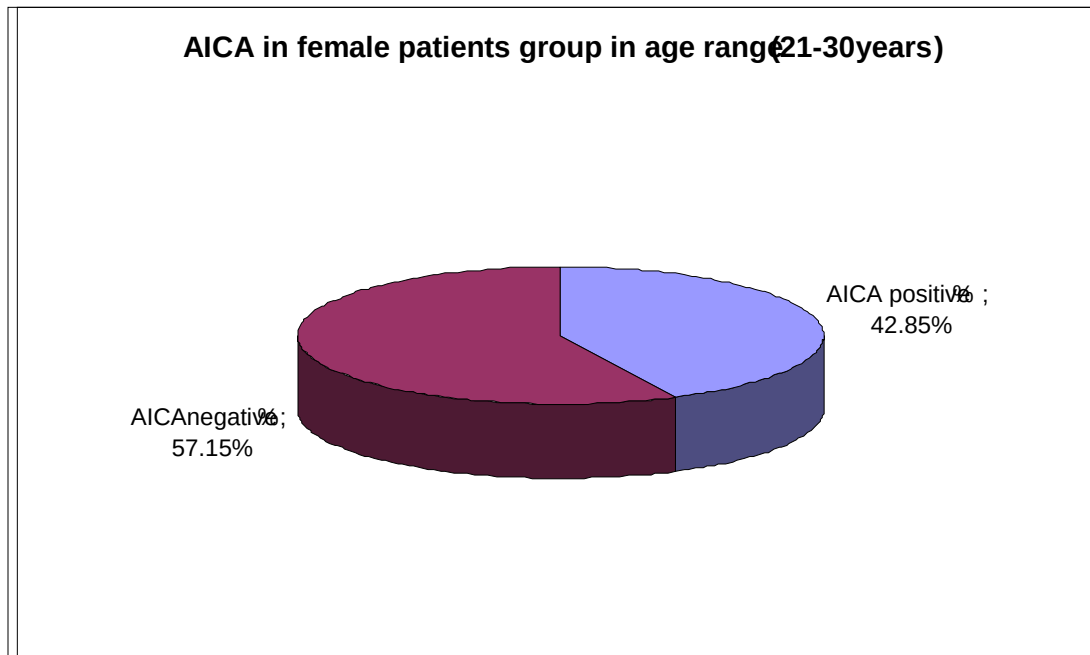
This result leads to clear fact (increasing and lowering of anti CMV IgG antibody effect on level of anti GAD antibody as well as ICA).

Table(3-3D) specific AICA in age group range(21-30y)

Patients groups	Positive AICA%	Negative AICA%
Male	40.57%	59.43%
Female	42.85%	57.15%



Figure(3-3D) Positive percent of AICA for male patient in age group range(21-30y)



Figure(3-3E) Positive percent of AICA for female patient in age group range(21-30y)

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These results for this age group show significant association between chronic infection and formation of auto antibodies (GAD,ICA) in those newly diagnosisT1D.

This result agrees with (Espinola- Kellin et al,2002) who suggested that 40% of patient with T1D newly diagnosis in adolescent and young causes by viral infection resulting by different mechanisms one of them direct infection to β -cell or cross reacting antibody and molecular mimicry in correlation with level of anti CMV IgG antibodies for virus. Our results in this age group explains when titer of anti CMVIgG but not anti CMVIgM antibody decreases this effect on auto antibodies (GAD,ICA) as in male patient. When titre of anti CMV IgG decreased, it reduces antiGAD antibody in addition to ICA, while in female patient an elevation caused elevation in ICA and anti GAD antibody.

4.4 ROLE OF CMV INFECTION IN TYPE 1 AUTOIMMUNE DIABETES IN AGE GROUP(31-40).

Fourth age group shows significant difference between anti CMV IgM in sera of male and female with T1D (0.286 ± 0.143 i.u/ml), (0.581 ± 0.2905 i.u/ml) respectively in comparison with healthy control .Female patient have titre of anti CMV IgM antibody slightly more than male patient as in Table and Figure (3-4)A.

Table(3-4A) Specific anti CMV IgM antibody in age group (31 – 40 y)

Groups	Sex	No.	Age(Years) mean $\pm$ SD	P	IgM(I.U/ML) mean $\pm$ SD	P
Control	male	9	33 $\pm$ 1.93		0.013 $\pm$ 0.0074	
	female	9	35 $\pm$ 3.44		0.02 $\pm$ 0.0131	
Patients	male	9	35 $\pm$ 2.69	P $\leq$ 0.05	0.286 $\pm$ 0.143	P* $<$ 0.05
	female	9	37 $\pm$ 3.58	P $>$ 0.05	0.581 $\pm$ 0.2905	P** $<$ 0.05

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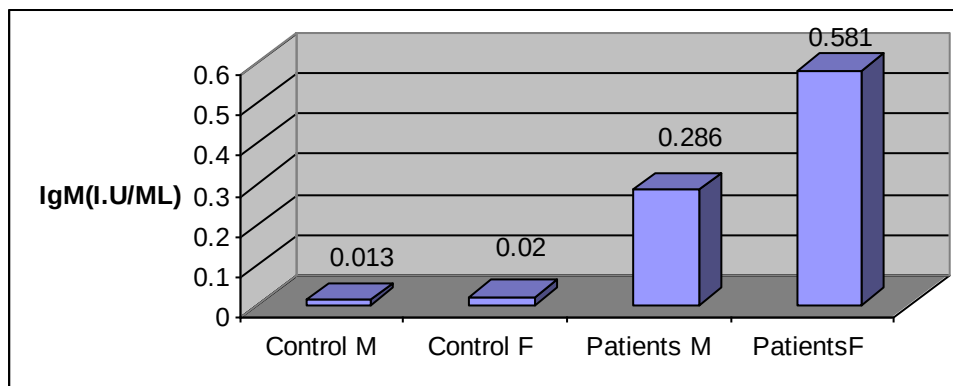


Fig. (3-4A) Level of anti CMV IgM antibody in age group (31 – 40 y)

Table and Figure(3-4)B, which explains level of anti CMV IgG antibody . There is decrease in titre of anti CMV IgG antibody in female patient (0.719 ± 0.3595 i.u/ml) while in male patient (6.188 ± 3.094 i.u/ml) and shows significant increasing when compared with healthy control ,male patient showed significantly high titre than female patient.

Table (3-4B) Specific anti CMV IgG antibody in age group (31 – 40 y)

Groups	Sex	No	IgG(I.U/ML) mean± SD	P
Control	male	9	0.059±0.038	
	female	9	0.170±0.085	
Patients	male	9	6.188±3.094	P* <0.05
	female	9	0.719±0.3595	P** <0.05

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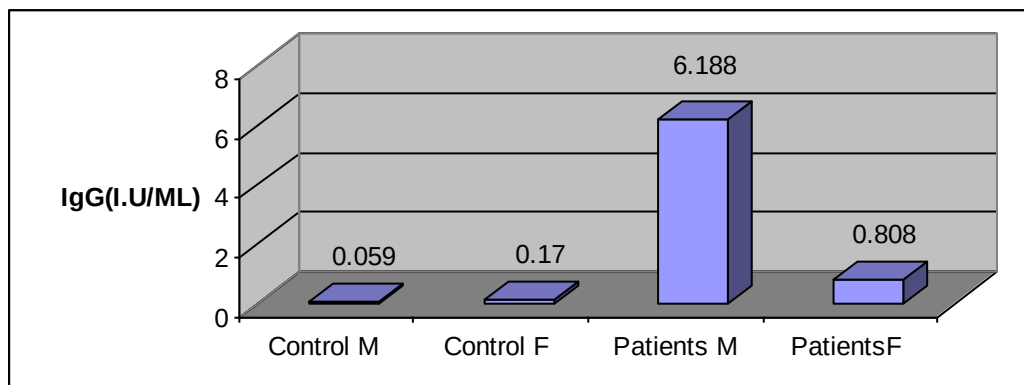


Fig. (3-4B) Level of anti CMV IgG antibody in age group (31 – 40 y)

This difference in the titer of anti CMV IgG antibody appear effective on Table and Figure (3-4) C, anti GAD antibody titer in sera of female patient with T1D (1.1024 ± 0.819 Unit /ml) while in male patient (1.5028 ± 1.066 unit /ml), however in both results there is a significant increase in anti GAD antibody titre from sera of

healthy control but male patient affected more in anti GAD antibody than female.

Table (3-4C) Specific anti GAD antibody in age group (31 – 40 y)

Groups	Sex	No	GAD (Unit /ML) mean± SD	P
Control	male	9	0.03±0.033	
	female	9	0.26±0.216	
Patients	male	9	1.5028±1.066	P* <0.05
	female	9	1.1024±0.819	P** <0.05

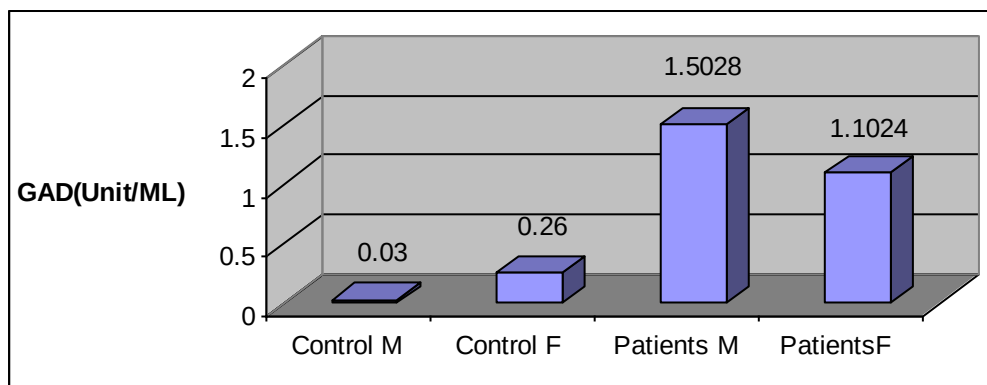
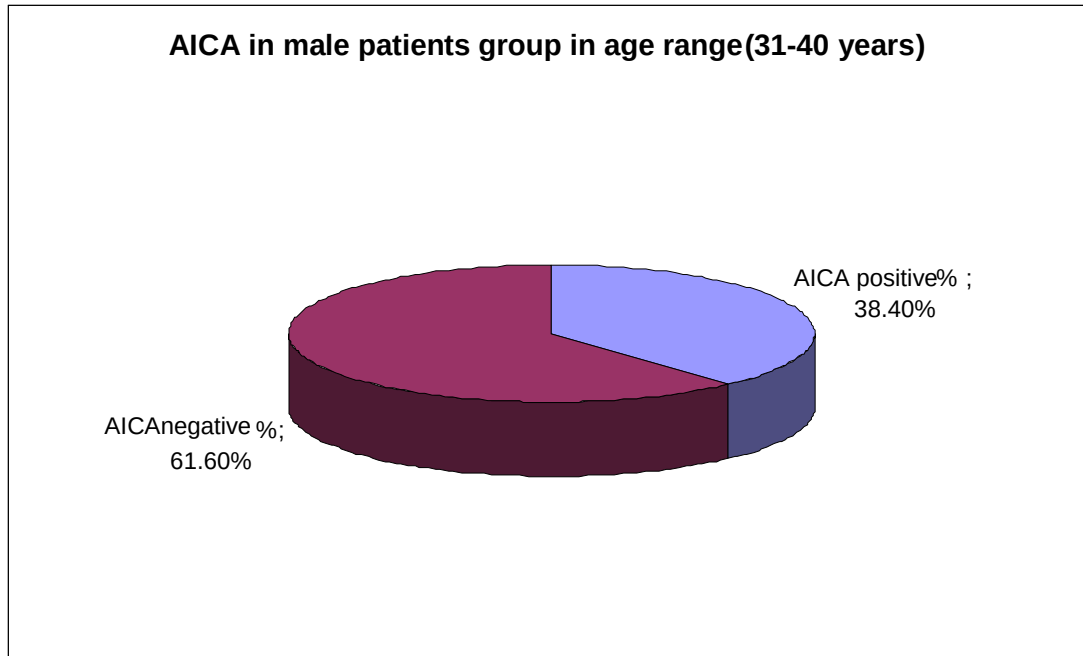


Fig. (3-4C) Level of anti GAD antibody in age group (31 – 40 y)

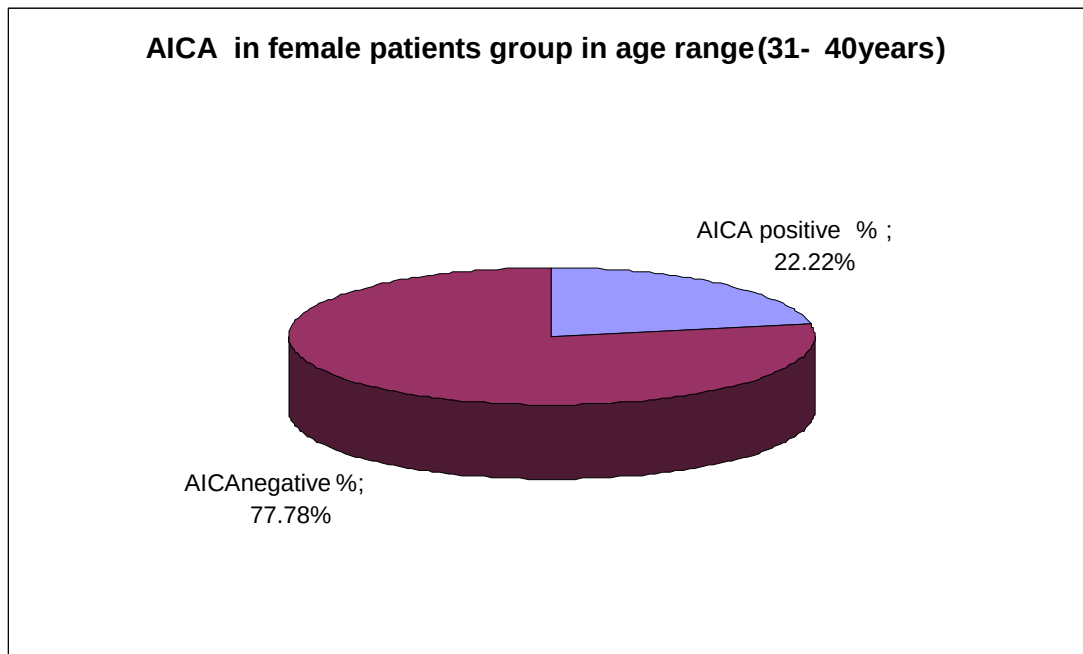
Positive anti islet cell antibody in female patient 22.22% is less than male patient 38.4% in correlation with titer of anti CMV IgG antibody.

Table(3-4D) specific AICA in age group range(31-40y)

Patients groups	Positive I CA%	Negative ICA%
Male	38.4%	61.6%
Female	22.22%	77.78%



Figure(3-4D) Positive percent of AICA for male patient in age group(31-40y)



Figure(3-4E) Positive percent of AICA for female patient in age group range(31-40y)

The titre of anti GAD antibody and anti islet cell antibody began to decrease in middle age group (31-40y) in correlation with decrease. In total titre of anti CMV IgG antibody especially in female patient seems very clear in this age group.

This agreement with (Visseren et al , 1997), The association between viral infection and T1D decreases in middle age and old age with decrease of chronic viral infection in studied group of newly diagnosis T1D patient. When titre of anti CMV IgG antibody is decreased, this reduces cross reacting antibody or proinflammatory cytokines in addition to reactive T-cell with GAD enzyme and reduces expression of MHC class 1.

4.5 ROLE OF CMV INFECTION IN TYPE 1 AUTOIMMUNE DIABETES IN AGE GROUP (41-50y).

There is significant increase in anti CMV IgM antibody in both male and female sera (0.63 ± 0.315 i.u/ml), (0.415 ± 0.2075 i.u/ml) respectively. When compared with health control sera as seen in the following Table and Figure(3-5A).

Table (3-5A) Specific anti CMV IgM antibody in age group (41 – 50 y)

Groups	Sex	No.	Age(Years) mean $\pm$ SD	P	IgM(I.U/ML) mean $\pm$ SD	P
Control	male	8	46 $\pm$ 2.13		0.0705 $\pm$ 0.0058	
	female	8	41 $\pm$ 0.92		0.0475 $\pm$ 0.0046	
Patients	male	8	42 $\pm$ 7.814	P>0.05	0.63 $\pm$ 0.315	P* <0.05

	female	8	46±3.105	P≤0.05	0.415±0.2075	P**<0.05
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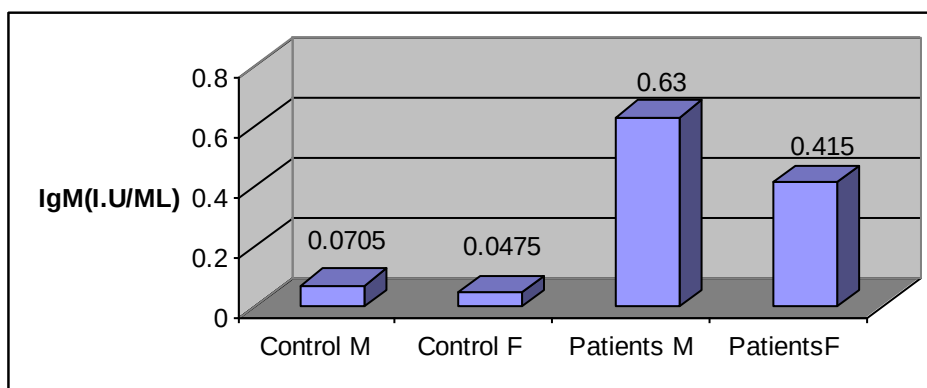


Fig. (3-5A) Level of anti CMV IgM antibody in age group (41 – 50 y)

Table and Figure(3-5)B specific anti CMV IgG antibody totally lower than previous age group(31-40y),but in male patient sera anti CMV IgG antibody there is no significant increasing when compared with healthy control ,titer for male patient (1.317 ± 1.207 i.u/ml) $P \geq 0.05$.while in female patient sera titer of anti CMV IgG antibody (3.816 ± 1.908 i.u/ml), there is a significant increasing as compared with female healthy control sera $P \leq 0.05$.

Table (3-5B) Specific anti CMV IgG antibody in age group (41 – 50 y)

Groups	Sex	No	IgG(I.U/ML) mean± SD	P
Control	male	8	0.5187±0.453	
	female	8	0.0013±0.0005	
Patients	male	8	1.317±1.207	P*>0.05
	female	8	3.816±1.908	P**<0.05

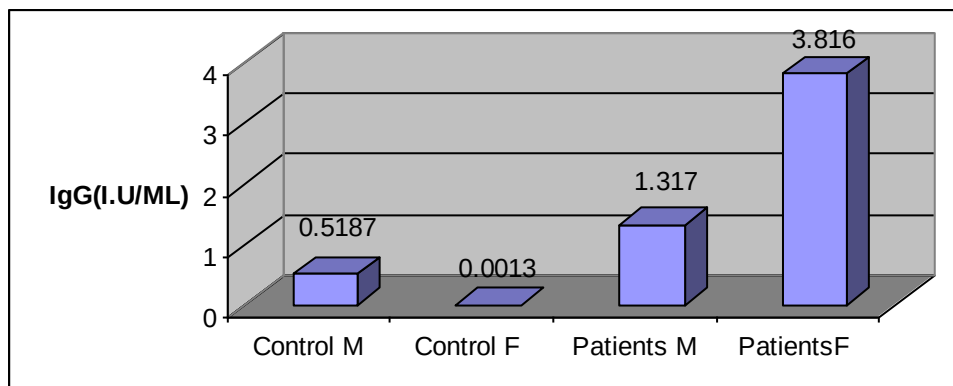


Fig. (3-5B) Level of anti CMV IgG antibody in age group (41 – 50 y)

Table and Figure(3-5C), also show effective by difference in anti CMV IgG antibody titer in sera of patient in this age group, anti GAD antibody titer (0.9418 ± 0.615 unit/ml), (1.228 ± 0.2877 unit/ml) for male and female frequently and this result shows significant elevation in comparism with healthy control.

Table (3-5C) Specific anti GAD antibody in age group (41 – 50 y)

Groups	Sex	No	GAD (Unit /ML) mean $\pm$ SD	P
Control	male	8	0.0415 ± 0.0122	
	female	8	0.043 ± 0.0010	
Patients	male	8	0.9418 ± 0.615	$P^* < 0.05$
	female	8	1.228 ± 0.2877	$P^{**} < 0.05$

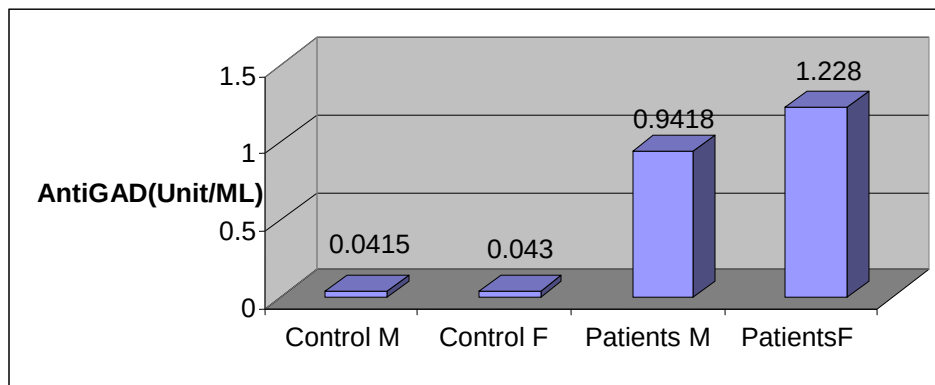
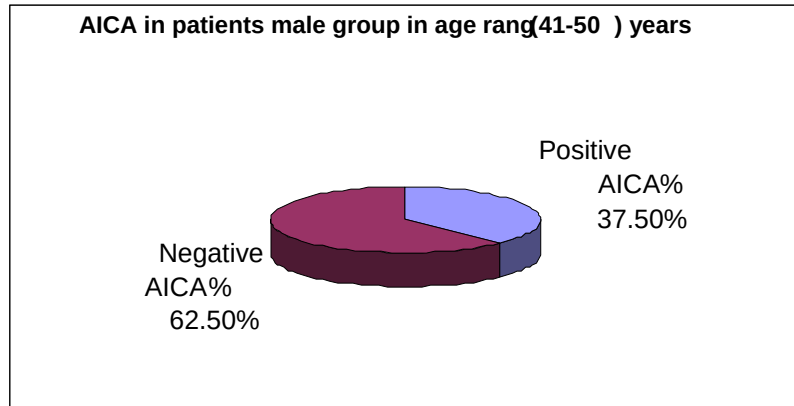


Fig. (3-5C) Level of anti GAD antibody in age group (41 – 50 y)

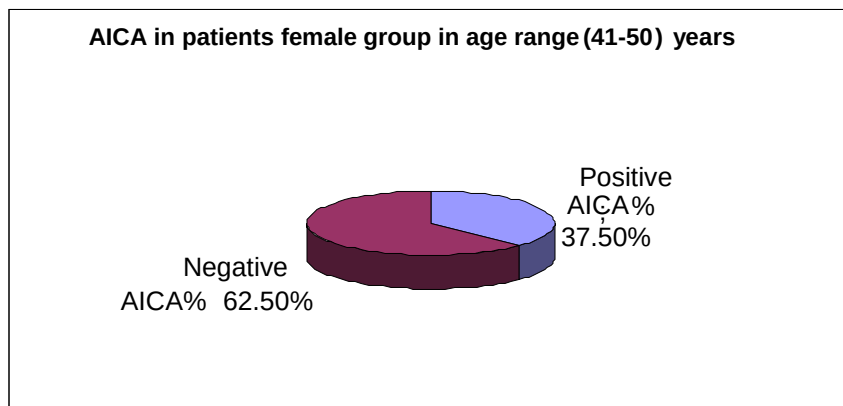
There is equal positive percentage of anti islet cell 37.5%, for both sex as seen in table(3-5D), Figure(3-5D),(3-5E), female show increasing in positive ICA in compared with previous age group (31-40y) in correlation of increasing in anti CMV IgG antibody, this showed in table (3-5D), Figure(3-5D),(3-5E).

Table(3-5D) specific AICA in age group range(41-50y)

Patients groups	Positive AICA%	Negative AICA%
Male	37.5%	62.50%
Female	37.5%	62.5%



Figure(3-5D) Positive percent of AICA for male patient in age group range(41-50y)



Figure(3-5E) Positive percent of AICA for female patient in age group range(41-50y)

The Above mentioned result showed association between chronic infection with CMV and occurrence of T1D remain in all age groups. Although decrease in total anti CMV IgG antibody, antiGAD antibody, anti islet cell antibody this reduction in last two parameter back to reduced in titer of anti CMV IgG antibody except in female patient, and there is an elevation in anti GAD antibody in addition to anti islet antibody in correlation with increasing titer of anti CMV IgG antibody in this age group.

This finding is also proved by (Imagawa et al,2000) who mentioned the association of chronic viral infection and occurrence

of T1D may occur at any age but level decrease with increasing in age this back to nature of T1D which affect children and young age

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more commonly and reduce occurrence of chronic infection.

3.6 ROLE OF CMV INFECTION IN TYPE 1 AUTOIMMUNE DIABETES IN AGE GROUP (51-60y).

These patient show marked increasing in anti CMV IgM antibody titer in sera of patient in Table and Figure(3-6)A, (1.462 ± 0.731 i.u/ml), (3.44 ± 1.72 .u/ml) for male and female in comparison with healthy control sera, and this may result from decrease of immune responses in old age which exposed them to different viral and bacterial infection.

Table (3-6A) Specific anti CMV IgM antibody in age group (51 – 60y)

Groups	Sex	No.	Age(Years) mean $\pm$ SD	P	IgM(I.U/ML) mean $\pm$ SD	P
Control	male	11	52 $\pm$ 0.46		0.071 $\pm$ 0.0004	
	female	11	56 $\pm$ 0.809		0.086 $\pm$ 0.0024	
Patients	male	11	56 $\pm$ 2.97	P $\leq$ 0.05	1.462 $\pm$ 0.731	P* $>$ 0.05
	female	11	55 $\pm$ 1.29	P $\leq$ 0.05	3.44 $\pm$ 1.72	P** $<$ 0.05

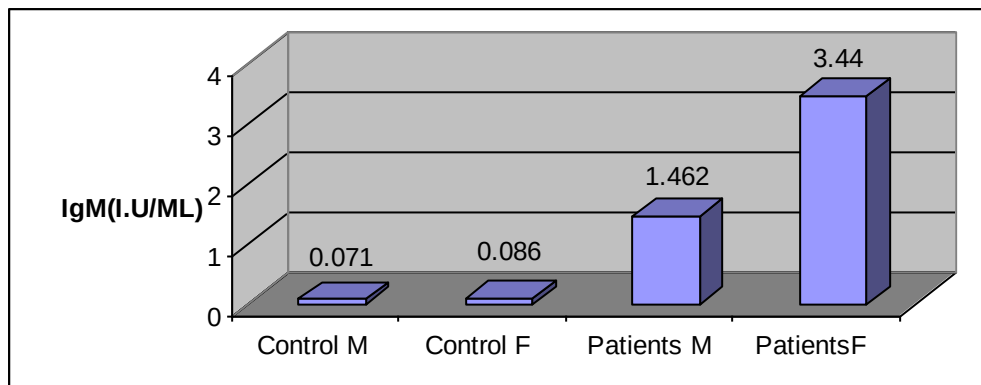


Fig. (3-6A) Level of anti CMV IgM antibody in age group (51 – 60 y)

Second Table and Figure for this age group(3-6)B, titre of anti CMV IgG antibody in sera of patient with T1D show significant increasing (2.595 ± 1.2975 .u/ml), (0.358 ± 0.179 i,u/ml) in male and female respectively more than healthy control male patient showed more affected in chronic infection.

Table (3-6B) Specific anti CMV IgG antibody in age group (51 – 60y)

Groups	Sex	No	IgG(I.U/ML) mean± SD	P
Control	male	11	0.008 ± 0.00046	
	female	11	0.0075 ± 0.0005	
Patients	male	11	2.595 ± 1.2975	$P^* < 0.05$
	female	11	0.358 ± 0.179	$P^{**} < 0.05$

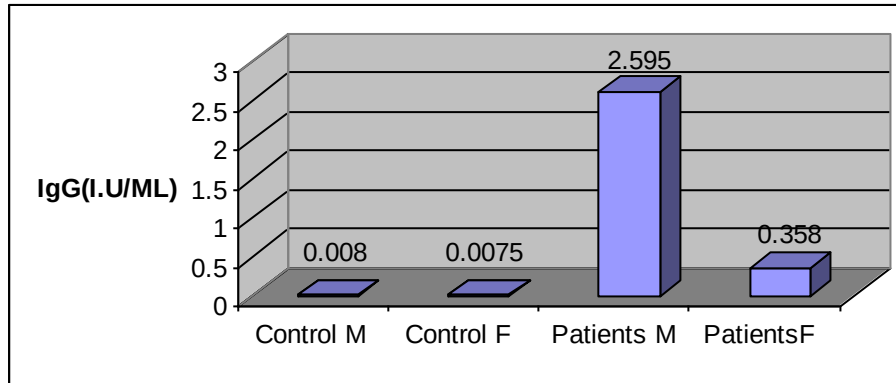


Fig. (3-6B) Level of anti CMV IgG antibody in age group (51 – 60 y)

The following results of antiGAD antibody titer (0.955 ± 0.745 unit/ml), (0.968 ± 0.329 unit/ml) for male and female respectively with little effective in anti CMV IgG antibody titer especially in male patient as seen in Table and Figure (3-5C).

Table (3-6C) Specific anti GAD antibody in age group (51 – 60y)

Groups	Sex	No	GAD (Unit /ML) mean $\pm$ SD	P
Control	male	11	0.197 ± 0.0005	
	female	11	0.196 ± 0.0011	
Patients	male	11	0.955 ± 0.745	$P^* < 0.05$
	female	11	0.968 ± 0.329	$P^{**} < 0.05$

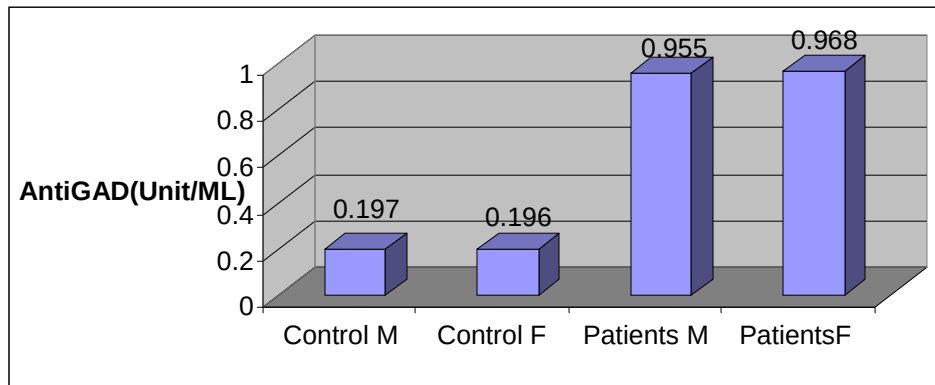
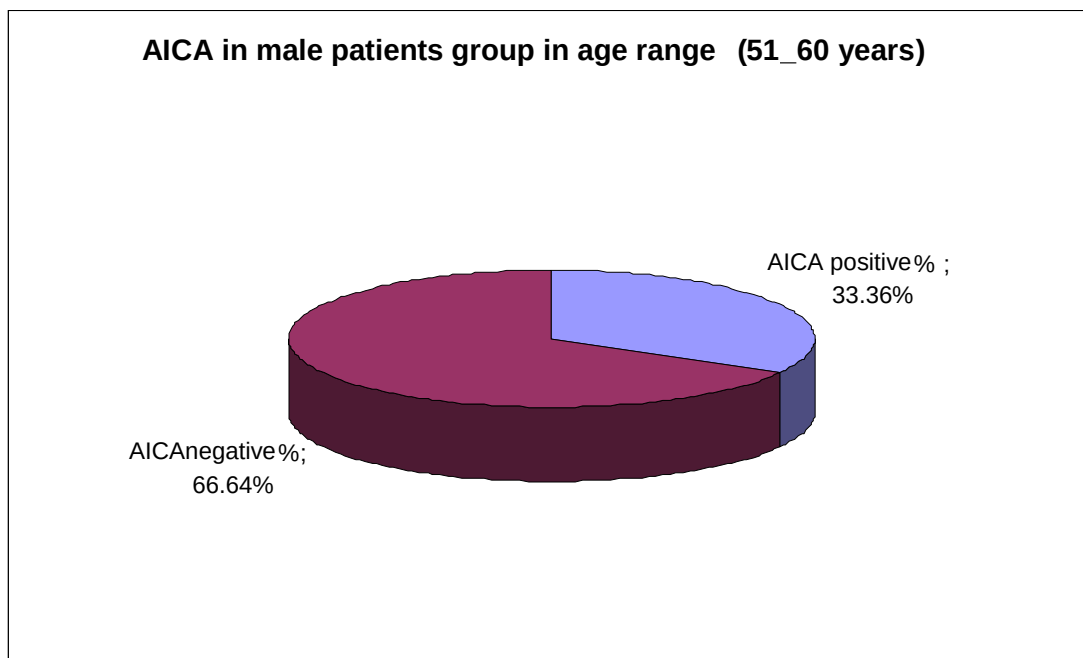


Fig. (3-6C) Level of anti GAD antibody in age group (51 – 60 y)

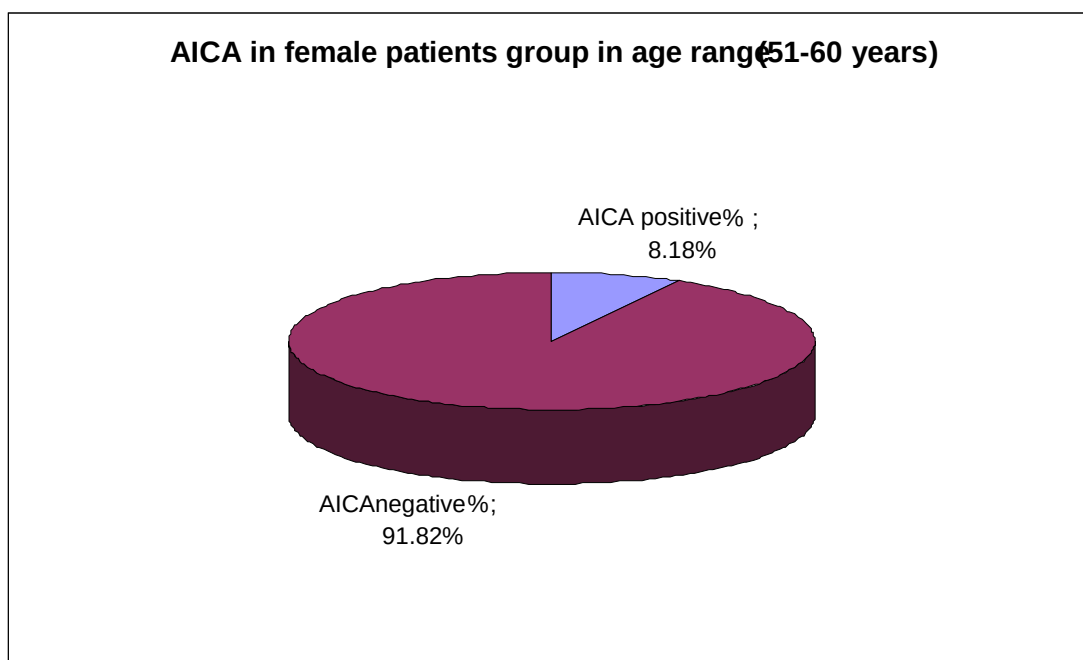
While positive anti islet cell antibody in male was 33.36%, and in female 8.18% with male show more affective in ICA than female.

Table(3-6D) specific ICA in age group range(51-60y)

Patients groups	Positive AICA%	Negative AICA%
Male	33.36%	66.64%
Female	8.18%	91.82%



Figure(3-6D) Positive percent of AICA for male patient in age group range(51-60y)



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This result explains low association between chronic CMV infection and occurrence of T1D as mentioned previously. The association decreases with increase in age as well as anti GAD antibody titer level decline within old age, however, this age group explain some association with anti islet cell antibody and titer of anti CMV IgG antibody. This result may return to the fact level of GAD enzyme decrease with age but auto antibodies to anti islet cell antibody may occur at any age so children and young age remain more exposure to these auto antibodies.

This result is correlated with (Bonifacio et al,1998) which demonstrated the association between occurrence of T1D in old age induced by viral infection was low. In other views, there is an elevated titre of anti islet cell in both sexes in comparism with health control sera. This mean that some association remains between titer of anti CMV and anti islet cell especially in male patient except anti GAD antibody in this age group of newly diagnosis T1D but this low association may return to a number of factors, and one of them is physiological change in pancreas gland that may lead to breaking of immune tolerance.

Finally, occurrence of T1D in old age group explains role of virus infection in predisposition to disease in old age that rarely occurs in age group over 45y according to result of may study.

4.7 EFFECT OF AGE ON CMV INFECTION IN DIFFERENT AGE GROUP.

Acute infection or reactivation of infection with CMV indicates by level of anti CMV IgM antibody which is done in this study only to explain role of chronic infection(anti CMV IgG antibody)In autoimmunity that is considered one cause of T1D and this processes take some time, noted total anti CMV IgM

antibody increase with age to reach maximal titer in old age with alteration in immune

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response . This acute infection did not show any association with autoimmune processes and occurrence of T1D.

Our result agrees with (Horikawa,et al,2000) seroprevalence of anti CMV IgM which is high in old age group.

Chronic CMV infection (anti CMV IgG antibody) increased from first age group (6m-10y) to reach maximal titer in adolescence and young age then begins to decrease in middle age group (31-40y)to the next old age.

These results agree with (Visseren et al,1997) who suggested that CMV infection is more common between children and young age and is considered one of the important environmental factors for T1D in these age groups.

This finding may back to nature of infection with CMV that transmmited from person to person, by secretion (saliva, urin, breast milk, semen) or through close contact for this infection is most common in children of young age owing to increase exposure to virus during this age.

This result disagree with (Bissinger et al,2002) who explained that chronic CMV infection is most common in old age as in correlation with decrease immune responses between older persons, in the United states antiCMV IgG antibody have been reported in 10-15% of the adolescence population, 40-50% of population by the age 35y and this may exceed 60-70% in population older than 65y.

This difference may be attributed to genetic difference, sensitivity of method used in the test, difference in style of life and personal health measurement, chronic CMV infection and

induction of T1D remain in all age group but this association decreased with increase in age while acute infection (anti CMV IgM Ab) increase with age.

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4.8 EFFECT OF SEX ON CMV INFECTION IN DIFFERENT AGE GROUP.

Different age groups with new onset T1D generally show that there is a correlation between male and female infected with CMV. Only there is a little difference except in age group (31-40y) in titre of antiCMV IgG antibody and this result is also explained by (Robert and Cech,2005)who found increased in seroprevalence anti CMV IgG antibody between female from 25-40y as result of pregnancy, hormonal state, sexual activity. This differs from one community to another, in other words difference in gender does not effect on CMV infection, our results are also mentioned by (Vercellotti ,2001) who said that difference in gender does not effect on CMV infection while difference in economic status, personal hygien, sexual activity is more likely to be associated with infection.

Personal hygiene is still the most effective measurement that helps to ovoid CMV infection and ovoid crowded living in addition to health education.

4.9 EFFECT OF AGE ON ANTI GAD ANTIBODY TITRE IN DIFFERENT AGE GROUP.

GAD enzyme found in pancreas gland, anti GAD antibody my form as a result of cross reactive antibody with virus protein or

CMV specific T-cell with GAD enzyme that stimulates β -cell to produce autoantibodies.

First age (6m-10y) the level of anti GAD antibody with clear significant increase when compared with control group but level remains not high because most common anti GAD antibody from IgM group in children is less than 10y years when continues to the next age group (11-20y) there is a marked significant increase in anti GAD antibody titer more than control group and first age group.

These age groups show maximal elevation in titer of anti GAD antibody and these findings correlated with (Leslie and Castelli,2004) who proved that anti GAD antibodies were more prevalent during childhood and adolescent and young age .The level decrease in next age group(21-30y) and this reduction may back to difference in level of anti CMV IgG antibody . This titer was high in comparism with healthy control, antiGAD antibody needed to predisposition factors and one of them is chronic viral infection.

Our study found that level of GAD auto antibodies in these age groups are effected by level of anti CMV IgG antibody more than effect of age.

Age group (31-40y) titer of anti GAD antibody is greater than titer of healthy control and the result is statistically significant $P \leq 0.05$, but this titer was less than previous age groups (21-30y).

When continues to the next age group (41-50y) there was a reduction in the titer of anti GAD antibody in responses to factor of titer anti CMV IgG antibody and increase in age.

This above result was correlated with (Sabbah et al,2000) who suggested that viral infection is considered one of the important environmental factor for T1D by formation of GAD auto antibodies. These auto antibodies decrease with reduced of viral antibodies as well as increase age of patient.

This means that age is considered one of the important parameters for level of GAD auto antibodies as explained in last age group(51-60y).The level of anti GAD antibodies is low in comparism with other age groups, although there is a significant titer of anti CMV IgG antibody when compared with healthy control .The explanation of this finding may be due to the fact that those patients who develop diseases T1D before age of 40y have HLA-DR3,DR4 show significant higher frequencies of anti GAD antibodies as well as anti islet cell antibodies while those who begin diseases (T1D) after 40y were HLA-DRB1,DRQB1 which show much less evidence of autoimmunity (Pozilli and Mario,2001).

4.10 EFFECT OF SEX ON ANTI GAD ANTIBODY LEVEL IN DIFFERENT AGE GROUP.

When considering the results, different age groups that are included in our study saw level of anti GAD antibody decrease or increase according to change in level of anti CMV IgG antibody especially in children and young age group in addition to factor age. These results gave good indication that the difference in sex does not effect on level of anti GAD antibody.

Pardni et al,(1999) found that there was similar range of anti GAD antibody in male and female patient with new onset T1D causes by CMV infection and difference in sex was not effecting on GAD auto antibodies formation. These auto antibodies form in responses to environmental factor in equal percentage in both sex.

This above result disagrees with (Blonas et al,1996)over 80% of females with new onset IDDM have anti GAD antibody, whereas the frequency in males was as low as 50-60% under the age of 10years,with an increase to 75-90%there after, at 20 years of age there was no longer difference between both sexes. The reason for such differences in the prevalence of these autoantibodies may be attributed to the difference in assay used, difference in sensitivity levels of procedure used, added to that difference in patient genetics and environmental condition.

4.11 EFFECT OF AGE ON ANTI ISLET CELL ANTIBODY (AICA) IN DIFFERENT AGE GROUP.

AICA is considered as an important marker for T1D which appears with anti GAD antibody prior to appearance of the clinical symptoms. (Verge et al., 1996)

There is a correlation between total positive anti islet cell and anti GAD antibody titer with anti CMV IgG antibody titer total anti islet cell antibody is consider high in all age groups when compared with healthy control which all of them gave negative result by immune fluorescent technique.

Age group (11-20y),(21-30y), shows maximal elevation in total positive anti islet cell antibody and these percentage decreased with increase of age to reach lower percentage in age group(51-60y) ,ICA positive percent correlated with anti CMV IgG antibody titer.

This result agree with (Maclaren et al,1999) who suggested that there is a difference in total percentage of anti islet cell in young age with peak elevation in this percent which decrease with age.

Hemstra et al, (2001) found that the level of AICA fluctuated according to level of anti GAD antibodies and these studies explain that there is an association in formation OF (GAD.ICA) autoantibody with CMV, Coxsakie B Virus, Mumps Virus in new onset IDDM, also explain in following age group (31-40Y), (41-50Y), (51-60Y) found total positive percent for ICA

decrease respectively with fall in titer of anti GAD antibody and anti CMV IgG antibody.

Important note in age group(51-60y),AICA shows more association with anti CMV IgG antibody than GAD auto antibodies and this may back to as mentioned previously . Level of GAD enzyme decreases with age for this ICA gave good indicator to autoimmunity during CMV infection in new onset T1D in old age better than GAD autoantibody as explained by (Lernmark et al, 1995), who noted that ICA show association with viral infection in new onset IDDM than anti GAD antibody during onset of diseases in over 50 years age when level of enzyme decrease .

4.12 Effect of sex on anti islet cell antibody (AICA) in different age group.

Age group (6m-10y),(11-20y) positive anti islet cell antibody in male patient with new onset T1D was more than female patient but this result is correlated with (Accilli,2003) which mentioned male patient with new onset diabetes have titer of anti islet cell antibody more than female patient. This difference between both sexes seen is in association with difference in anti CMV IgG antibody titer.

Young age group (21-30y) positive anti islet cell percent in female patient decrease to 42.85% from previous age group while in male patient ,there was little decrease in positive percent of anti islet cell antibody to reach 40.57% as when compared with

previous age group 44.44 %. This difference results from change in titer of anti CMV IgG antibody when increased in sera of female

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Patient. While in male patient there is little decrease in this titer and this result agreed with (sridher,1996) who found that females are affected earlier than males by autoimmunity process of type 1 DM. The most accepted reason for sex difference in those who developed the diseases at 20-40years old was peak occurrence of IDDM, partially may result from high susceptibility at this age since sex steroids hormone lead to decreased insulin sensitivity so females affected by these autoantibodies earlier when there is an environmental factor as (viral infection) the mechanisms of autoimmunity accelerated in female faster than male by viral antigen expression on β -cell antigen altered in addition to expression of cytokine or HLA antigen. However, this does not agree with finding in next age group (31-40y) that found male patient have positive percent of anti islet cell 38.4% more than female patient that decreased to 22.22%, but this difference is correlated with anti CMV IgG antibody and anti GAD antibody titre change is not affected by hormonal change. It means that ICA antibody in young age and middle age have association with CMV infection or anti GAD antibody titer but the effect by difference in sex (male and female) as that noted by Barker et al, (2004)when noted there is difference in level of anti islet cell antibody between male and female patient with newly diagnosed T1D with some difference according to difference in sex but remain correlation with antiGAD antibody and anti CMV IgG antibody titer, Our result shows an association between positive AICA with CMV infection in young and middle age groups.

Age group (51-60y) when level of GAD enzyme decreases in this age group, anti islet cell antibody gave clear association with anti CMV IgG antibody titer in male patient (2.595 ± 1.2975 i.u/ml) while positive anti islet cell antibody percent 33.36%, female patient (0.358 ± 0.179 i.u/ml) and positive anti islet cell 8.18% for female patient.

From the above results, we can conclude that anti islet cell antibody in old age gives better picture for autoimmunity induced by CMV infection than GAD auto antibodies.

CONCLUSIONS

According to this study, we can conclude the following

- 1- Cytomegalovirus infection may be association with the development of type 1 autoimmune diabetes in different age groups.
- 2-Association between chronic cytomegalovirus infection and type 1 Diabetes Mellitus was high in children and young age.
- 3-Anti GAD antibody titer decreases with the age.
- 4-Total positive anti islet cell antibody decreases with the age.
- 5-Sex has no effect on CMV infection.
- 6-Titre of anti CMV IgM antibody does not effect on autoimmune processes.
- 7-Difference in sex does not effect on anti GAD antibody titre.
- 8-In old age group(51-60y)anti islet cell shows more association with anti CMV IgG antibody than anti GAD antibody.
- 9- anti GAD antibody shows more correlation with level of anti CMV IgG antibody than anti islet cell antibody in newly diagnosed T1D.

RECOMMENDATIONS

- 1-Clinical trial and cohort studies through vaccination against CMV may clarify the situation.
- 2-Make CMV antibody test as one of routine laboratory test especially in children and young and treatment of positive cases effectively.
- 3-Test for Anti GAD antibody and Anti islet cell antibody may help to diagnosis before appearance of clinical symptoms and change of blood sugar value.
- 4-Clinical trial and concern of the researches on other types of virus that are considered environmental factors for type 1 autoimmune diabetes to make control on the diseases.
- 5-Further study and researches on used GAD antibody as vaccine. Could be carried out.

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Appendix (1) : Questionnaire chart for patients

Name of patient:

Age:

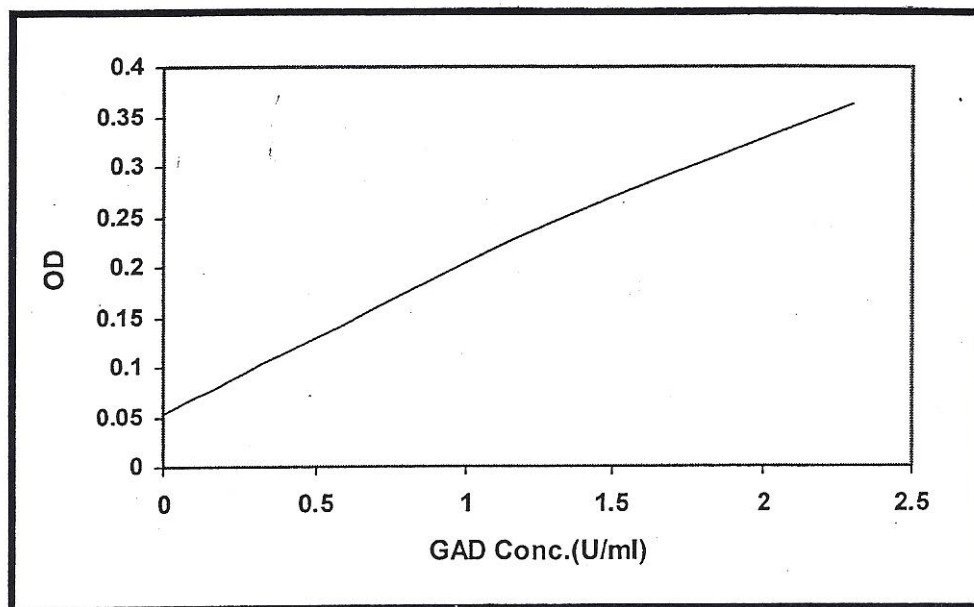
Sex:

Clinical Diagnosis:

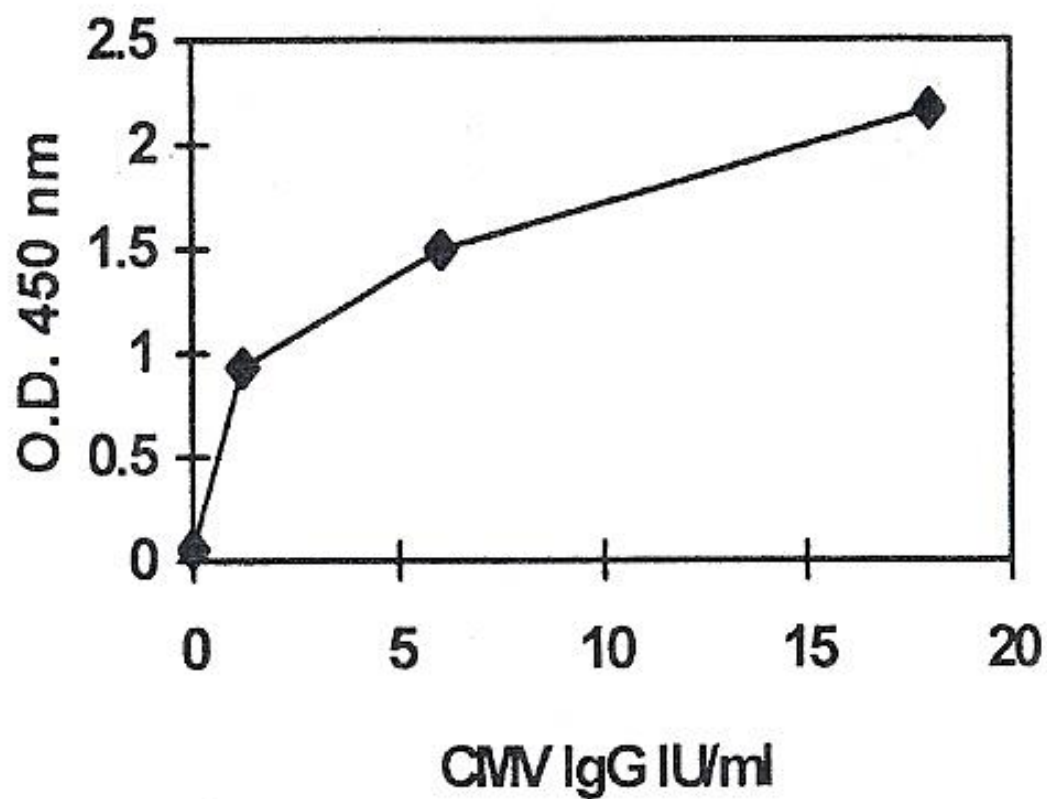
Familial history:

Time of diagnosis:

Note:



Appendix (2) : Standard curve of enzyme immunoassay for detection anti-GAD autoantibodies



Appendix (3) : Standard curve of enzyme immunoassay for detection anti-CMV IgG antibodies

الخلاصة:

ان فيروس انتفاخ الخلية قد تم ادراجه ضمن العوامل المسببة لداء السكري (النوع الاول) في كثير من الدراسات ولذلك فان الدراسات الوبائية افترضت الاصابة المزمنة بهذا الفيروس واحدة من الاسباب للنوع الاول من داء السكري.

الهدف من الدراسة هو للتحري عن دور فايروس انتفاخ الخلية بانه واحد من العوامل البيئية للنوع الاول من داء السكري في مجاميع عمرية مختلفة مع مجموعة ضابطة من الاصحاء.

الدراسة أجريت في مركز الغدد الصماء والسكري والمركز الوطني للسكري التابع إلى دائرة صحة الكرخ للفترة من الاول من اب الي غاية كانون الاول عام 2008. اخذت العينة التي تتكون من 120 شخص (60ذكور, 60اناث) مصابين بداء السكري النوع الاول حديثا, 120 شخص (60ذكور و60اناث) مجموعة ضابطة.

كل الأشخاص تم فحصهم لوجود الاجسام المضادة لفيروس انتفاخ الخلية في مصولهم متزامنا مع وجود الاجسام المضادة إلى انزيم (GLUTAMIC ACID DECARBOXYLASE) والأجسام المضادة إلى (ISLET CELL) في البنكرياس تم فحص نسبة السكر في الدم بعد الصيام الي مجموعة المرضى والمجموعة الضابطة.

كانت هنالك علاقة بين الاشخاص المصابين بالاصابة المزمنة بفايروس انتفاخ الخلية وحدوث النوع الاول من السكر وعرف بواسطة قياس الاجسام المضادة الي انزيم GLUTAMIC DECARBOXYLASE وخلايا ISLET CELL في البنكرياس في مختلف الفئات العمرية .

الاطفال والشباب اظهروا علاقة قوية بين الاصابة المزمنة بفايروس انتفاخ الخلية وحدوث النوع الاول من داء السكري مقارنة مع المجاميع العمرية المختلفة.

الاجسام المضادة الي انزيم GLUTAMIC ACID DECARBOXYLASE اظهرت حساسية عالية الي مستوى الاجسام المضادة المزمنة الي فايروس انتفاخ الخلية اكثر من الاجسام المضادة ISLET CELL في البنكرياس ماعدا في الفئة العمرية الكبيرة (51-60 سنة) الاجسام المضادة ISLET CELL في البنكرياس اوضحت علاقة مع الاجسام المضادة لفايروس انتفاخ الخلية اكثر من الاجسام المضادة الي GLUTAMIC ACID DECARBOXYLASE.

الاجسام المضادة الي الاصابة الحادة (anti CMV IgM Ab) لا تؤثر على حدوث الاجسام المضادة الي (ISLET CELL, GLUTAMIC ACID DECARBOXYLASE) في حدوث النوع الاول من داء السكري. الاختلاف بالجنس لا يؤثر على الإصابة بفايروس انتفاخ الخلية.

تلك الدراسة أوضحت دور الإصابة المزمنة بفايروس انتفاخ الخلية بانه واحد من العوامل البيئية للنوع الاول من داء السكري.

دراسات أكثر نحتاج لتحديد دور فايروسات اخرى بانها من العوامل البيئية للنوع الاول من داء السكري.

CHAPTER ONE

Introduction and Literatures Review

CHAPTER TWO

Material and Method

CHAPTER THREE

Results and Discussion

Conclusion and Recommendation

APPENDIC ES