تقييم المناعة الخلطية ضد حمة الحصبة في محافظة النجف

رسالة مقدمة إلى

مجلس كلية الطب / جامعة بابل

وهي جزء من متطلبات نيل شهادة الماجستير في

الأحياء المجهرية الطبية

من قبل

أنور جاسم محمد سارة

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Evaluation of Humoral Immunity Against Measles Virus in Al-Najaf Governorate

A thesis

Submitted to the Council of College of Medicine – Babylon University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Medical Microbiology

By

Anwar J. M. Sarah

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بسم الله الرحمن الرحيم

﴿يُؤْتِي الْحِكْمَةَ مَنْ يَشَاءُ وَمَنْ يُؤْتَ الْحِكْمَةَ فَقَدْ أُوتِيَ خَيْرًا كَثِيرًا وَمَا يَذَّكَّرُ إِلَّا أُولُوا الْأَلْبَابِ﴾

صدق الله العلي العظيم

سورة البقرة

(الآية 269)

Dedication

To my parents

and all my family members

with love and respect

Anwar

We, the examining committee, certify that we have read the thesis entitled **(Evaluation of Humoral Immunity Against Measles Virus in Al-Najaf Governorate)** have examined the student **Anwar J. M. Sarah** in its contents, and that in our opinion it is accepted as thesis for **the Degree of Master of Science in Medical Microbiology.**

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الخلاصة:

أعدت هذه الدراسة لتقييم عيارات أضداد حمة الحصبة صنف (IgG) في أشخاص ملقحين وغير ملقحين ضد حمة الحصبة في محافظة النجف. أستخدم اختبار الألايزا المصلي لفحص 375 عينة مصل تم جمعها خلال فترة الدراسة (نيسان إلى تشرين الثاني 2006). تراوحت أعمار الأشخاص اللذين شملتهم الدراسة بين 1-30 سنة. كان منهم 335 ملقحين و 40 غير ملقحين. تم تقسيم الأشخاص الملقحين إلى خمس مجاميع عمرية:

مجموعة A (1<7) ومجموعة B (7<13) ومجموعة C (13<19) ومجموعة D (19<25) وأخيراً مجموعة E (25<31). في حين تراوحت أعمار الأشخاص غير الملقحين بين 1-3 سنوات لذا قسموا إلى ثلاث مجاميع عمرية (1 و 2 و 3 سنة). تم أخذ المعلومات المتعلقة بالعمر والجنس ومكان الإقامة وتأريخ التلقيح ضد حمة الحصبة لكل شخص. أظهرت نتائج الدراسة أن أعلى عيار لل(IgG) كان في المجموعة العمرية الأولى وهو (234.82) ولوحظ أيضاً أن العيار يتناقص مع الزيادة بالعمر وصولاً إلى أوطأ عيار لل(IgG) في المجموعة العمرية E وهو (6.6087). سجل أعلى عيار لل(IgG) (1024) في عينات مصل من أشخاص بعمر 6 سنوات واللذين لقحوا بجرعة منشطة من لقاح الحصبة المختلطة (MMR) الحاوي على عترة (شوارز). بينما سجل أوطأ عيار لل(IgG) في عينات مصل من أشخاص بعمر 29 سنة وكان مقداره (4). أظهرت النتائج المتعلقة بمكان الإقامة أن هنالك اختلافات بين عيار ال(IgG) لكل من سكنة الريف والمدينة حيث كان متوسط عيار ال(IgG) لسكنة المدينة (92.4211) أعلى من متوسط عيار ال(IgG) لسكنة الريف (83.6667). أيضا أظهرت النتائج المتعلقة بالجنس أن هنالك اختلافات بين عيار ال(IgG) لكل من الذكور والإناث حيث كان متوسط عيار ال(IgG) للإناث (94.366) أعلى من متوسط عيار ال(IgG) للذكور (84.7253). بينما أظهرت مجموعة السيطرة (غير الملقحين) اختلافات غير ملحوظة في معايير ال(IgG)

للسنوات العمرية المختلفة. كذلك استخدم في هذه الدراسة اختبار الانتشار المناعي الإشعاعي المفرد لتقييم التراكيز الكلية غير الخاصة لل(IgG, IgM, IgA, C3, C4) لستين شخص مختار (50 شخص ملقح و 10 أشخاص غير ملقحين) حيث أظهرت النتائج أن التراكيز الكلية لكل من ال(IgG, IgM, IgA, C3, C4) في الأشخاص الملقحين في الثلاث سنين الأولى من المجموعة العمرية A هي أعلى من الأشخاص غير الملقحين (مجموعة السيطرة) في الأعمار المماثلة.

Summary

This study was conducted to evaluate the anti-measles IgG antibody titers in measles vaccinated and non vaccinated normal individuals in Al-Najaf Governorate. The serological test "ELISA" was applied to evaluate the 375 collected sera samples during April to November 2006. Individuals included with age range (1-30) years; 335 of them were vaccinated, and 40 non vaccinated (control group). The vaccinated individuals were divided into five age groups (A (1<7), B (7<13), C (13<19), D (19<25), and E (25<31)), while the non vaccinated individuals (control group) were from the age of 1-3 years. Age, sex, residency and history of measles vaccination were recorded for each individual.

The results of this study revealed; that the highest IgG titer (234.82) as detected by ELISA had been seen in age group (A) and the titer was decreasing with increasing of age and reaching to the lowest IgG titer (6.6087) in age group (E). The highest IgG titer (1024) was recorded in sera samples of six years individuals who had got the booster dose of Measles-Mumps-Rubella (MMR) vaccine (Schwarz strain). The lowest IgG titer (4) was detected in sera samples of 29 years individuals.

The residency of individuals was showed differences between IgG titers of rural and urban individuals. The total mean of IgG titer of urban was (92.4211) higher than the IgG titer (83.6667) of rural.

The sex of individuals also showed differences between IgG titers of males and females. The mean of IgG titer (94.366) of females was higher than the IgG titer (84.7253) of males. While the control group was showed neglectable differences in IgG titers of the different age years.

The single radial immunodiffusion (SRID) tests for estimation of the nonspecific total concentrations of IgG, IgM, IgA, C3 and C4 of 60 (50 vaccinated and 10 control) selected individuals were used in this study. The results revealed that the total concentrations of IgG, IgM, IgA, C3 and C4 in the vaccinated individuals of the first three years of age group A were higher than the unvaccinated individuals (control group) of the same ages.

Contents

Chapter One

Introduction

1.1: Introduction

Measles is a highly contagious viral disease, it remains a major cause of worldwide infant mortality accounting for almost one million deaths every year globally (Green *et al.*, 2001; WHO, 2005; CDC, 2006b). Measles virus (MV) is generally transmitted by aerosolized secretions (Boon *et al.*, 2006). Most children recover uneventfully from the illness, but serious complications can occur, including pneumonia and involvement of the central nervous system (Sips *et al.*, 2007). Despite the highly contagious nature of the disease, MV has been effectively controlled during the past four decades by immunization with live attenuated vaccines. Measles is typically a self-limiting disease, whereas MV in general does not cause a persistent infection, no animal reservoir for measles virus exists, no vector is involved in its spread and the human is the only natural reservoir of MV (Hasegawa *et al.*, 2006; Sellin *et al.*, 2006). MV is rarely cause persistent infection mainly in individuals who are immunocompromized (Moss *et al.*, 2002; Russell *et al.*, 2004; D'Souza *et al.*, 2006). MV has only one serotype (Hunt, 2006), the virus is antigenically stable and vaccination with the currently used live attenuated vaccines proved to be highly effective in preventing disease (Stittelaar *et al.*, 2002; and Permar *et al.*, 2006), but MV infection is still a major health problem in developing countries and is the highest cause of mortality among children from a vaccine-preventable disease in

the world (WHO, 2005). The current measles vaccines are safe and efficacious for children over the age of 9 months, but due to the less developed immune system and the presence of transplacental maternal antibody, the vaccine is less effective in infants. Another problem associated with the current attenuated measles vaccines is they do not raise as high or as long-lived, neutralizing antibody responses as wild-type MV infection (Green *et al.*, 2001). The WHO has sent the objective of eradicating measles by the year 2010 as part of its expanded programme of immunization (EPI) (WHO, 2005; Boon *et al.*, 2006), whereas substantial progress towards measles control has been made since 1989. In 1998 the estimated global number of cases and deaths had declined to 31 million and 875000 respectively (i.e., reductions exceeding 63% and 83% in comparison with the pre-vaccine era) (WHO, 1999).

Recent studies about incidence of measles infection in Iraqi population indicated that there was marked increase in measles cases during 1997 and also during the period of 1998-1999, the total number of measles cases were 454 during 2003 and there was marked increase in the cases during the period extended from January till June 2004 and the total number of reported cases were 8253 especially in Basra and other southern governorates, while 101 cases of measles were reported during September 2004, and 56 cases were reported in October 2004 (Al-Khafaji, 2006).

1.2: Aims of the Study

1. To assess the effectiveness of measles vaccination program.
2. To assess the level of specific anti-measles antibody (IgG) titer and their protective activity.
3. To assess the social and residential factors and their roles in the levels of Ab specific titer.

Chapter Two

Review of Literatures

2.1: Measles

Measles is an acute, highly contagious viral disease capable of producing epidemics. It characterized by high fever, cough, coryza, conjunctivitis, and Koplik's spots, which precede the appearance of a florid, generalized macular and popular rash (Cooper, 1999; WHO, 2007). Measles, which is more common in winter and spring, is one of the most readily transmitted communicable diseases (CDC, 2006b). It is frequently complicated by middle ear infection or diarrhea. The disease can be severe, with bronchopneumonia or brain inflammation (encephalitis) leading to death in approximately 1 of every 1000 cases in developed countries. In developing world, case-fatality rates often exceed 150 deaths per 1000 cases (Maldonado, 2004; CDC, 2006a).

2.2: History of Measles

Measles is probably as old as civilization itself. The written history of measles is classically traced to the writings of the physician Rhazes, also known as Abu Becr, who lived during the 10^{th} century. However, the disease was apparently recognized as early as the 7^{th} century by such ancients as Hebrew physician Al Yehudi (Bernstein and Schiff, 1998; Redd *et al.*, 1999). Rhazes referred to measles as **Hasbah**, which means "eruption" in Arabic (Redd *et al.*, 1999). *Rubeola* and *morbilli* are descriptive Latin words first used in the middle ages. The later is a diminutive of *morbus*, meaning "disease"

which was reserved to refer to bubonic plague; *morbilli* referred to a minor disease. "Measles" is probably derived from *mesels*, the anglicized form of *misellus*, which in turn is a diminutive of Latin word *miser*, meaning miserable and referring to the sufferer of various eruptions or sores. The presence of nonspecific leprous sores was incorrectly identified with the disease called *morbilli* in Latin. Thus, *mesels* came to be equated with the disease and not the sufferer of ill-defined skin lesions. It was probably not until the severe epidemics of measles during the seventeenth century when annual bills of mortality in London in 1629 listed the two diseases separately (CDC, 1998; Cooper, 1999; Redd *et al.*, 1999).

Danish physician Peter Panum in 1846 not only confirmed that measles was contagious but also defined the 14 days interval between exposure and appearance of exanthem, recognized the higher mortality at extremes of age, and observed that infection provided life long immunity (CDC, 2006b).

In 1911, using infected material from acute cases, Goldberger and Anderson transmitted human measles infection to monkeys, clearly demonstrating the existence of an infectious agent or substance responsible for measles. This finding antedated the technology to isolate and culture the measles virus. In 1954, Enders and Peebles successfully isolated the measles virus in human and monkey kidney tissue culture (Redd *et al.*, 1999).

In 1957, Milovanovic and his colleagues made adaptation of the measles virus to the chicken embryo, whereas Katz in 1958 cultivated the virus in chicken embryo tissue culture. This successful cultivation of the virus enhanced the virologist and paved the road to the development and licensure of Edmonston B Live attenuated measles vaccine in 1963 (Redd *et al.*, 1999; Latif, 2002).

Dramatic effect on the incidence of measles and its associated complication occurred as a results of wide spread vaccination program in western hemisphere (Redd *et al.*, 1999).

Reductions in morbidity and mortality have been so great that global eradication of the disease has been proposed and judged feasible (CDC, 1997; Redd *et al.*, 1999).

2.3: Etiology

Measles virus (MV), the cause of measles, is an RNA virus of the genus *Morbillivirus* in the family *Paramyxoviridae* that belong to the order Mononegavirales (Hahm *et al.*, 2003; Maldonado, 2004; Allen *et al.*, 2006). It infects only humans and nonhuman primates, while the humans are the only natural reservoir of MV (Hasegawa *et al.*, 2006; Sellin *et al.*, 2006).

MV is closely related to *canine distemper* virus (CDV), *rinderpest* virus, *peste des petits ruminants* virus, *phocine distemper* virus of seals, and porpoise *morbillivirus* in addition to recently isolated virus which infects horses and accidentally humans. All these virus exhibit antigenic similarities and all

produce similar disease in their host species (Sibylle and Volkerter, 2000; Greenwood *et al*., 2004).

MV is a stable, monotypic virus, making it an excellent candidate for eventual eradication (Ilonen *et al*., 1990; Permar *et al*., 2006).

2.3.1: Morphology and Virus Structure

MV is highly pleomorphic with an average size of 120-250 nm, and both filamentous and irregular form are known (Norrby, 1985; Redd *et al.*, 1999).

The virion shown in an electron micrograph is bounded by a lipid envelope which bears a fringe or spike-like projection (peplomers) 5-8 nm long. The envelope below the spikes is 10-20 nm in thickness and encloses the helical viral ribonucleoprotein core which has a diameter of 17 nm and a regular pitch of 5 nm. Immediately below the envelope, M proteins appear as a shell of electron dense material (Norrby, 1985).

There are six structural proteins, three complexed with the RNA genome and form the nucleocapsid: The phosphoprotein (P), the large protein (L), and nucleoprotein (N) (Redd *et al.*, 1999). Other three structural proteins are complexed with the envelope: Fusion protein (F), hemagglutinin protein (H) and the matrix protein (M) (Norrby, 1985; Redd *et al.*, 1999).

The F and H proteins are responsible for membrane fusion and attachment, respectively (Yanagi *et al.*, 2006; Ohno *et al.*, 2007; Tahara *et al.*, 2007).

The F and H envelope proteins are glycosylated; the innermost of the three envelope proteins, the M protein, is not. Both viral transmemberane proteins (fusion (F) and hemagglutinin (H)) are present on the envelope surface and appear as projections from the particle. They extend through the virion envelope (transmembrane) and appear on its inside surface (Redd *et al.*, 1999).

The H protein is the receptor-binding and hemagglutinating monkey erythrocytes (Green *et al.*, 2001), its molecular weight 76000-80000 Dalton (Casili *et al.*, 1981). The fusion protein precursor (FO) splits under the effect of host enzyme to yield two disulfide-linked polypeptides, a non glycosylated protein (denoted F1) of a molecular weight 40000 Dalton, and other heterogeneous low molecular weight 16000-23000 Dalton glucosamine rich protein (denoted F2) (Jawetez *et al.*, 1995). The nucleoprotein (N) of a molecular weight 6000 Dalton forms the helical nucleocapsid and plays a central role in replication of genomic RNA, and its domains are required for binding phosphoprotein (P) molecular weight 66000-73000 Dalton and large protein (L) molecular weight 100000-200000 Dalton. The three proteins are involved in viral polymerase activity that

functions in transcription and RNA replication (Bankamp *et al*., 1996).

The matrix protein (M) of a molecular weight 34000-39000 Dalton underlies the viral envelope. It has an affinity for both nucleoprotein (N) and the virus surface glycoproteins, and it is important in virion assembly and budding (Tyrrell *et al*., 1980).

The viral genomic RNA is fully encapsulated by (N) protein to form the ribonucleoprotein (RNP) core structure that resist RNAase degradation (Rota *et al*., 1994).

Although MV is considered as a stable virus, sequence analysis of the N, H, P, and M genes has identified differences among wild virus strains as well as differences among wild and vaccine strains (Rota *et al*., 1992; Na *et al*., 2003).

2.3.2: Resistance to Physical and Chemical Agents

MV is not a highly resistant virus outside the body, e.g. at room temperature, it looses 60% of its infectivity in 3-5 days, and killed in half an hour at 56°C (Jawetez *et al*., 1995; HC, 2001).

MV preserves well in cold protein medium and survives at -70°C for up to 5.5 years; and at refrigerator temperature 4-6°C, it survives for 5 months (Redd *et al*., 1999).

The particle is dependent upon the integrity of the envelope for infectivity and is inactivated by any procedure which disrupts this structure. Hence, the virus is sensitive to detergents or other lipid solvents such as ether and acetone. It is killed by

20% ether within 10 minutes at room temperature and by 50% acetone, within 3 minutes. Due to the sensitivity of MV to ether and temperature, it is possible to ether treatment and thermal inactivation to differentiate between MV activities such as hemagglutination and hemolysis, whereas hemagglutinin shows greater stability than hemolysin which in turn is more thermostable than virus infectivity (Norrby and Falksveden, 2005).

MV is also sensitive to beta-propiolactone, it looses its infectivity within 2 hours at 0.01% concentration and a temperature at 37°C, but it does not alter complement-fixing activity (Christie, 1974).

The virus is acid labile, and rapidly inactivated at pH value below 4.5 although they remain infective in the range 5-9 it also inactivated by proteolytic enzymes, this virus sensitivity to acid and enzyme preclude measles infection via the stomach and intestine. However, the ability of the virus to survive desiccation in the form of micro droplets enables it to spread as an aerosol (Black, 1982).

2.3.3: Pathogenesis

Measles is a systemic infection. It is transmitted via direct person-to-person contact and causes a symptomatic viral prodrome culminating in the hallmark maculopapular measles rash (Permar *et al.*, 2006). The primary site of infection is the respiratory epithelium of the nasopharynx, and possibly of the

conjunctivae, with spread to regional lymphatics. Specifically, 2-3 days after exposure, there is a primary viremia with further replication of virus at the site of inoculation as well as in regional and distant reticuloendothelial tissue. Then, 5-7 days after exposure, there is an intense secondary viremia of 4 to 7 days duration that leads to infection of and further replication in the skin, conjunctivae, respiratory tract, and other distant organs (Redd *et al.*, 1999).

MV is shed from the nasopharynx beginning with the prodrome until 3-4 days after rash onset (Redd *et al.*, 1999).

The characteristic rash is probably a manifestation of a hypersensitivity reaction and preceded by systemic viremia and lymphopenia, and clearance of the rash is followed by a transient suppression of T-lymphocyte responses that lasts several weeks, leaving the infected individual susceptible to other infections (Permar *et al.*, 2006). Therefore, the measles rash may not be seen in person with suppression of the cell-mediated immune system (Redd *et al.*, 1999).

The pathogenesis of measles infection indicates that prevention through immunization could be accomplished by inhibiting replication and dissemination from the nasopharynx or by inhibiting the viremia that occurs during the incubation period. The first approach requires the presence of local secretory IgA antibody or transudated IgG; the second approach requires circulating antibody, either actively or passively

acquired, to neutralize the virus. Although infection can be prevented solely after administration of antibody, induction of cellular immunity would also seem to be desirable (Redd *et al.*, 1999). Measles virus infection of B-cells results in marked alterations in proliferation and immunoglobulin production. Very little is known about the changes of gene expression, if any, during acute measles virus infection, therefore children with primary agammaglobulinemia do not have more severe measles infections than do children with normal immune systems, and both develop long-lasting immunity after infection. These observations indicate that the cell-mediated immune system alone is adequate to prevent measles (Redd *et al.*, 1999; Wang *et al.,* 2003).

2.4: Clinical Symptoms

The first clinical symptoms of measles (characteristic red, blotchy rash beginning on the face and becoming generalized) occur after 14 days (range, 7-18 days) incubation period that follows airborne or droplet exposure (around the third day of illness). If infection occurs after parenteral exposure, the incubation period is shortened by 2 to 4 days, whereas immunosuppressed persons may have a prolonged incubation period (Berggren *et al.,* 2005; Boon *et al.*, 2006).

The prodromal stage is heralded by onset of fever, malaise conjunctivitis, coryza, and tracheobronchitis (manifesting as cough) and lasts 2 to 4 days. Koplik spots, a rash (enanthem)

present on mucous membranes, are considered to be pathognomonic for measles. It occurs 1-2 days before rash to 1-2 days after the rash, and appears as punctate blue-white spots on the bright red background of the buccal mucosa. (Bellini and Rota, 1998; Perry and Halsey, 2004).

The measles rash is a maculopapular eruption that usually lasts 5 to 6 days. It begins at the hairline, then involves the face and upper neck. During the next 3 days the rash gradually precedes downward and outward, reaching the hands and feet (CDC, 2006b). Sometimes the rash ending with fine desquamation that may go unnoticed in children who are bathed daily. The duration of exanthema rarely exceeds 5 to 6 days. An exaggerated desquamation is commonly seen in malnourished children (Perry and Halsey, 2004). Measles is frequently complicated by middle ear infection or diarrhea (Watson *et al.*, 1998; AAP, 2003).

Mild or asymptomatic measles infections are probably very common among measles-immune persons and may be the most common manifestation of measles during outbreaks in highly immune populations (Helfand *et al.*, 1998).

2.5: Complications

MV causes transient but profounds immunosuppression resulting in increased susceptibility to secondary bacterial and viral infections (Schneider-Schaulies and Dittmer, 2006). Due to the development of these opportunistic infections, measles

remains the leading vaccine-preventable cause of child death worldwide (Kerdiles *et al.*, 2005).

The complications associated with measles infection have been the subject of much description and review (Redd *et al.*, 1999) these include the following:

a. Both primary measles (giant cell) pneumonia and secondary bacterial pneumonia occur. Bacterial otitis media is quite common (Levinson, 2004).
b. MV may play a part in the development not only of Crohn's disease (Elliman *et al.*, 2001; Ghosh and Afzal, 2001) but also of ulcerative colitis (Redd *et al.*, 1999; Thjodleifsson *et al.*, 2002).
c. Diarrhea is a frequent cause of death because it may persist long after the acute insult and further aggravate a preexisting malnourished state (Redd *et al.*, 1999).
d. Postinfectious encephalitis occurs in approximately 0.1% of reported cases (CDC, 2006b). The mortality rate of encephalitis is 10%, and there are permanent sequelae, such as deafness and mental retardation, in 40% of cases (Levinson, 2004; Sips, 2007).
e. Subacute Sclerosing Pan Encephalitis (SSPE) is a rare degenerative central nervous system disease (Shingai *et al.*, 2003; Bellini *et al.*, 2005). Onset occur occurs an average of 7 years after measles (range 1 months - 27 years), and occurs in

five to ten cases per million reported measles cases (Singer *et al*., 1997; CDC, 2006b).

The clinical features of SSPE are personality changes, mental deterioration, involuntary movements, and muscular rigidity, invariably ending in death (Leland, 2002; Hunt, 2006).

f. Other described complications include laryngotracheo-bronchitis, hepatitis, Appendicitis and ileocolitis, pericarditis, glomerulonephritis, hypocalcaemia and Stevens-Johnson Syndrome (Redd *et al.*, 1999; Bellini and Icenogle, 2003).

2.6 Atypical Measles Infection (AMI)

Atypical measles infection occurs only in persons who received inactivated (killed) measles vaccine (KMV) and are subsequently exposed to wild type MV. KMV sensitized the recipient to MV antigens without providing protection (Bellini and Icenogle, 2003; CDC, 2006b). Patients with atypical measles lack antibodies to MV F-protein, and had exaggerated cellular responses to measles antigen (Norrby, 1985; Polack *et al.,* 2000). Subsequent infection with MV leads to signs of hypersensitivity polyserositis. The illness is characterized by fever, pneumonia, pleural effusions, and edema. In contrast to acute measles, the rash develops on distal extremities and spreads centripetally. Atypical measles may be prevented by revaccinating with live measles vaccine (AAP, 1997).

2.7: Modified Measles (MM)

Modified measles occurs primarily in patients who received immunoglobulin as postexposure prophylaxis and in young infants who have some residual maternal antibody. It is usually characterized by prolonged incubation period, mild prodrome, and sparse, discrete rash of short duration. Similar mild illness has been reported among previously vaccinated persons (CDC, 1997; CDC, 2003; Bellini *et al.*, 2005).

2.8: Measles in Immunocompromized Persons

The pathogenicity of MV is intimately linked to the immune status of the infected individual. Measles is typically a self-limiting disease; however, individuals who are immunocompromized (defects in cell-mediated immunity as a result of lymphoma, chronic infection, malnutrition, or immunosuppressive therapy) (Moss *et al.*, 1999; Redd *et al.*, 1999), or at the extremes of age are at risk for severe, progressive MV infection. Such individuals can develop atypical measles rashes. These rashes can be severe and desquamating, although sometimes they are absent (Redd *et al.*, 1999; Okamura *et al.*, 2001; Moss *et al.*, 2002). Complications of measles that occur in individuals with impaired cellular immunity include MV giant-cell pneumonia and measles inclusion body encephalitis. In addition, prolonged MV shedding has been reported for malnourished children (Redd *et al.*, 1999).

2.9: Epidemiology

Measles occurs throughout the world. In the absence of an immunization it is a ubiquitous, highly contagious, seasonal disease affecting nearly every person in a given population by adolescence (Redd *et al.*, 1999).

Although measles vaccination has been successful in developing countries, the WHO estimates that around 45 million cases with 1.2 million deaths still occur annually, with the majority of cases arising in less developed countries (Loo *et al.*, 2003; Berggren *et al.*, 2005; WHO, 2005).

Measles is transmitted primarily from person to person by large respiratory droplets. Airborne transmission via aerosolized droplet nuclei has been documented in closed areas (Watson *et al.*, 1998; AAP, 2003; Institute of Medicine, 2004).

Humans are the only natural reservoir of MV, although some laboratory animals can be infected (Redd *et al.*, 1999; Song *et al.*, 2005; Ohno *et al.*, 2007).

Before the introduction of vaccines in most developed countries, school-aged children had the highest risk of infection and accounted for the largest proportion of cases (Redd *et al.*, 1999).

However, in dense urban areas, transmission among preschoolers took an greater importance (NVAC, 1991).

Hospital-based studies in Sudan and Iraq showed that, 45% and 60% respectively of admitted cases were younger than 2 years of age (Al-Khafaji, 2006).

Before widespread vaccination, in many developing countries, poor nutrition and rapid loss of maternal antibody may explain why a greater proportion of infants are susceptible at an earlier age than are those in developed areas (Redd *et al.*, 1999). Malnutrition, especially vitamin A deficiency, may also be an important factor leading to the marked severity of measles in the developing world because of defects in cellular (and possibly humoral) immunity (Redd *et al.*, 1999; WHO, 2005).

In Iraq, a prominent increase in the incidence of measles was observed during 1991-1992. This could be attributed to the malnourishment and inadequate vaccine shortage due to the economic sanction (Muhieldeen, 1992).

Measles vaccination in Iraq started at 1973-1974 on irregular bases; however during 1985, MV vaccine is included in EPI (Al-Khafaji, 2006).

There is only one serotype of measles and a single natural infection gives lifelong protection (Schneider-Schaulies and Meulen, 2002; Hunt, 2006).

2.10: Immune response to MV infection

It has been mentioned that natural MV infection generates long-lasting immunity that includes both MV-specific antibody and memory T-lymphocyte responses (D'Souza *et al.*, 2006).

Long-term protection from reinfection occurs without a requirement for reexposure, while nonspecific innate immune mechanisms may be important in the control of MV during the first days following infection, adaptive MV-specific immune responses mediate viral clearance and provide protection against subsequent MV infections (Russell *et al.*, 2004).

In communities, epidemic usually occurs every 2-3 years, last for 3-4 months (Levinson, 2004). Babies obtain protection from their mothers through maternal antibodies which persist for 6 months (Boon *et al*., 2006). The age at which measles occurs varies according to environment. In developing countries measles occurs very early in life as maternal antibody decreases (Garenne *et al.*, 1991; Williams *et al*., 1995; Levinson, 2004).

Acute infection is followed by a substantial antibody response which is initially IgM and subsequently IgG. Anti-measles IgM is occasionally found in persons with multiple sclerosis, but it seems to be an abnormal phenomenon in late immunity. The IgG anti-measles response persists for years without apparent reinfection (Hunt, 2006).

Cell-mediated immunity plays a very important role in the protection of host from measles (Levinson, 2004; Permar, 2006). A moderate cellular response, as measured by lymphocyte proliferation, has been demonstrated during the early convalescent period, but 3 to 4 months after infection only very low T-cell responses can be demonstrated in most

individuals. Similarly, specific T-cell killing of MV-infected target cells has not been demonstrated years after natural infection or immunization with virus. In contrast to the majority of low responder (LR) normal individuals, a few seropositive individuals who are high responders (HR) have been identified. The HR individuals have substantial responses long after MV infection and have been previously studied in an effort to examine the nature of this persistent response and to provide some insight into the normally low response (Rose *et al*., 1984, Levinson, 2004).

2.11: Measles Morbidity and Mortality

MV infection and the profound immunosuppression it causes are currently responsible for nearly one million deaths annually. The reports among regions with measles elimination goals shows different incidence levels, the American regions reported the lowest incidence (1.6 per 100.000) in 1998. In 2003 a total of 828 cases in USA had been reported three of them were dead (Welstead *et al*., 2005; Shingai *et al*., 2005).

Among all regions, Africa region reported the highest number of measles cases and incidence, whereas the number of cases reported during 1997-1998 in Eastern Mediterranean region increased by 58% and the outbreaks were reported in Syria, Iran, Saudi Arabia, and Morocco. On the other hand the number of cases reported from European region declined 59%, and the number of countries reporting, measles cases declined

from 45 in 1997 to 31 in 1998 of all the cases reported, more than half were reported from Africa (CDC, 1999).

In 2002-2003, the recent measles outbreaks were reoccurred in Eastern Mediterranean region (Tunisia, Libya, and Syria) and Iran (Djebbi *et al*., 2005).

Recent studies about incidence of measles infection in Iraqi population indicated that there was marked increase in measles cases during 1997 and also during the period of 1998-1999 and according to the Center of Disease Control reports, the total number of measles cases were 454 during the year 2003 and there was marked increase in the cases during the period extended from January till June 2004 and the total number of reported cases were 8253 especially in Basra and other southern governorates, while 101 cases of measles were reported during September 2004, and 56 cases were reported in October 2004 (Al-Khafaji, 2006).

In 1996, approximately 1.67 million measles-associated deaths were prevented because of the impact of vaccination program in a glob burden of disease study by Murray and Lopez (1997) provides data on the public health importance of measles worldwide. It's included 107 cases of death among all age groups, measles ranked eight overall, accounting for 1.1 million deaths worldwide in 1990 and 1 million in 1996 (Murray and Lopez, 1997).

Although the mechanism of sex-related mortality following high-titer immunization is unknown, it has been postulated that vaccine-induced, prolonged immunosuppression leads to increased susceptibility to disease. Both measles infection and immunization cause transient immunosuppression, and measles case fatality rates may be highest among females (Garenne, 1994; Forthal *et al*., 1994; Forthal *et al*., 1995; Atabani *et al*., 2000).

2.12: Treatment

There is no specific antiviral therapy for measles, and the basic treatment consists of providing necessary supportive therapy such as hydration and antipyretics and treating complications such as pneumonia (Maldonado, 2004).

2.13: Prevention

2.13.1: Prevention of Spread of MV

Persons who have a generalized rash and fever and persons who have fever and respiratory symptoms following exposure to a person with measles may be infectious with measles. Persons who are potentially infectious with measles should minimize the risk of spread of the disease by limiting contact with other people who may be susceptible to measles. Contact should be limited until a medical diagnosis has been established excluding measles, or the symptoms resolve completely, or 4 days have passed since the onset of the rash. Persons who are potentially infectious with measles should especially avoid public

transportation (including commercial airlines) and crowded indoor areas (CDC, 2006a).

2.13.2: Measles Eradication

The success of vaccination against measles in developed countries has significantly reduced the incidence of measles-related morbidity and mortality. However, measles is still the leading cause of mortality in children in underdeveloped countries due to low vaccination coverage, high transmissibility of the measles virus as well as primary and secondary vaccine failure (Levinson, 2004; WHO, 2005).

(Orenstein *et al*., 2000) reported that the feasibility of eradicating measles was according to four criteria:

a. The critical role of humans in maintaining MV transmission.
b. The availability of accurate diagnostic tests.
c. The existence of effective and safe vaccines.
d. The need to demonstrate elimination from a large geographic area.

The potential impediments for eradication include the following:

a. Lack of political will in some industrialized countries.
b. Transmission among adults.
c. Increasing urbanization and population density.
d. The human immunodeficiency virus epidemic.

e. Waning immunity and the possibility of transmission from sub-clinical cases.

f. Risk of unsafe injections.

Despite these challenges, a compelling case can be made in favor of measles eradication and was believed that it will be in the near future. However, the timing of measles eradication is less certain (Orenstein *et al*., 2000; Dhiman *et al*., 2004; CDC, 2006b).

2.13.3: Prevention Through Passive Immunization

Administration of immunoglobulin has the disadvantage that the immunity conferred is only temporary (approximately 3 to 4 weeks) assuming that neither modified nor typical disease occurs. However, certain situation require prophylaxis immunoglobulins against measles; these situation include exposure of children younger than 1 years, pregnant woman, immunocompromized patients, and other susceptible persons with a contraindication to the receipt of live vaccine. Immunoglobulin administered in a doses ranging from 0.05 to 0.5 ml/kg within 6 days after recognized exposure to a case of measles is effective in preventing or modifying subsequent disease (Reeder *et al*., 1992; Cunningham *et al*., 1997; Redd *et al*., 1999). The maximum dose in all cases is 15 ml administer intramuscularly. The vaccine should be administered 5 to 6 months later in the absence of the disease (CDC, 1994).

Intravenous immunoglobulin (IVIG) preparation may be especially important for persons with the acquired immunodeficiency syndrome (AIDS) who are exposed to measles (Redd *et al.*, 1999).

IVIG can be administered in the same dose as immunoglobulin expressed as milligrams of protein / per kg B.W. (41.25 mg/kg [165 mg/ml × 0.25 ml/kg] for normal individuals and 82.5 mg/kg [165 mg/ml × 0.5 ml/kg] for immunocompromized persons) (Redd *et al.*, 1999).

2.13.4: Prevention Through Active Immunization

A. Vaccine Strains and Preparation

After the isolation and propagation of MV in tissue culture by Enders and Peebles in 1954, numerous attenuated measles vaccines, most derived from Edmonston strain (the strain named after the youth from whom the virus was isolated) are currently produced worldwide. Four vaccines of non-Edmonston derived strain are in active use, these including Leningrad-16, Shanghai-191, (CAM-70 and TD97 which are derived from Tanabe strain). In most cases, the virus was cultured in chicken embryo cells, but few vaccines were attenuated in human diploid cells, of these Edmonston-Zagreb vaccine, used extensively in Yugoslavia since 1969, was derived from the Edmonston strain and underwent additional passage in WI-38 cells. Other vaccine strains have been adapted to MRC-5 and R-17 human diploid cells in Iran and in China (Redd *et al.*, 1999).

Most vaccines contain small doses of antibiotics (e.g. 25 μg of neomycin per dose), but some do not; sorbitol and gelatin are used as stabilizers. A combined live vaccine of Measles, Mump and Rubella (MMR) were licensed in United States since 1971 this type of vaccine contains at least 1000 $CCID_{50}$ = $TCID_{50}$ (Cell or tissue culture infectious dose 50%) of MV (potency test) (Redd *et al.,* 1999; Dashefsky, 2003). Recently oral delivery plasmid DNA vaccines encapsulated in biodegradable particles is an attractive rout of immunization currently under evaluation. Experimentally plasmid encapsulated DNA expressing the MV nucleocapsid protein administered orally to mice induced increasing titers of N-specific serum IgG antibodies (Shallcross *et al.*, 1999).

Formalin-inactivated alum-precipitated vaccine derived from the Edmonston strain was also licensed since 1963 and used in United States until 1967. This vaccine was also used in some provinces in Canada. Usually, three doses of killed vaccine or two doses of killed and one dose of live vaccine were administered at monthly intervals with few side effects. Use of killed vaccine was eventually not recommended when became apparent that this vaccine produced short-lived immunity and placed many recipients at risk for atypical measles infection (Redd *et al.*, 1999; CDC, 2006b).

B. Dose and Route of Administration

Measles vaccine must contain at least 1000 median $TCID_{50}$. This dose is administered in 0.5ml (Louis, 2003). The minimum dose required to immunize a seronegative child has been found to be as low as 20 $TCID_{50}$ in some studies but higher in others (WHO-EPI, 1992; Redd *et al.*, 1999). Although there are only limited data on the intramuscular route, it appears to be as effective as subcutaneous vaccination (Redd *et al.*, 1999).

More recently, studies have been undertaken to determine whether aerosol administration of measles vaccine could overcome maternal antibody and immunize younger infants. Many of these studies have found the Edmonston-Zagreb vaccine strain to be more immunogenic than the Schwarz strain when it is administered by aerosol. However, whereas some investigators reported high seroconversion rates after administration by this route in young infants. The aerosol route is not being used routinely (Redd *et al.*, 1999; Atabani *et al.*, 2000).

C. Immune Response as a Result of Vaccination

Immunization induces both humoral and cellular immunity and the production of interferon. Laboratory evidence of immunity is most conveniently documented by use of antibody assays because test for cell-mediated immunity are not standardized. However, even with antibody assays, results of studies on vaccine-induced immunity may vary depending on

the sensitivity of the antibody assay method used (Redd *et al.*, 1999).

The administration of vaccines to transplant candidates earlier and more rapidly than in the healthy child will improve vaccination rates among transplant recipients while not compromizing immunogenicity (Campbell and Herold, 2005; Wood *et al*., 2006; Verma and Wade, 2006)

IgG, IgM and IgA antibodies can be detected in both serum and nasal secretion. IgM antibody can be detected in the serum between 3 and 4 weeks after vaccination and disappears soon thereafter (Helfand *et al*., 1998; Redd *et al.*, 1999).

Although only small amounts of IgA have been detected in serum, IgA is the predominant antibody found in nasal secretions. Although detectable serum IgA and IgM antibodies are transient, IgG antibodies generally persist for many years. Vaccine-induced antibody titers are typically lower than those induced by natural infection. Vaccine-induced immunity is subject to boosting on challenge, by either vaccine or wild virus; likewise, similar boosting can be observed after natural infection. Thus immunization usually provides immunity as solid as that induced by natural infection (Redd *et al.*, 1999).

Measles-specific cell-mediated immunity (CMI) after live attenuated vaccine has seldom been studied because of the lack of a simple *in vitro* assay. With the importance that CMI plays in natural infection, it would seem that successful vaccination

would stimulate such immunity. In studies that have been conducted, CM immune responses after live attenuated vaccine appear to be similar but less pronounced than those after natural infections. It was found that there was a good correlation between antibody titers and lymphoproliferative responses in 124 children receiving their first dose of MMR vaccine. Also in revaccinated children, it was found that there were good lymphoproliferative responses after revaccination, even among those whose antibody titers dropped to low levels (Redd *et al.*, 1999).

In vitro cytotoxic T lymphocyte responses have been detected after vaccination but are lower than those after natural infection (Redd *et al.*, 1999; Permar *et al*., 2006). It appears that both vaccine and wild infection result in a biphasic immune response beginning with transient production of interleukin-2 (IL-2) and interferon-γ (IFN-γ) followed by more sustained production of IL-4. First, $CD8^+$ T cells are activated, which are important for viral clearance. Later, beginning about the time of rash onset, $CD4^+$ T cells are activated and are involved in antibody production. These responses by the cell-mediated immune system correspond to an initial Th1-type response with a shift to a Th2-type response. The mechanisms underlying the profound suppression of CMI accompanying measles are unclear. Interleukin-12 (IL-12), derived principally from

monocytes and macrophages, is critical for the generation of CMI (Redd *et al.*, 1999).

The high measles antibody titers interfere with the humoral response in subjects who receive a booster immunization, whereas the cellular response is boosted at least transiently, after revaccination (Wong-Chew *et al*., 2003).

2.14: Diagnosis

The diagnosis is usually apparent from the characteristic clinical picture. Measles should be suspected in children with an acute erythematous rash and fever, preceded by a 2 to 4 day prodrome of cough, coryza, conjunctivitis and photophobia. Clinical features that support the diagnosis of measles include the following: The presence of Koplik spots, the characteristic 2 to 4 days of intensifying prodromal symptom, the progression of the rash from the head to the trunk and out to the extremities, and the lysis of fever shortly after the appearance of rash (Redd *et al.*, 1999).

Laboratory tests are necessary to confirm the diagnosis, especially when measles is rare. In the United States, it is recommended that clinicians obtain a blood or other suitable specimen for laboratory confirmation from all patients suspected of having measles, unless the patient is part of an already documented measles outbreak (Redd *et al.*, 1999). Anyhow, there are several laboratory diagnostic methods used for estimation of the virus:

2.14.1. A: Virus isolation

MV isolation from patients is possible during prodromal phase but difficult in the later stage due to the development of specific antibody. Nasopharyngial secretion, conjunctiva, blood and urine are good sources for isolation (Bellini and Icenogle, 2003; CDC, 2006b).

MV can be isolated by tissue culture in human or rhesus monkey kidney cells (Redd *et al.*, 1999). The virus has been adapted to a variety of tissue cultures and established cell lines such as human embryonic lung, human ominion, human carcinoma cells, green monkey kidney cell (Vero cells) and chick embryo cells (Tikhonova *et al*., 1992).

It has not been possible to isolate MV directly from brain of patient with subacute sclerosing panencephalitis (SSPE), isolation can be achieved by *in vitro* cultivation of brain cells, by co cultivation, and by fusion of brain cells with either human or simian permissive cell (Latif, 2002).

2.14.1.B: Polymerase Chain Reaction (PCR)

Reverse transcription polymerase chain reaction (RT-PCR) of measles virus RNA was applied only in specific instances, such as suspected infection of the immunosuppressed in the absence of rash when only limited antiviral immunoglobulins might be expected, developing pneumonia without a rash or unexplained encephalitis (Sibylle and Volkerter, 2000; Bellini and Icenogle, 2003).

RT-PCR analysis using measles virus N-and / or F-gene specific primers are mostly performed on serum, nasopharyngeal aspirates and urine sediments cells; the test can also be applied to tissue samples such as brain material. This technique requires an RNA extraction step prior to reverse transcription and subsequent PCR amplification (Esolen *et al*., 1993; Shimizu *et al*., 1993; Sibylle and Volkerter, 2000; Leland, 2002).

2.14.2: Serological Diagnosis

Diagnosis of measles may be made if anti-measles antibody titers rise by more than four fold between the acute and the convalescent phases (Sibylle and Volkerter, 2000; Levinson, 2004) and as measles has been targeted for global eradication (Basch, 1994) and to achieve this goal, highly sensitive and specific test may be required to assess the susceptible population and to monitor vaccine efficacy (Scott *et al*., 1984; Latif, 2002).

A. Hemagglutinalion Inhibition Test (HAIT)

Hemagglutination Inhibtion test for detection of measles virus depends on the specific antibody to the MV-hemagglutinin antigen which is responsible for agglutination of monkey erythrocytes so binding of these specific antibody will prevent the monkey erythrocytes agglutination when added and incubated at a proper temperature (Bellini and Icenogle, 2003).

This test used for comparing acute and convalescent sera during which four fold or more increase in antibody titer (Redd *et al.*, 1999).

This test is relatively simple, reproducible, and reliable, however, it is not sensitive enough to assess immunity status against measles after infection or vaccination (Neumann *et al*., 1985). The other disadvantage of this test is the necessity to remove non-specific inhibitors and agglutinin which may affect the sensitivity and reliability of HI, besides this test depends strictly on monkey erythrocytes (Boteler *et al*., 1983; Latif, 2002).

B. Passive Hemagglutination Test (PHAT)

This test is one of the most sensitive serological procedures available for the estimation of antibody and it has been used commonly for diagnosis of viral infections. Under ideal conditions the sensitivity of this test reached as little as 0.003 μg of antibody and will yield a positive results, and both IgG and IgM antibodies can be function equally well in PHAT (Barrett, 1983).

This test is highly related to hemagglutination inhibition test but it is more sensitive and reliable than it (Li, 1990; Zhang, 1990), and the test has also been found to show a high correlation with enzyme immunoassay (Nagieva *et al*., 1991). One of the advantages of PHAT is that it does not involve

simian erythrocytes in diagnosis of MV (Sakata and Sugiura, 1988).

C. Avidin-Biotin Latex Agglutination

The avidin-biotin latex agglutination assay is a simple, rapid test well suited to detection of viral antibody. The assay is based on the fact that the latex particles, when coated with a ligand such as an antigen, assume the properties of the ligand and form stable lipophilic colloids. The particles remain in suspension when stored or when mixed with heterologous serum but aggregate when mixed with homologous serum. The procedure permits control of the amount of antigen attached to the latex and eliminates the need for highly purified antigens by using the strong affinity of avidin for biotin (Shekarchi *et al.,* 1988; Tuokko, 2005).

D. Indirect Immunofluorescence Antibody Technique (IFAT)

Indirect immunofluorescence techniques have been widely used to stain cell shed in nasal secretions, although it may be necessary to remove antibodies which already coat virus antigens with low pH buffer. Stained cells include macrophages and ciliated cells as well as giant cells (Sibylle and Volkerter, 2000; CDC, 2006b).

The test is also used for detection of MV-antigen in skin rash's (Stenkvist and Bjorvatn, 1976). Urinary sediment cells have also been examined with high degree of success (Boyd,

1983; Bellini and Icenogle, 2003). Immunofluorescence-positive cell may be shed in the urine from 2 days before up to 5 days after the appearance of the rash. Such cells may also be present in the urine 4-16 days after vaccination with live vaccine, so this method may therefore be more applicable in later stages than examination of nasophyrangeal secreation (Sibylle and Volkerter, 2000).

IFAT is used for detection of measles antibody on a narrow scale, although it is less sensitive than plaque reduction neutralization test and more sensitive than hemagglutination test (De-Souza *et al*., 1990).

E. Plaque Reduction Neutralization Test (PRNT)

The neutralization test is the most sensitive and specific test used for detection of measles neutralizing antibodies and the plaque reduction neutralization method is the most sensitive one (Johnson *et al.*, 1994; Kong *et al.*, 1996). It is remain the standard against which other tests are measured, but because this test is expensive, time limited, personal intensive and difficulty to interpret due to variability in the titration end point and the possible non-specificity of the neutralization, it is limited to the research work (Jawetez *et al*., 1995; Redd *et al.*, 1999).

F. Complement Fixation Test (CFT)

Complement fixation test has traditionally employed assay to detect antibody to measles virus. The test is designed for quantitative and qualitative estimation of antibodies, and it has

received widespread application in both research and clinical laboratory practice (Stites and Rogers, 1987).

The test is simple to perform, reproducible, and is suitable for testing large number of sera, but it has been shown to be less sensitive. The complement fixing antibodies can be detected fifteen days after inoculation of live attenuated measles vaccine and reach its peak at twenty-five to twenty-eight days but can not persist for longer periods of time (Redd *et al.*, 1999).

G. Radioimmunoassay (RIA)

This test is one of the sensitive techniques used for estimating measles virus antibodies, and it was used to discriminate between anti-measles isotypes in human sera and cerebrospinal fluids (Aristila *et al.,* 1977). Although this test is reliable, sensitive and specific, its disadvantage is expensive, hazardous, requiring adequate facilities and high experience for proper store and dispose of radioactive material (Helfand *et al.*, 1997).

H. Enzyme Linked Immunosorbent Assay (ELISA)

ELISA is sensitive, widely available and may be diagnostic if done at the appropriate time. ELISA tests were used to assay antibody to a variety of infectious agents (Bellini and Icenogle, 2003; CDC, 2006b), including MV antibody (O'beirn *et al.*, 1983; Rice *et al.,* 1983). It was found that there was a good correlation between ELISA and other serological methods for the diagnosis of acute measles (Neumann *et al*., 1985; Ratnam *et*

al., 1995; CDC, 1998). ELISA is an alternative test for other tests for detection of low measles antibodies (De-Souza *et al.*, 1991).

Generally, a previously susceptible person exposed to either vaccine or wild-type MV will first mount an IgM response and then an IgG response (Halsey and Hyman, 2001). The IgM response will be transient (1-2 months), and the IgG response should persist for many years (Vitek *et al.*, 1999). Uninfected persons should be IgM negative and will be either IgG negative or IgG positive, depending upon their previous infection history (Bellini *et al.*, 2005).

Only a single serum specimen is adequate to detect the presence of IgM antibody (Lievens and Brunell, 1986; Rossier *et al.,* 1991; Mayo *et al.,* 1991; and Bellini and Rota, 1998).

Indirect ELISA tests for IgM require that IgG antibodies be removed before testing for IgM can be done to avoid false negative results (CDC, 2006b). However, IgM capture ELISA have recently been developed that they do not require removal of IgG and are not affected by rheumatoid factors (Hummel *et al.,* 1992). IgM capture tests for measles are often positive on the day of rash onset. However in the first 72 hours after rash onset, up to 20% of tests for IgM may give false-negative results (CDC, 1998). Tests that are negative in the first 72 hours after rash onset should be repeated. IgM is detectable for at least 28

days after rash onset and frequently longer (Atkinson *et al.*, 1992).

IgG testing for acute measles requires demonstration of a rise in titer of antibody against MV, so two serum specimens are always required. The first specimen should be drawn as soon after rash onset as possible. The second specimen should be drawn 10-30 days later. The tests for IgG antibody should be conducted on both specimens at the same time. The same type of test should be used on the both specimens (CDC, 2003; Institute of Medicine, 2004; CDC, 2006b).

Chapter Three

Materials and Methods

3.1: Materials

3.1.1: Blood Sample

In this study, 375 serum samples were collected from individuals with age range (1-30) years. Those individuals were admitted to different hospitals, clinics and schools as illustrated in (Table 3-1);

Table (3-1): Distribution of study samples according to place, sex and vaccination status.

Place	No.	M	F	V	N-V	Age range (y)
Al-Zahra'a Maternity & Children Hospital	62	35	27	44	18	1-10
Al-Furat Al-Awsat General Hospital	20	11	9	12	8	1-7
Al-Nasir Health Clinic	24	16	8	10	14	1-6
Central Health Laboratory in Al-Najaf	57	32	25	57	-	14-30
Waleed Al-Ka'abah Primary School for Boys	11	11	-	11	-	7, 8
Al-Qasim Primary School for Boys	23	23	-	23	-	6-13
Zein Al-A'abdin Primary School for Girls	24	-	24	24	-	6-15
Al-Furdos Primary School for Girls	5	-	5	5	-	7, 9
Al-Jawadain Primary School for Coeducation	60	35	25	60	-	6-12, 20-29

Place	No.	M	F	V	N-V	Age range (y)
Al-Sadeer Secondary School for Boys	30	30	-	30	-	13-18
Al-Lathiqiyah Secondary School for Girls	12	-	12	12	-	13-17
Thiqar Secondary School for Coeducation	32	20	12	32	-	13-18
Al-Najaf Teachers Institute for Girls	15	-	15	15	-	17-22
Total	**375**	**213**	**162**	**335**	**40**	**1-30**

M = Male, F = Female, V = Vaccinated, N-V = Non-Vaccinated, y = year.

The vaccinated individuals were divided into five age groups table (3-2).

Table (3-2): Distribution of vaccinated individuals on the age groups (A, B, C, D, and E).

Age Group (y)	Descriptive Name	Sex		Residency		Total
		M	F	R	U	
1-6	A	46	54	34	66	100
7-12	B	37	27	28	36	64
13-18	C	65	42	47	60	107
19-24	D	18	23	12	29	41
25-30	E	16	7	5	18	23
Total		**182**	**153**	**126**	**209**	**335**

R = Rural, U = Urban.

The control individuals (non vaccinated) were included in the following table (3-3):

Table (3-3): Distribution of non vaccinated individuals (control group) on the three ages (1, 2 and 3 years).

Age (y)	Sex		Residency		Total
	M	F	R	U	
1	10	6	7	9	16
2	7	5	7	5	12
3	5	7	5	7	12
Total	**22**	**18**	**19**	**21**	**40**

Age, sex, residency and history of measles vaccination were recorded for each individual.

3.1.2: Antigen (Measles Virus Vaccine)

Attenuated measles virus vaccine (Schwarz strain) (Aventis Pasteur SA – France) was used as coating agent in this study. The vaccine contains approximately 1000 CCID 50 (cell culture infectious dose 50 %) with traces of antibiotics (1% neomycin) and human albumin for lyophilization.

3.1.3: Chemicals and Reagents

A. Chloroxylenol 5%

This antiseptic was used in concentration of 2% which prepared by adding 2cc of Chloroxylenol to the 98 cc of water and used for disinfecting the skin before collection of blood sample.

B. Coating Buffer, 0.05M Carbonate-Bicarbonate Buffer

This buffer was prepared according to the method of Al-Khafaji (2006) as follows:

Sodium carbonate	1.59 gm (0.015M)
Sodium bicarbonate	2.93 gm (0.035 M)

Dissolved in 1000 ml distilled water and pH adjusted to 9.6 by pH meter.

It was prepared freshly, and filtered by 0.22 µm membrane filtration with vaccum.

C. Washing Buffer Solution

Phosphate buffer containing 1% tween 20 and 0.01% thimerosal (Biokit - Spain). It was used as washing solution after each step of ELISA test.

D. Concentrated Conjugate

Rabbit anti-human IgG antibodies conjugated with peroxidase. It contains red dye, preservatives and protein stabilizers. To be diluted 1/51 with the conjugate diluent before use, by adding 300 µl of concentrated conjugate to the bottle containing 15 ml of conjugate diluent (Biokit – Spain).

E. Conjugate Diluent

Tris buffer containing yellow dye, additives and preservatives. It was used to dilute the concentrated conjugate (Biokit – Spain).

F. Substrate Solution

Citrate-acetate buffer containing hydrogen peroxide (Biokit – Spain).

G. Chromogen

3,3', 5,5'-Tetramethylbenzidine (TMB) dissolved in dimethylsulphoxide (DMSO) (Biokit – Spain).

H. Anti-Measles Virus Positive Control Group

It consists of five specific hyperimmune serum samples, which were obtained from College of Medicine, Al-Kufa University. They were titrated and the IgG titer was 2048.

I. Anti-Measles Virus Negative Control Group

A total of 5 serum samples were obtained from College of Medicine, Al-Kufa University. They were titrated and the IgG titer was negative.

J. Stopping Solution

1N sulphuric acid (Biokit – Spain).

K. Single Radial Immunodiffusion (SRID) Endoplates for IgG, IgM, IgA, C3 and C4 (Biomaghreb – Tunis).

3.2: Laboratory Equipments and Apparatuses

Equipment	Manufacturing Company
Syringe (5 ml)	Medeco – UAE
Sterile plastic test tube (10 ml)	Afco-Dispo – Jordan
Centrifuge	BioActivea – Germany
Refrigerator	LG – Korea
Freezer	Sanyo – Japan
Micropipette	Transferpette – Germany
Microtiter plate (U-shaped bottom)	Biokit – Spain
Adhesive seals	Biokit – Spain
Incubator	Memmert – Germany
ELISA washer	Tecan Austria GmbH – Austria
ELISA reader	Tecan Austria GmbH – Austria
Ocular lens	Olympus – Japan
Electronic balance	Sartorius – Germany
pH meter	Hoeleze and Cheluis, KG – Germany
Water distillator	GFL – Germany
Millipore filter paper	Sartorius membrane filters GmbH – Germany

3.3: Methods

3.3.1: Preparation of Serum Samples

Blood samples (3-5) ml were drawn aseptically in 5 ml disposable syringe from each individual included in this study. Blood samples were left to clot at room temperature for 30 minutes. Then, the blood samples were put in refrigerator at 4°C for 2 hours. After that clot was detached by sterile disposable inoculating loops and the tubes were centrifuged at 3000 rpm for 10 minutes. Cell-free sera were collected in sterile test tubes and stored at (-20°C) until used (Lewis *et al.*, 2001).

3.3.2: ELISA Test for Detection of Anti-Measles Virus IgG

This test was carried out by a micro-method; the measles virus was used as coating agent. The optimal dilution of the virus antigen was determined by Chequre board titration in which the virus suspension was diluted with carbonate-bicarbonate buffer (pH 9.6) and added in 100 µl volumes per well of microtiter plate (U-shaped bottom). Plates were incubated for 1 hour at 37°C and held for 16 hours at 4°C, then washed 3 times with washing buffer solution.

Then sera samples were added in a volume of 100 µl per well (positive and negative control sera sample, also included per each plate). The plates were incubated at 37°C for 2 hours followed by removing of excess serum by washing for 3 times as before. After that 100 µl of horse radish peroxidase conjugate [rabbit anti- human IgG antibodies (Biokit laboratory LTD)]

diluted 1:51 according to manufactory company instructions in conjugate dilution buffer, was added to each well, then plates were incubated for 1 hour at 37°C followed by washing step for three successive time with washing solution for removing of un-reacted horse radish peroxidase conjugated antibodies. After that 100 µl of ready to use substrate (TMB) is pipette in each well, then plates covered and incubated in dark place at room temperature for 20 minutes, followed by addition of 100 µl of 1N sulphuric acid (H_2SO_4) as stopping reagent to stop the substrate reaction, after mixing thoroughly, the color was stable and reading of the result was performed by using ELISA reader system at 450 nm. Absorbance of the serum sample was more than 10% above the cut-off value. The result was regarded as positive whereas the serum samples gave absorbance 10% below the cut-off value regarded negative and the results in between regarded as questionable.

The mean optical density of cut-off value**,** cut-off value = absorbance of the negative control +0.25; was calculated and it was found to be 0.440. So the higher the optical density, the higher level of anti-measles immunoglobulin in serum is present, which reflect the higher immune response of the vaccines.

3.3.3: Determination of Immunoglobulins and Complement Levels

Principles of single radial immunodiffusion (SRID) test:

Equal volumes of test serum samples were added to the wells in an agarose gel-containing a mono-specific antiserum. The sample diffuses radically through this gel and the substance being assayed (antigen) forms a precipitation ring with the mono-specific antiserum (Lowell, 2001). Ring diameters were measured by viewing device (ocular). Unknown concentrations were determined from the tables supplemented with each type of endoplate which contains 12 wells (Lewis *et al.*, 2001).

Procedure:

A. Endoplates and the serum were removed from refrigerator. Reagents were equilibrated to room temperature.
B. Plate was removed from ziplock bag. After lid removed, the wells were inspected for moisture. If moisture was present, plates were left uncovered to remain at room temperature (approximately 15 minutes) until moisture evaporated.
C. Sera were thoroughly shaken (in their own containers) by inversion. Each serum sample was dispensed into the appropriate wells. Each well required 5 µl of serum.
D. After lid was replaced, it was incubated at room temperature on a level surface. Incubation times were 48

hours for IgA, IgG, C3 and C4 tests and 72 hours for IgM test.

E. Immunopreciptin ring diameters were microscopically measured by ocular lens to the nearest 0.1 mm. The calculated diameters were compared to the standard diameter to calculate the concentrations of serum humoral factors (Lowell, 2001).

3.4: Statistical analysis

Mean, standard deviation and T-test were carried out according to Bowers (1997).

Chapter Four

Results

4.1: Evaluation of ELISA Anti-Measles IgG Activity in Vaccinated and Non Vaccinated Individuals

The efficacy of vaccination programs against measles virus were evaluated by ELISA test for estimation of anti-measles antibody (AMAb) titer. A total of (335) measles-vaccinated individuals their age ranging from 1 year up to 30 years were grouped into five (A, B, C, D and E) groups and forty non vaccinated individuals their age ranging from 1 to 3 years, were also included as control.

4.1.1: Evaluation of AMAb IgG Titer in Group A

A total of 100 child were included in this group; their age range from 1<7 years. Table (4-1) describes their ages and variable titer distribution.

The highest IgG titer (1024) was detected in 9% of sera samples of six years individuals whereas the highest frequency (mode) of IgG titer was (128) which was detected in 25% of samples followed by the IgG titers (256) and (512) which were detected in 22% and 8% of sera samples respectively, whereas 36% of sera samples were with AMAb of (≤64) IgG titers.

The total mean of IgG titers of this age group was (234.82).

Table (4-1): ELISA Anti-Measles IgG Antibody Titer in Group A.

Age (year)	Total no.	IgG titer			No. in each mean
		Mode	Mean	SD	
1	16	16	13	6	4
		256	256	0	9
		512	512	0	3
2	19	16	26.4	23.2551	5
		128	173.7143	63.6474	14
3	18	64	34.8889	29.0364	9
		128	128	0	8
		512	512	-	1
4	14	64	45.2	25.1608	10
		128	160	64	4
5	8	64	64	0	3
		128	153.6	57.2433	5
6	25	32, 64	39.2	25.3614	5
		256	237.7143	48.3795	7
		512	512	0	4
		1024	1024	0	9
Total 1-6	**100**	**128**	**234.82**	**283.2797**	**100**

SD = Standard Deviation.

4.1.2: Evaluation of AMAb IgG Titer in Group B

In this age group 64 child were included; their age range from 7<13 years. Table (4-2) describes their ages and variable titer distribution.

Two different IgG titer ranges were found in this age group. In 6 (9.4%) serum samples the titer (128) was the highest titer found or recorded, whereas the highest frequency (mode) of AMAb titer was (≤64) which was found in 58 (90.6%) serum samples. The pooled mean of group B IgG titer was (48).

Table (4-2): ELISA Anti-Measles IgG Antibody Titer in Group B.

Age (year)	Total no.	IgG titer			No. in each mean
		Mode	Mean	SD	
7	10	64	64	0	7
		128	128	0	3
8	10	32	35.2	16.5247	10
9	10	32	36.8	20.0266	10
10	11	32, 64	40	20.9489	8
		128	128	0	3
11	9	64	42.6667	21.166	9
12	14	32	30.8571	20.0571	14
Total 7-12	**64**	**64**	**48**	**32.3473**	**64**

4.1.3: Evaluation of AMAb IgG Titer in Group C

A total of 107 individuals were included in this group; their age range from 13<19 years. Table (4-3) expresses their ages and variable titer distribution.

The tested sera of this group revealed AMAb titer of (≤32) which is the same AMAb titer. The highest IgG titer with highest frequency (mode) was (32) which was found in 65.4% of sera samples. The IgG titer (16) was found in 32.7% of samples.

The mean of IgG titers was (26.3178).

Table (4-3): ELISA Anti-Measles IgG Antibody Titer in Group C.

Age (year)	Total no.	IgG titer		
		Mode	Mean	SD
13	21	32	28.9524	6.438
14	25	32	28.16	6.9742
15	12	32	26.6667	7.8779
16	13	16	23.3846	8.3020
17	21	32	25.9048	7.9618
18	15	32	22.4	9.657
Total 13-18	**107**	**32**	**26.3178**	**7.9249**

4.1.4: Evaluation of AMAb IgG Titer in Group D

This age group included 41 individuals; their age range from 19<25 years. Their ages and variable titer distribution is presented in table (4-4).

The majority of cases in this age group were with IgG titer (≤8). In this age group, the IgG titer (16) was the highest which was found in 9.8% of sera samples, whereas the IgG titer (8) was the highest frequency (mode) which was found in 75.6% of samples.

(8.1951) was the overall mean of IgG titers in this age group.

Table (4-4): ELISA Anti-Measles IgG Antibody Titer in Group D.

Age (year)	Total no.	IgG titer		
		Mode	Mean	SD
19	9	8	10.6667	4
20	9	8	8.8889	2.6667
21	3	8	8	0
22	11	8	6.9091	1.8684
23	2	8	8	0
24	7	8	6.2857	2.1381
Total 19-24	**41**	**8**	**8.1951**	**2.9599**

4.1.5: Evaluation of AMAb IgG Titer in Group E

In this age group 23 individuals were included; their age range from 25<31 years. Table (4-5) describes their ages and variable titer distribution.

In this age group the AMAb of (≤8) was the range in the tested sera samples. The IgG titer (8) was the highest with the highest frequency (mode) which was found in 65.2% of sera samples whereas (4) was the lowest IgG titer which was found in 34.8% of samples. The AMAb titer mean of this age group was (6.6087).

Table (4-5): ELISA Anti-Measles IgG Antibody Titer in Group E.

Age (year)	Total no.	IgG titer		
		Mode	Mean	SD
25	3	8	6.6667	2.3094
26	6	8	7.3333	1.633
27	5	8	6.4	2.1909
28	4	4, 8	6	2.3094
29	3	4	5.3333	2.3094
30	2	8	8	0
Total 25-30	**23**	**8**	**6.6087**	**1.9479**

4.1.6: Evaluation of AMAb IgG Titer in Measles Non Vaccinated Individuals (Control Group)

A total of 40 child were included in this group; their age range from 1<4 years. Their ages and variable titer distribution is revealed in table (4-6).

The tested sera of this group revealed a titer range of AMAb (≤16). The highest IgG titer in this age group was (16) which was detected in 10% of sera samples whereas the highest frequency (mode) IgG titer was (4) which was evaluated in 37.5% of sera samples, while the lowest IgG titer was (≤2) which was detected in 7.5% of sera samples. The total mean of this group was (5.05).

Table (4-6): ELISA Anti-Measles IgG Antibody Titer in Control Group.

Age (year)	Total no.	IgG titer		
		Mode	Mean	SD
1	16	4	5.375	4.8287
2	12	2, 4	4.6667	4.2923
3	12	4	5	4.0452
Total 1-3	**40**	**4**	**5.05**	**4.3439**

4.2: ELISA AMAb IgG Titer and Social Factors

4.2.1: AMAb IgG Titer and Age

A. AMAb IgG Titer and Age in Measles Vaccinated Individuals and Control Group

The behavior in AMAb titer in the studied groups by depending the mean is graphed in figure (4-1). The highest mean of IgG titer was noted in age group A (234.82), followed by group B (48), group C (26.3178), group D (8.1951) and finally the lowest titer was in group E (6.6087), while the total mean of AMAb IgG titer in control group was (5.05).

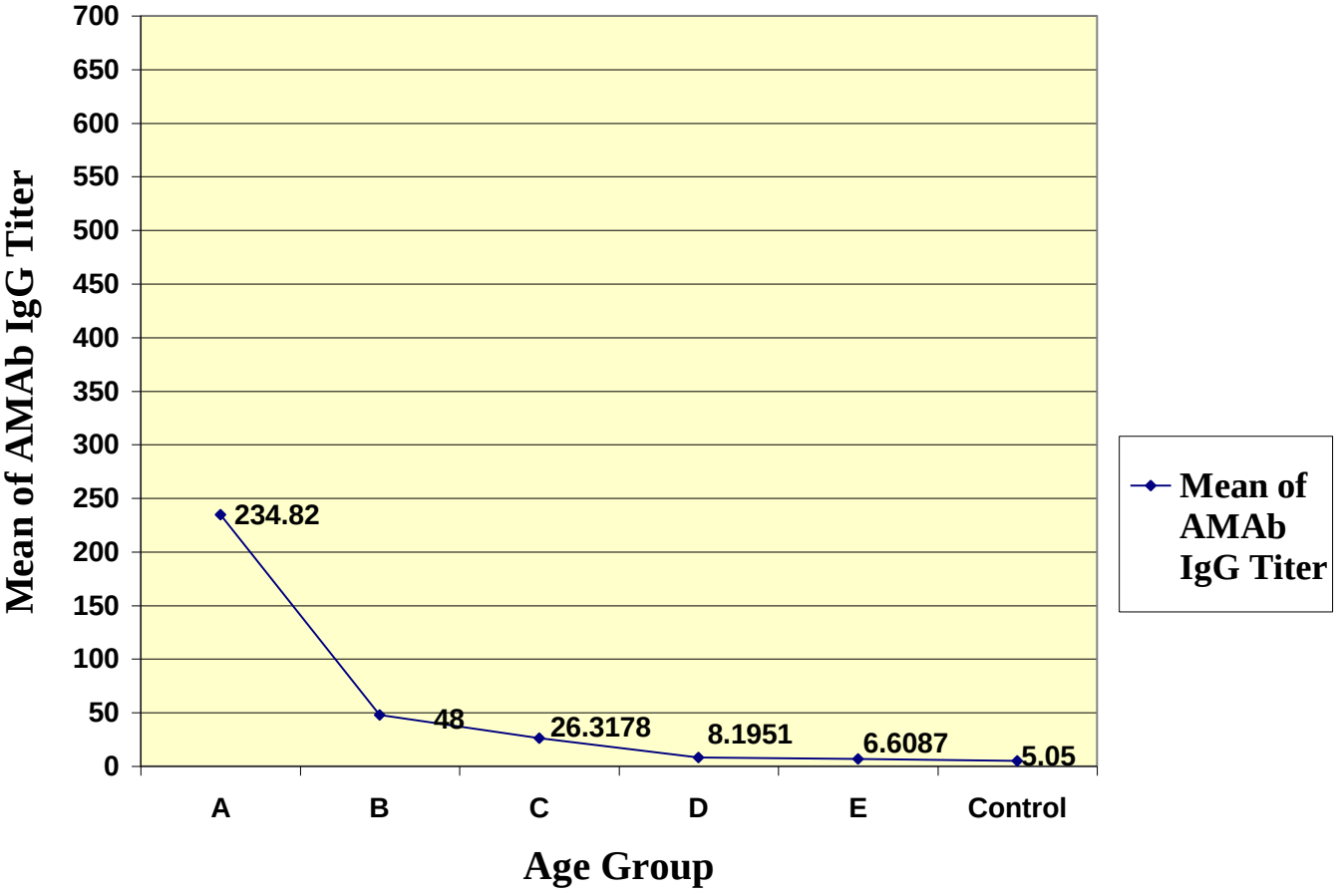

Figure (4-1): The mean of AMAb IgG titer in the studied vaccinated and control age groups.

B. AMAb IgG Titer and Age in Measles Non-Vaccinated Individuals (Control Group)

Figure (4-2) shows the AMAb IgG titers according to age of studied control group. In the first year of age the mean of IgG titer was (5.375) which was the highest IgG titer in this group, while in the second year of age the mean of IgG titer was (4.6667) which was the lowest titer in this group. In the third year of age the mean of IgG titer was (5) which was higher than the titer in the second year and lower than the titer in the first year.

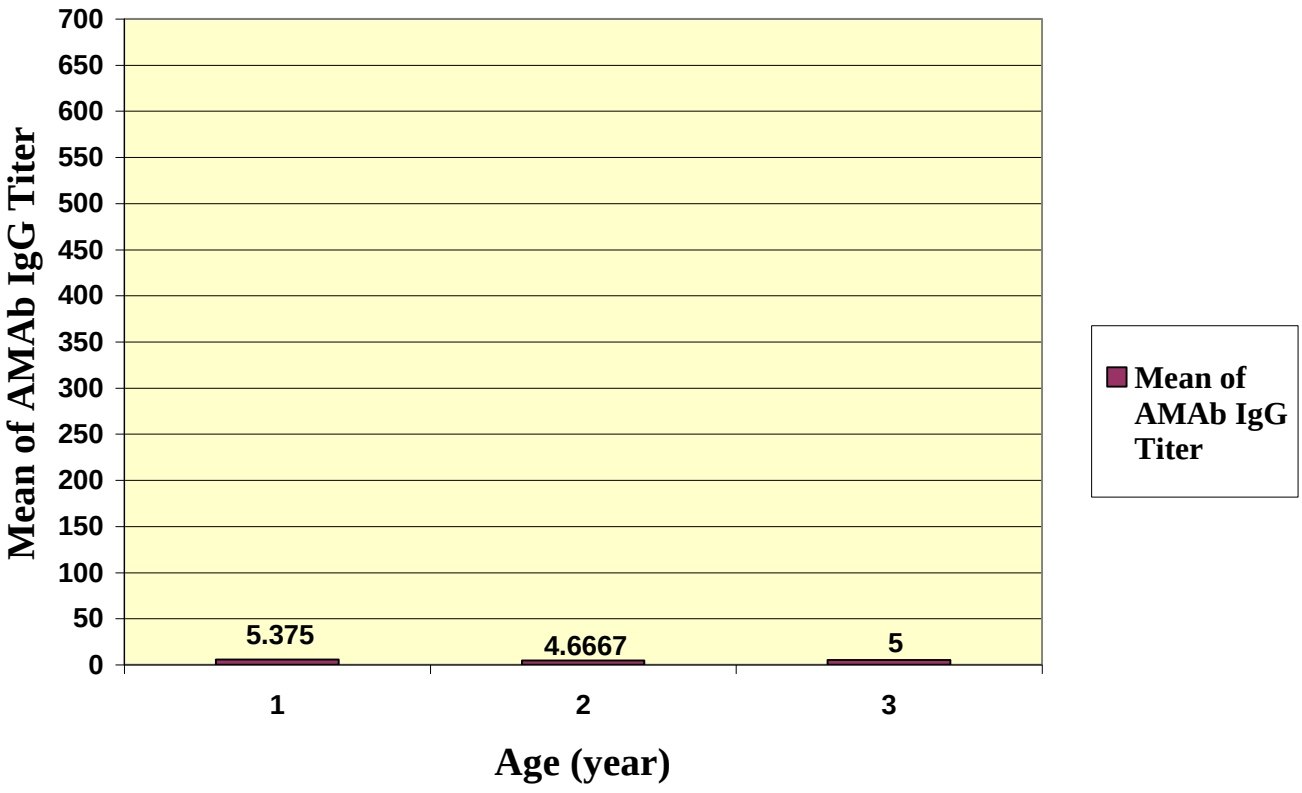

Figure (4-2): The mean of AMAb IgG titer in control group in different ages each year.

4.2.2: AMAb IgG Titer and Residency

The distribution of AMAb titers according to residency of studied measles vaccinated individuals and their ages each year, figure (4-3).

In rural group, the highest mean of IgG titer (632) was recorded at six years and the titer was decreasing with increasing of age and reaching the lowest mean of IgG titer (4) at 27 and 28 years, while in the urban group the highest mean of IgG titer (474.5882) was recorded at six years and the titer was decreasing with increasing of age and reaching the lowest mean of IgG titer (4) at 29 years.

Figure (4-4) reveals the distribution of AMAb IgG titers according to residency of studied measles vaccinated individuals and age groups.

In rural group, the highest mean of IgG titer was noted in age group A (235), followed by group B (42.5714), group C (26.0426), group D (8.6667) and finally the lowest mean of IgG titer was in group E (6.4).

In urban people, the AMAb mean of IgG titer was recorded at group A (234.7273) which was lower than IgG titer in age group A of rural. In age group B, the mean of IgG titer (52.2222) was higher than mean of IgG titer in age group B of rural, also the mean of IgG titers in C (26.5333) and E (6.6667) age groups of urban were higher than mean of IgG titer of rural C and E age groups respectively, but the mean of IgG titer in age group D (8)

of urban was lower than mean of IgG titer in age group D of rural.

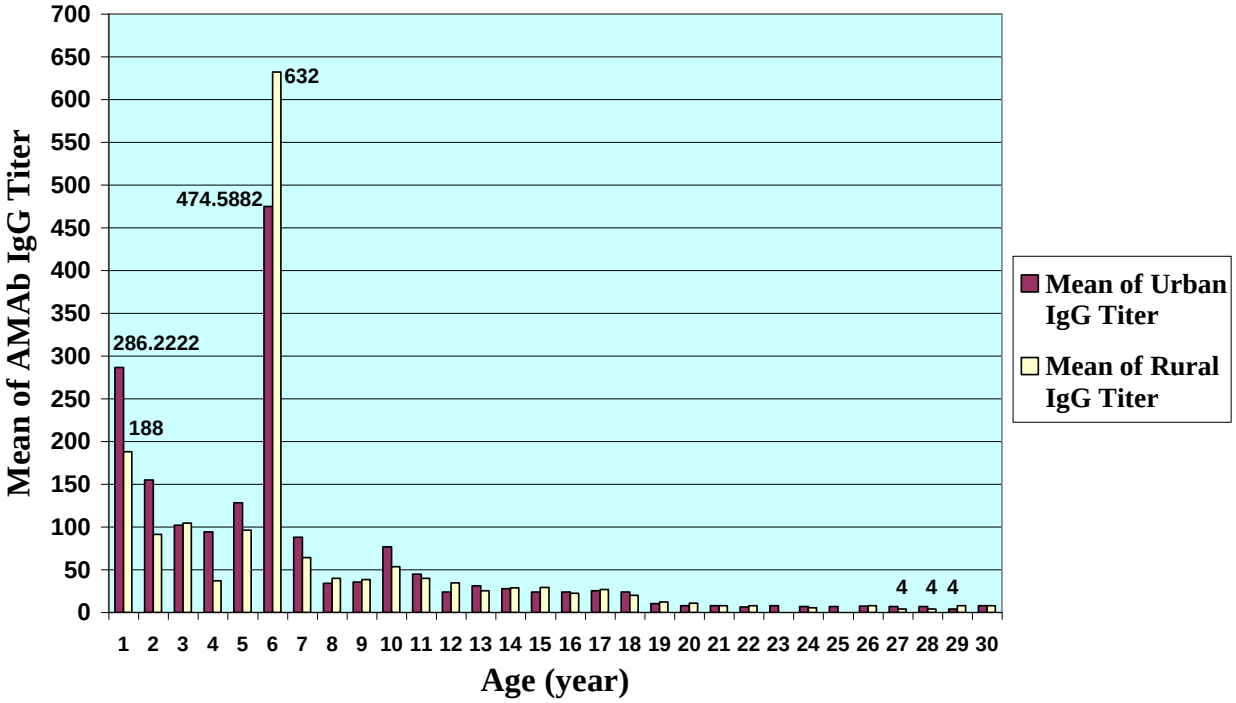

Figure (4-3): The mean of AMAb IgG titer in rural and urban vaccinated individuals in different ages each year.

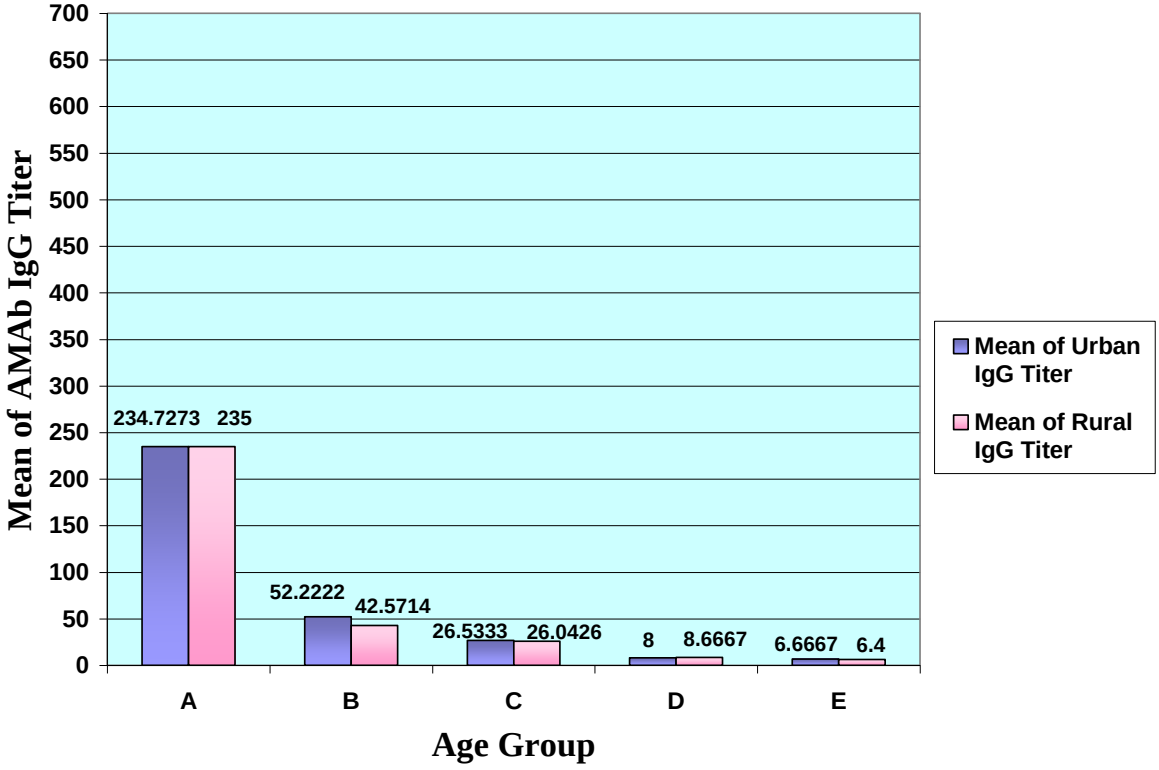

Figure (4-4): The mean of AMAb IgG titer in rural and urban vaccinated individuals in different age groups.

4.2.3: AMAb IgG Titer and Sex

The distribution of AMAb IgG titers according to sex of studied measles vaccinated groups is expressed in figure (4-5). In the males the highest mean of IgG titer (666) was estimated at six years and the titer was decreasing with increasing of age and reaching the lowest mean of IgG titer (4) at 29 years, whereas the highest mean of IgG titer (430.9333) was estimated at six years in the females and the titer was decreasing with increasing of age and reaching the lowest titer mean (6) at 29 years.

Figure (4-6) reveals the distribution of IgG titers according to sex of studied measles vaccinated individuals and age groups.

In males, the highest mean of IgG titer was recorded in age group A (251.0435), followed by group B (51.6757), group C (26.7077), group D (6.8889) and finally the lowest titer mean was in group E (6.25).

In females, the highest mean of IgG titer was recorded at age group A (221) which was lower than IgG titer mean in age group A of males. In age groups B and C, the IgG titers means (42.963) and (25.7143) respectively were also lower than IgG titer mean in age groups B and C of males, whereas the mean of IgG titers in age groups D (9.2174) and E (7.4286) were higher than the means of IgG titers of males age groups D and E respectively.

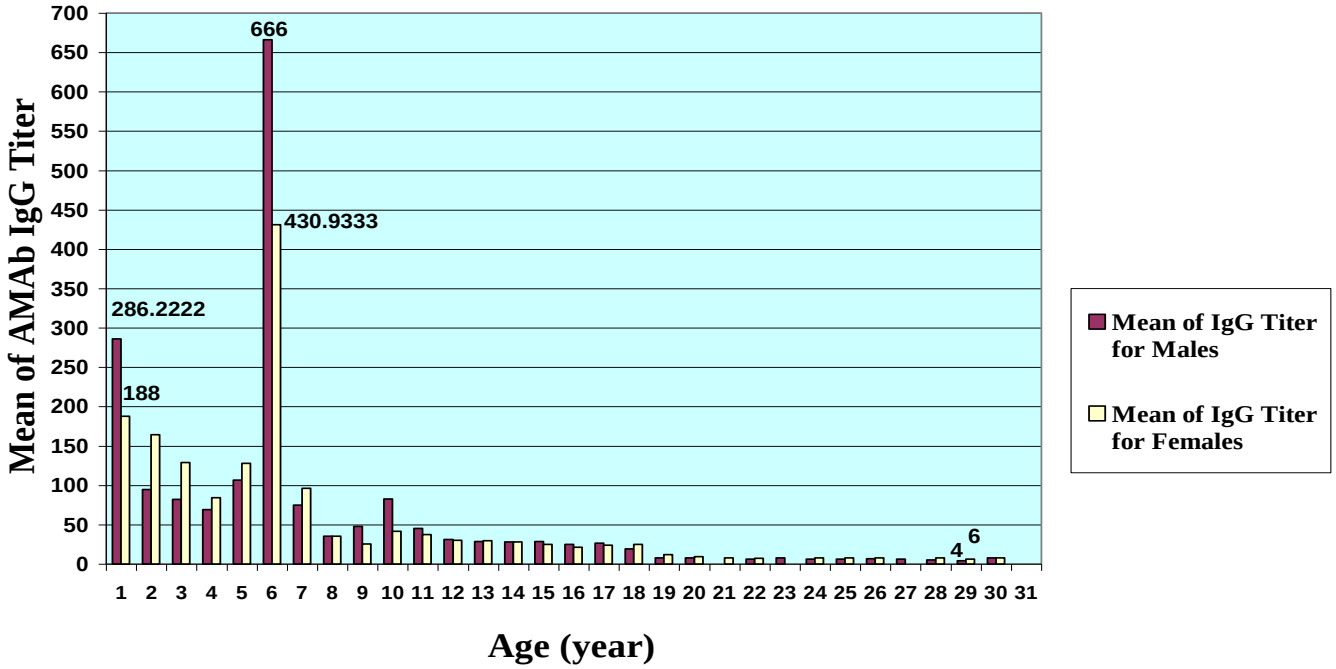

Figure (4-5): The mean of AMAb IgG titer in vaccinated males and females in different ages each year.

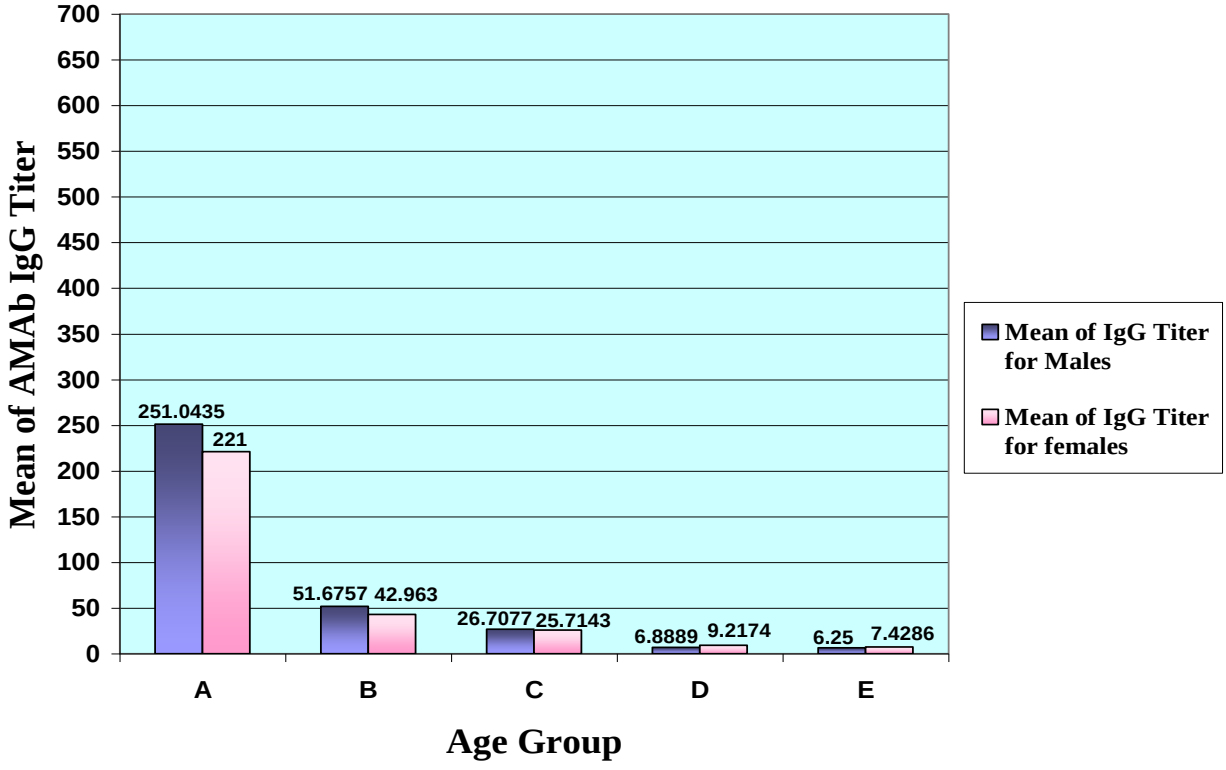

Figure (4-6): The mean of AMAb IgG titer in vaccinated males and females in different age groups.

4.3: Estimation of SRID (total non-specific) Complement Components and Immunoglobulins for the Studied Groups

4.3.1: Estimation of Immunoglobulins

A. Evaluation of IgG Concentration in Measles Vaccinated Individuals:

Figure (4-22) shows a photograph for SRID test for IgG.

Figure (4-7) reveals the results of SRID test for IgG concentration at the studied age groups. The lowest concentration was found in group A (996.56 mg/dl). The concentrations in groups B and C were (1098.35 mg/dl) and (1176.57 mg/dl) respectively, whereas the highest concentration of IgG was found in group D (1399.11 mg/dl) and lastly the IgG concentration in group E was (1016.28 mg/dl).

Figure (4-8) reveals the results of SRID test for IgG concentration at each year of the studied groups. The highest concentration of IgG (1653.9 mg/dl) was noticed at 23 years and 25 years while the lowest concentration (514.85 mg/dl) was noticed at 26 years whereas the other ages were revealed various IgG concentrations.

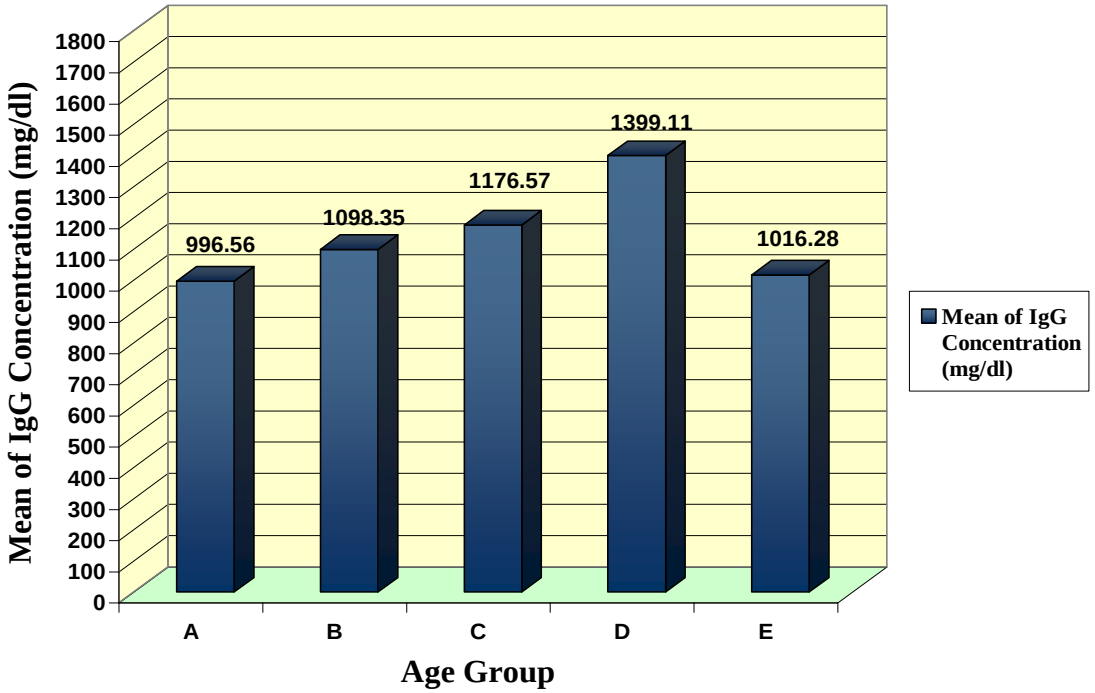

Figure (4-7): The mean of IgG concentration found in vaccinated individuals in different age groups.

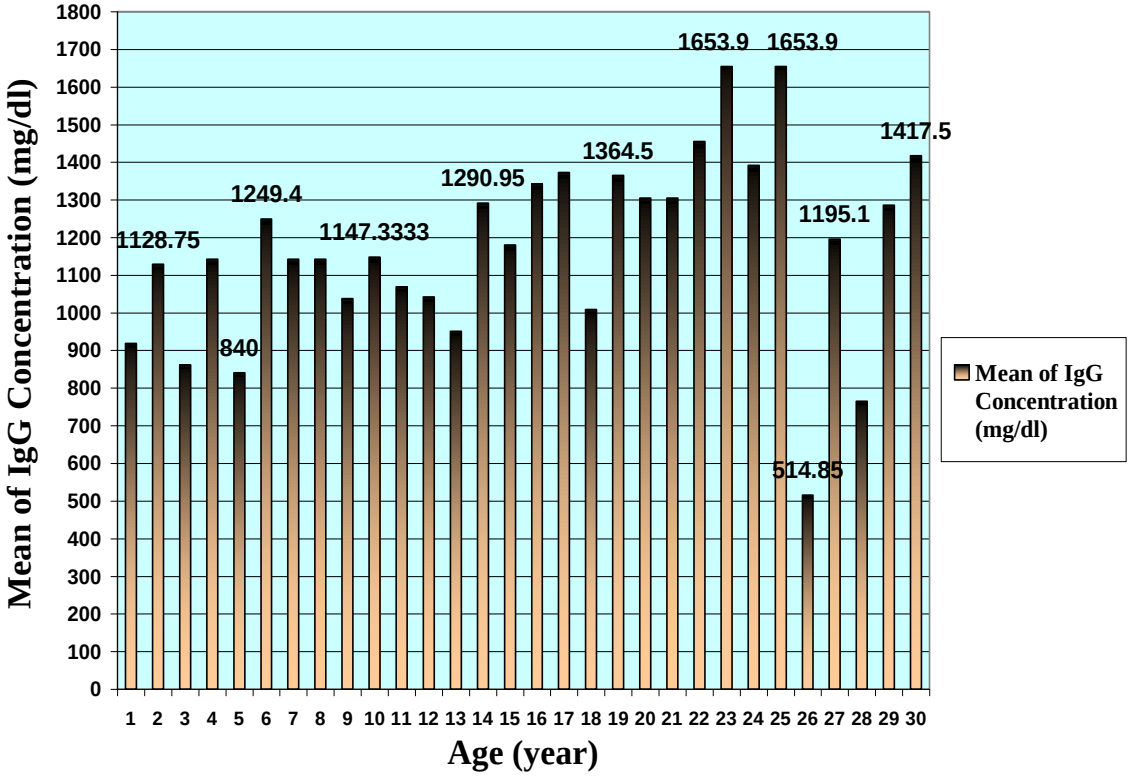

Figure (4-8): The mean of IgG concentration found in vaccinated individuals each year.

B. Evaluation of IgG Concentration in Control Group:

Figure (4-9) shows the results of SRID test for IgG concentration at each year of the studied control group. The lowest concentration (582.8667 mg/dl) was found in the first year of age. In the second year of age the concentration was (707.525 mg/dl) which was higher than the concentrations in the first year while in the third year of age, the concentration of IgG (734.6667 mg/dl) was the highest concentration in this group.

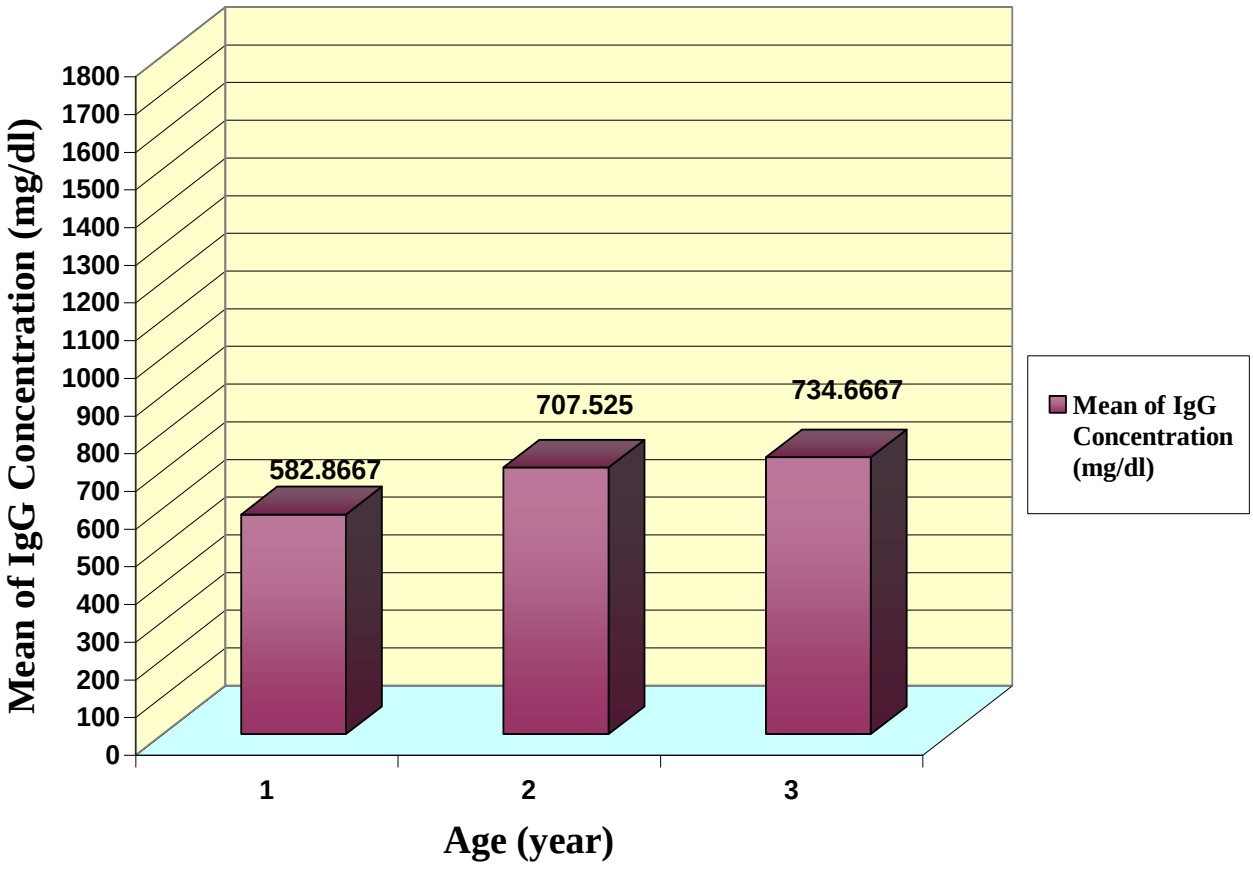

Figure (4-9): The mean of IgG concentration found in control group each year.

C. Evaluation of IgM Concentration in Measles Vaccinated Individuals:

Figure (4-23) shows a photograph for SRID test for IgM.

Figure (4-10) reveals the results of SRID test for IgM concentration at the studied age groups. The highest concentration of IgM (148.64 mg/dl) was noticed in group A. The concentrations in groups B and C were (135.15 mg/dl) and (138.73 mg/dl) respectively whereas in group D, the concentration of IgM was (142.76 mg/dl). Lastly the IgM concentration in group E (119.88 mg/dl) was the lowest concentration.

Figure (4-11) shows the results of SRID test for IgM concentration at each year of the studied groups. The highest concentration of IgM (257.6 mg/dl) was found at 5 years while the lowest concentration (34.2 mg/dl) was found at 8 years whereas the other ages were revealed different IgM concentrations.

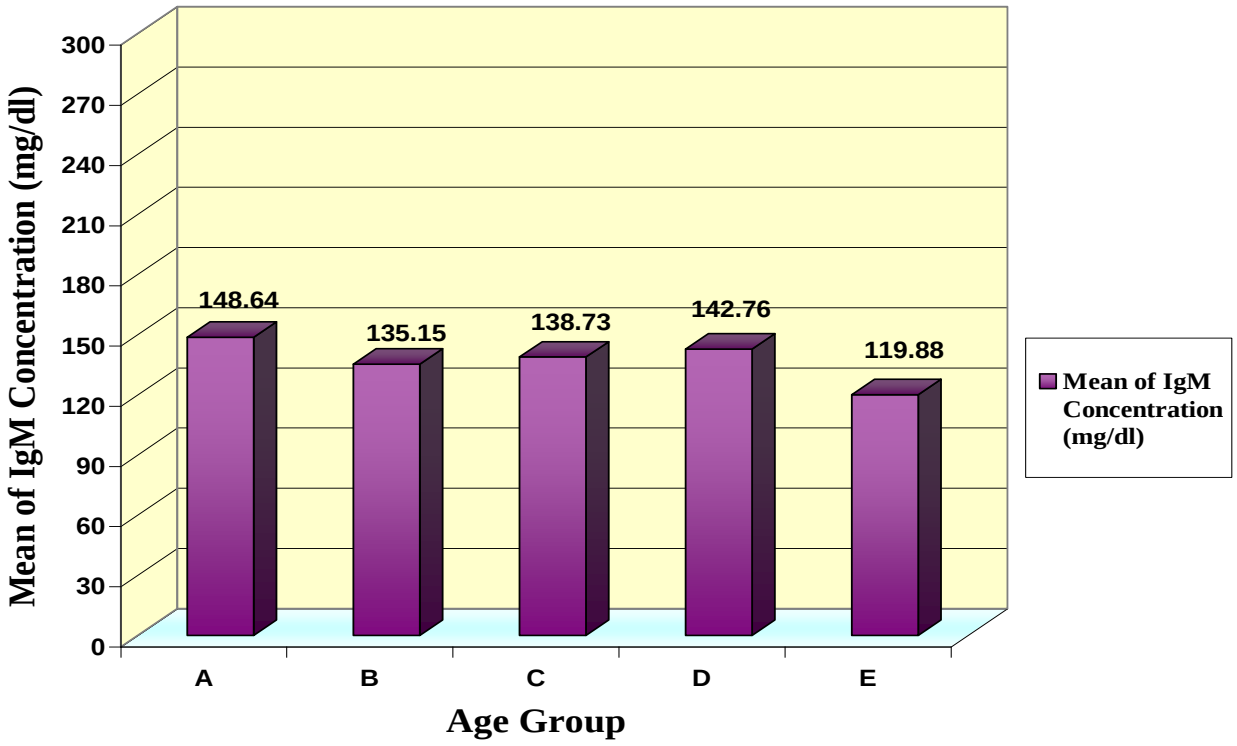

Figure (4-10): The mean of IgM concentration for measles vaccinated individuals in different age groups.

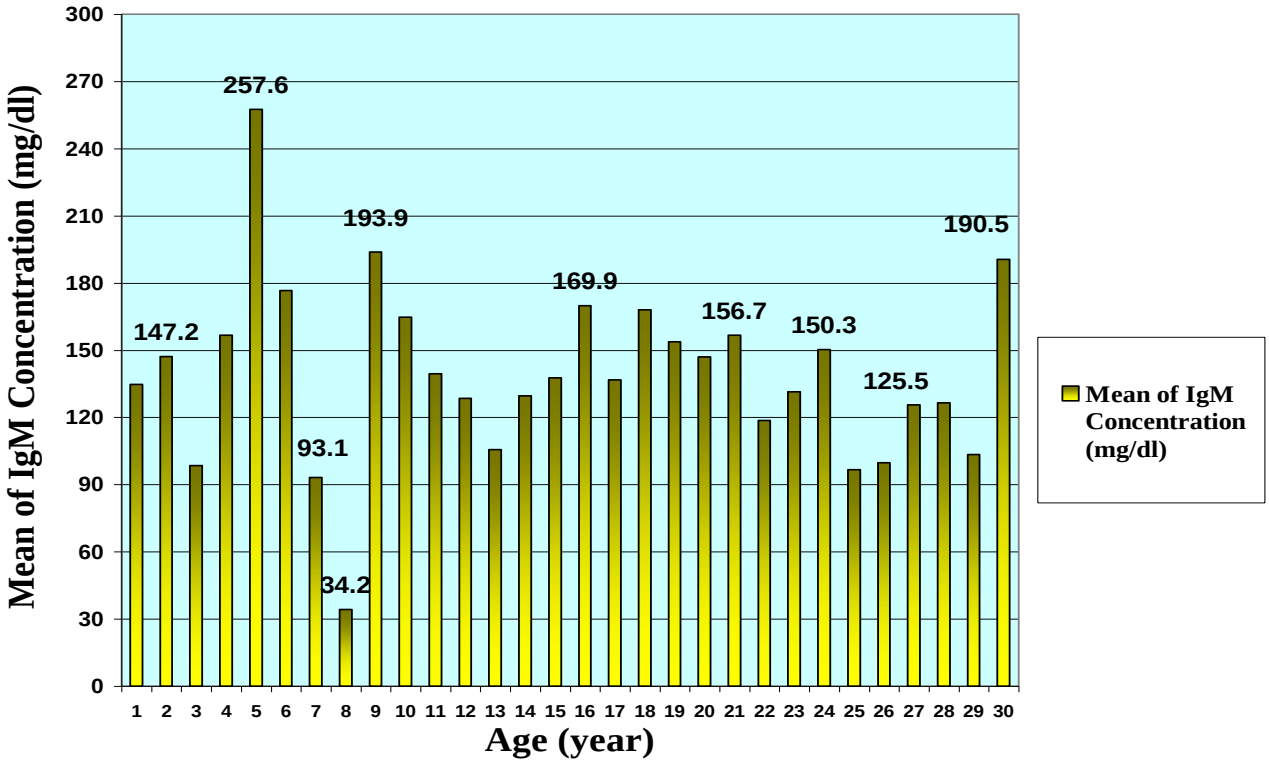

Figure (4-11): The mean of IgM concentration in measles vaccinated individuals each year.

D. Evaluation of IgM Concentration in Control Group:

Figure (4-12) reveals the results of SRID test for IgM concentration at each year of the studied control group. In this group there were three different IgM concentrations. The lowest concentration (89.0333 mg/dl) was recorded at the first year whereas the highest concentration (127 mg/dl) was recorded at the second year of age. In the third year of age, the concentration was (91.3 mg/dl) which was higher than the concentration in the first year and lower than the concentration in the second year.

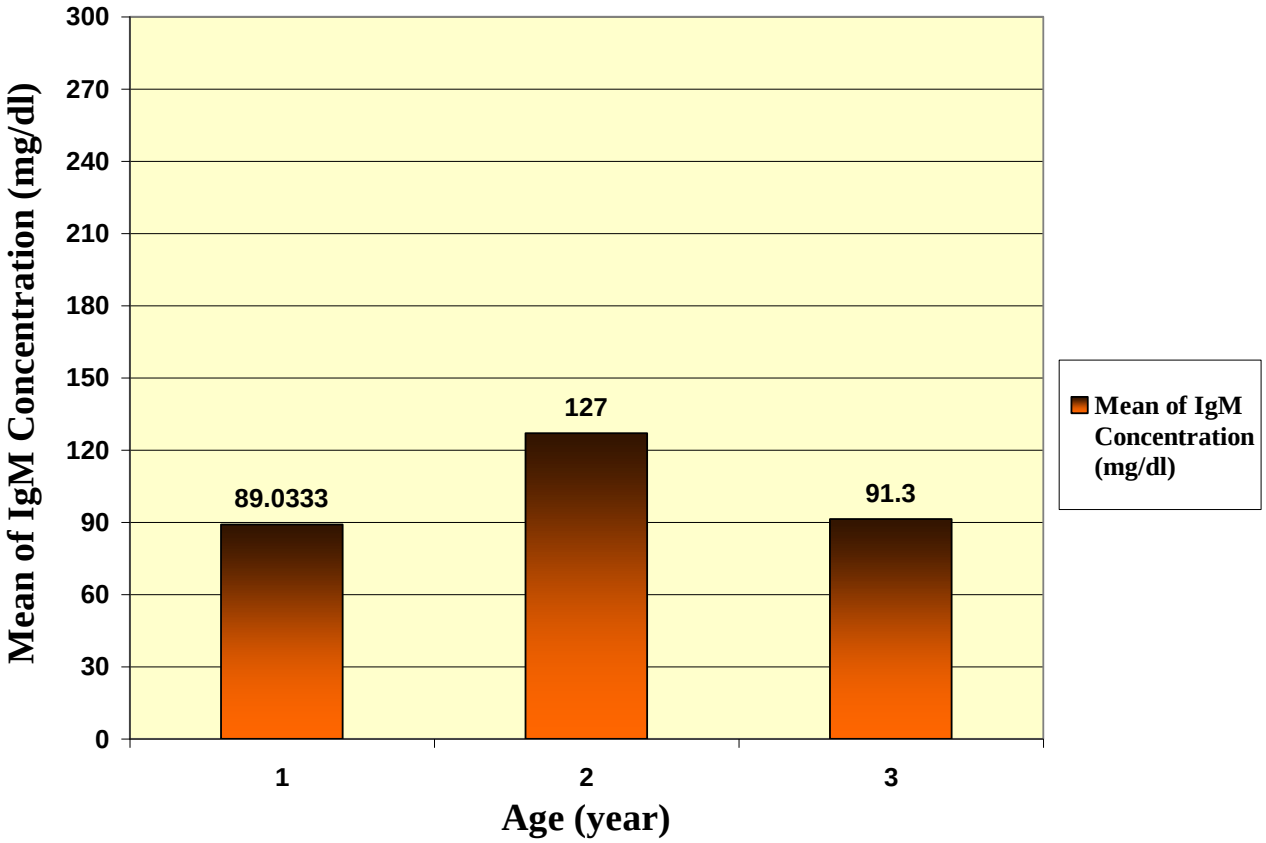

Figure (4-12): The mean of IgM concentration found in control group each year.

E. Evaluation of IgA Concentration in Measles Vaccinated Individuals:

Figure (4-24) shows a photograph for SRID test for IgA.

Figure (4-13) reveals the results of SRID test for IgA concentration among the studied age groups. There were various IgA concentrations among the studied age groups. Group A was revealed the lowest concentration (123.43 mg/dl) while the other age groups were showed different concentrations: Group B (161.58 mg/dl), group C (174.75 mg/dl), group D (223.46 mg/dl) which was the highest IgA concentration and lastly group E (216.82 mg/dl).

Figure (4-14) reveals the results of SRID test for IgA concentration at each year of the studied groups. Different IgA concentrations were recorded at the different years of age. At 3 years the lowest concentration (66.6 mg/dl) was noticed whereas the highest IgA concentration (338.6 mg/dl) was noticed at 21 years.

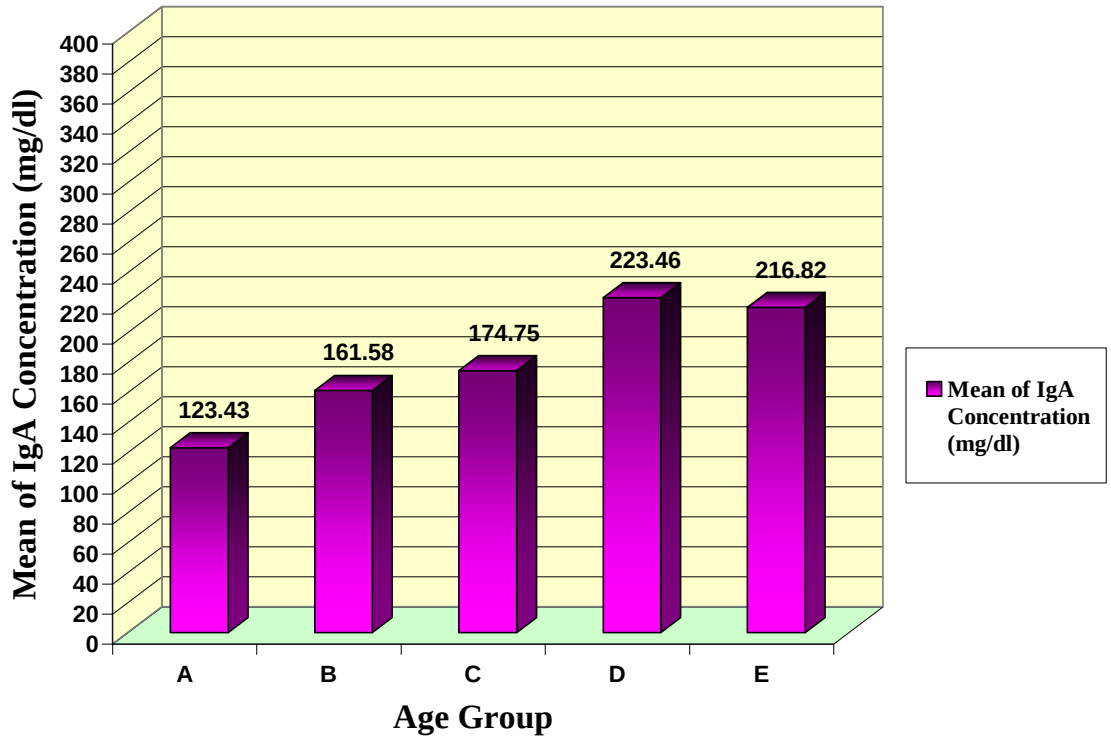

Figure (4-13): The mean of IgA concentration for measles vaccinated individuals in different age groups.

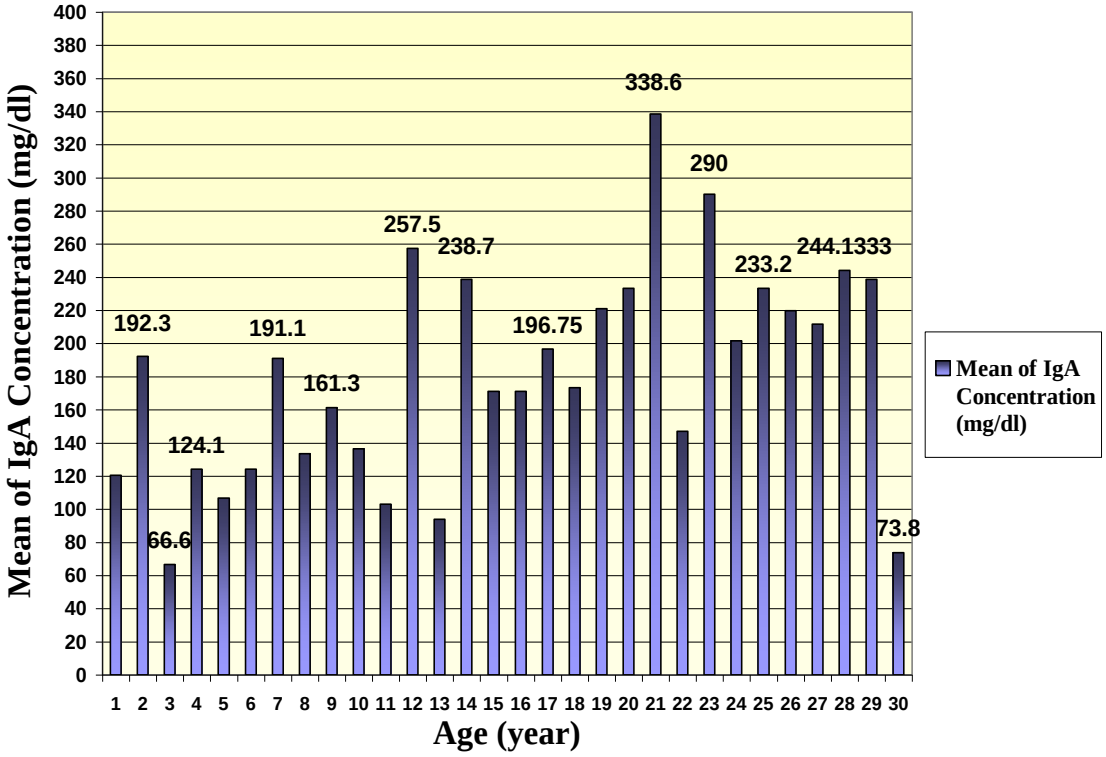

Figure (4-14): The mean of IgA concentration for measles vaccinated individuals at each year of age.

F. Evaluation of IgA Concentration in Control Group:

Figure (4-15) shows the results of SRID test for IgA concentration in each year of the age of the studied control group. The lowest IgA concentration (23.1 mg/dl) was appeared at the first year. In the second year increased to (34.9333 mg/dl), and to (94.5 mg/dl) was appeared at the third year.

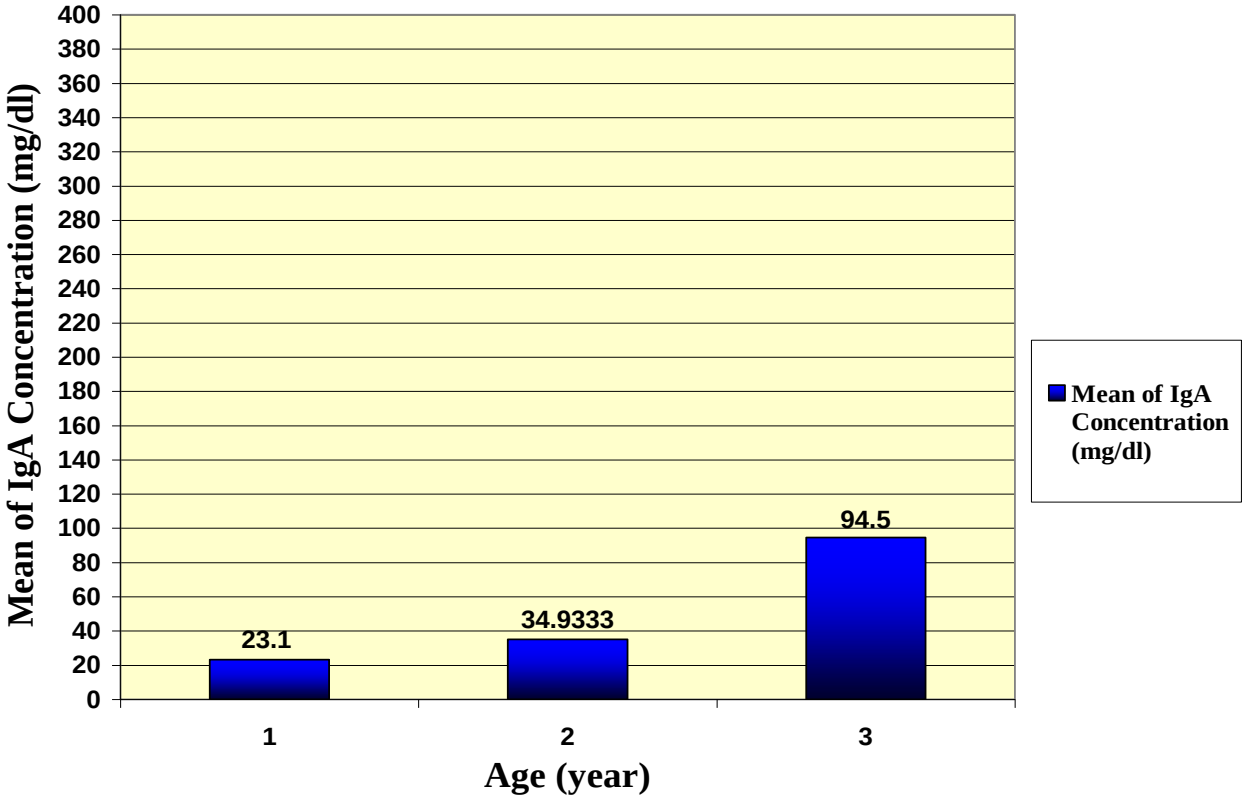

Figure (4-15): The mean of IgA concentration for control group at each year.

4.3.2: Estimation of Complement Components

A. Estimation of Complement Components in Measles Vaccinated Individuals:

Figure (4-25 a, b) shows a photograph for SRID test for C3 and C4 respectively.

Figure (4-16) shows the results of SRID test for C3 concentration at the studied age groups. In groups A and B, the C3 concentrations were (102.31 mg/dl) and (91.85 mg/dl) respectively whereas in group C, the lowest concentration (90.92 mg/dl) was found. The C3 concentration was (105.78 mg/dl) in group D and lastly in group E, the highest concentration (111.28 mg/dl) was noticed.

Figure (4-17) shows the results of SRID test for C3 concentration at each year of the studied groups. The lowest concentration (73.5 mg/dl) was noticed at 13 years whereas the highest concentration of C3 (137.7 mg/dl) was noticed at 27 years while the other ages were revealed various C3 concentrations.

Figure (4-18) reveals the results of SRID test for C4 concentration among studied age groups. Group A was revealed the highest C4 concentration (36.22 mg/dl) whereas group B was revealed the lowest concentration (24.37 mg/dl) followed by group C (25.98 mg/dl), group D (26.04 mg/dl) and finally group E (29.52 mg/dl).

Figure (4-19) shows the results of SRID test for C4 concentration in each year of the studied groups. The highest concentration of C4 (80.7 mg/dl) was estimated at 4 years whereas the lowest concentration (16.2 mg/dl) was estimated at 13 years while the other ages were showed different C4 concentrations.

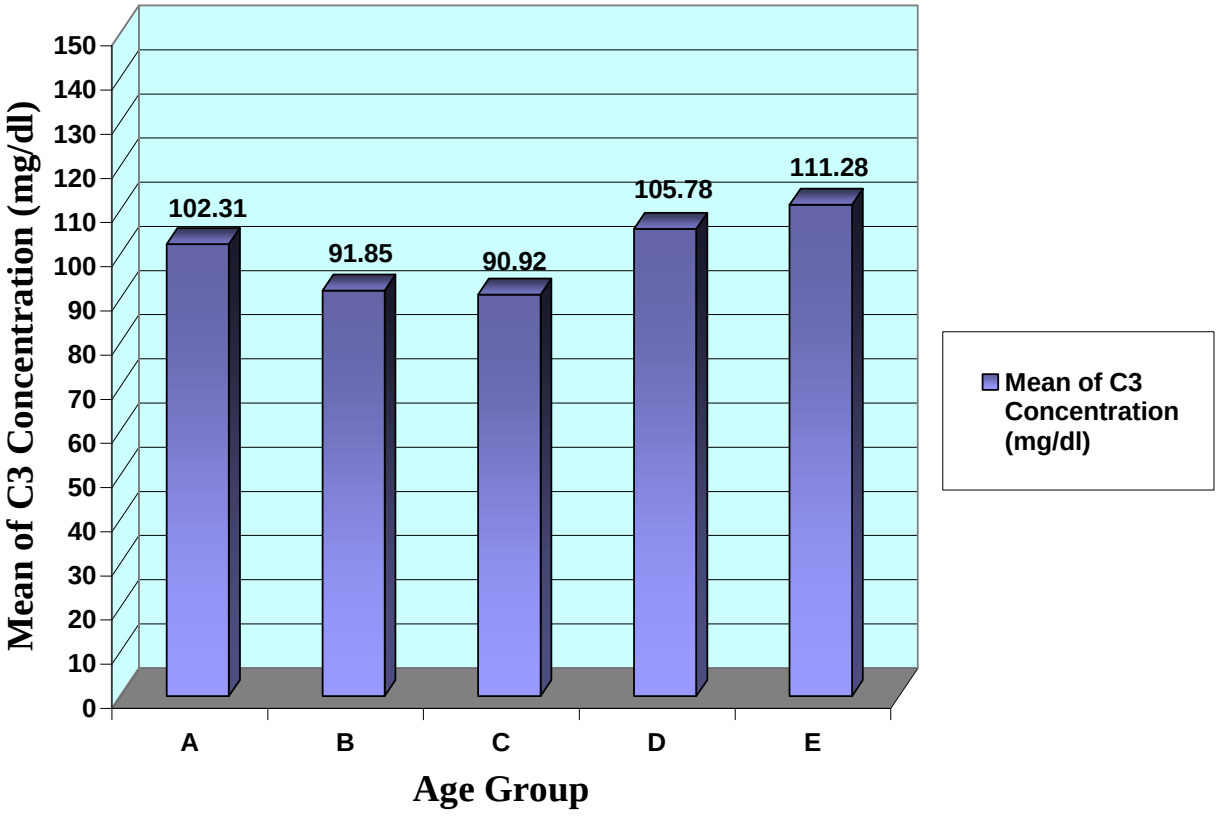

Figure (4-16): The mean of C3 concentration for measles vaccinated individuals in different age groups.

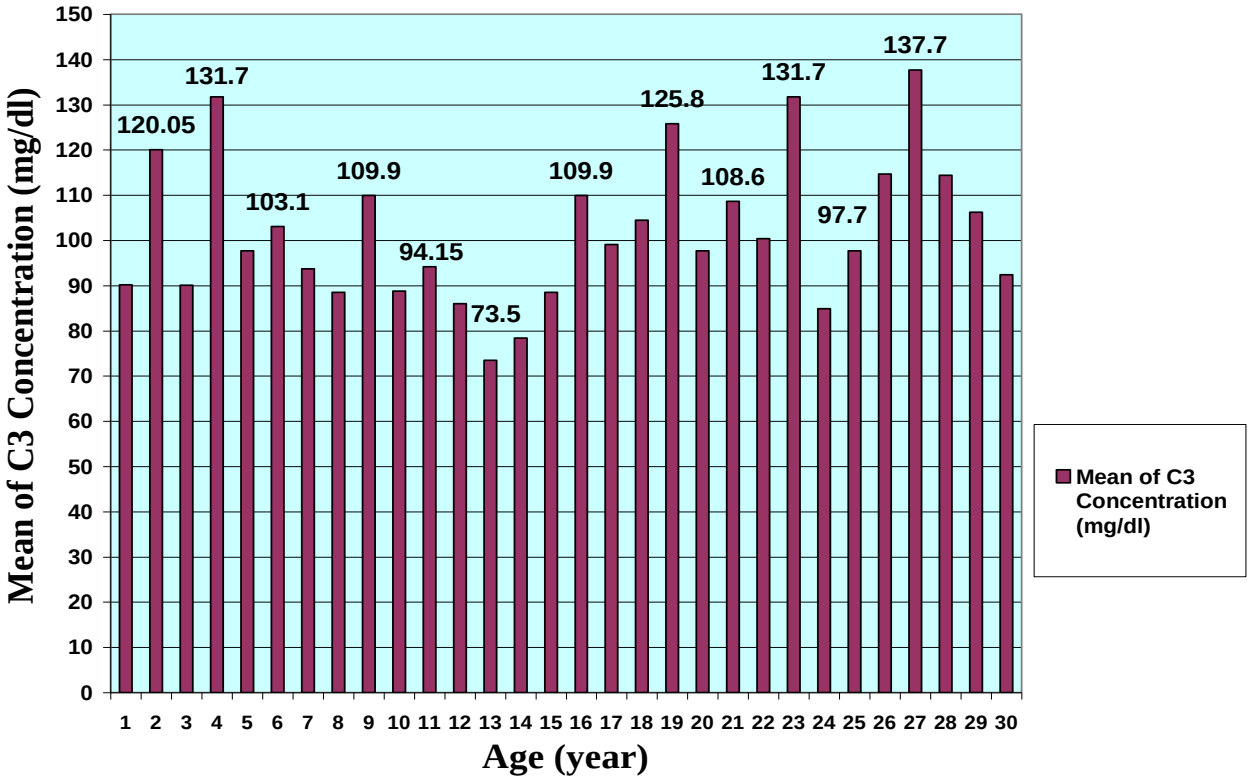

Figure (4-17): The mean of C3 concentration for measles vaccinated individuals at each year of age.

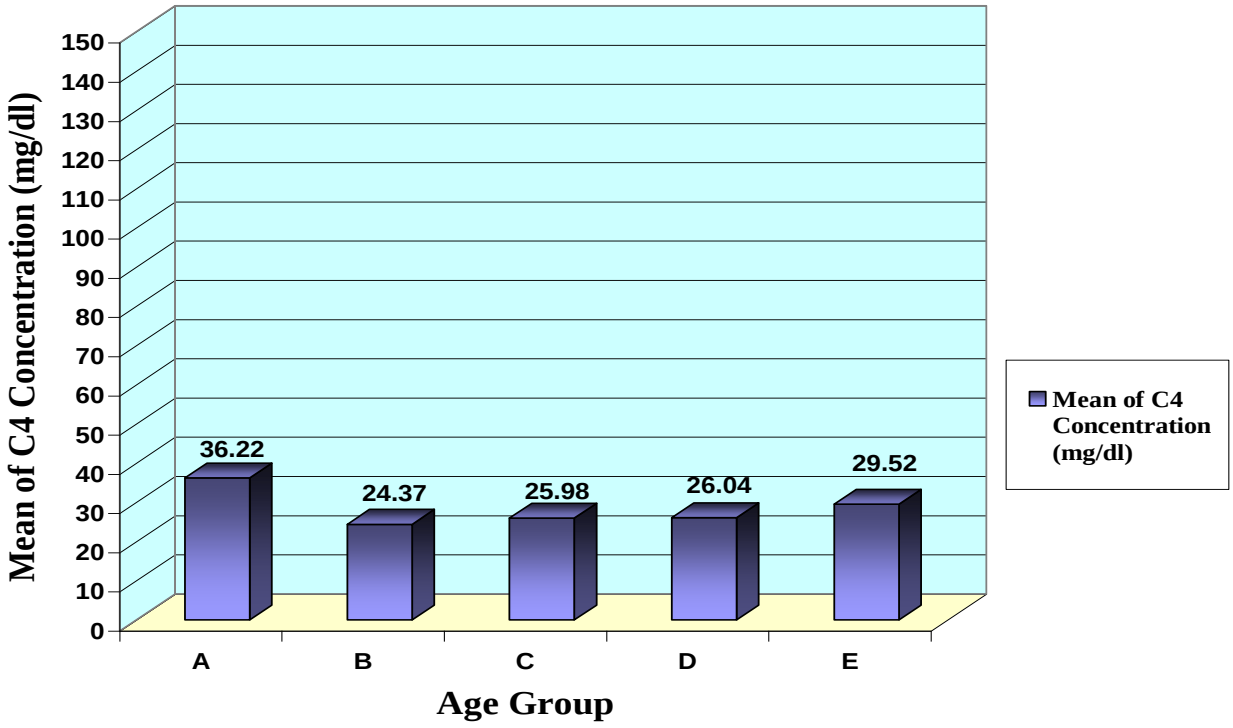

Figure (4-18): The mean of C4 concentration for measles vaccinated individuals in different age groups.

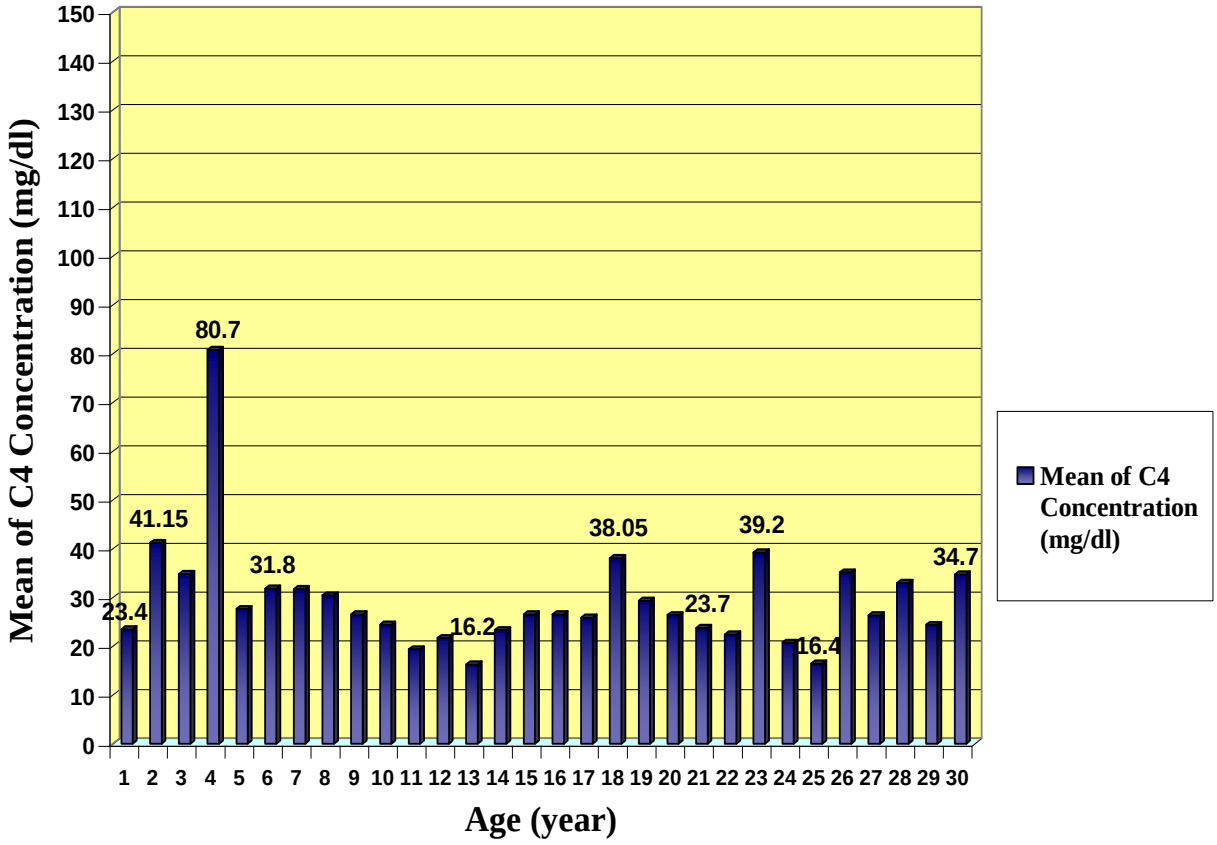

Figure (4-19): The mean of C4 concentration for measles vaccinated individuals at each year of age.

B. Estimation of Complement Components in Control Group:

Figure (4-20) reveals the results of SRID test for C3 concentration in each year of the age of the studied control group. The lowest C3 concentration (70.9667 mg/dl) was appeared at the first year. In the second year, the concentration was increased to (88.675 mg/dl), and to (93.0333 mg/dl) was appeared at the third year.

Figure (4-21) shows the results of SRID for C4 concentration found in each year of the age of the studied control group. At the

first year, the C4 concentration was (19.7667 mg/dl) which was the lowest concentration recorded in the control group and the concentration was increase with increasing of age and reaching to (23.825 mg/dl) in the second year and to (32.0333 mg/dl) at the three years old group which was the highest C4 concentration recorded.

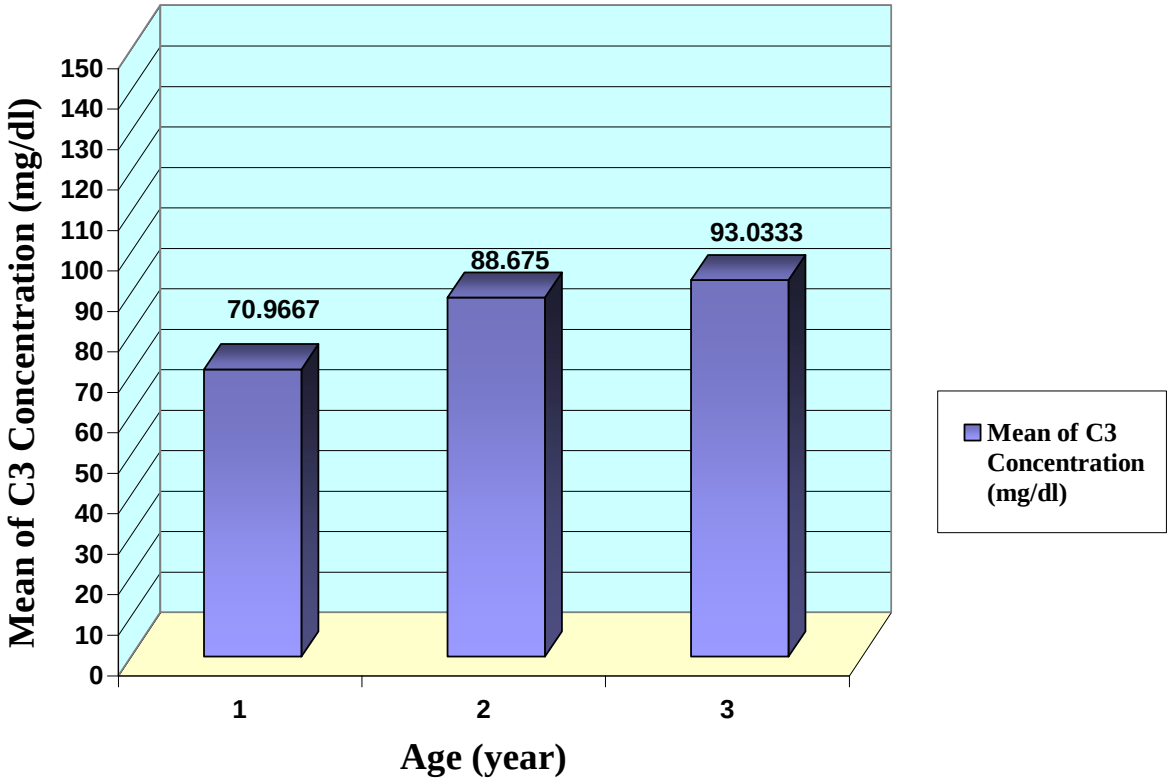

Figure (4-20): The mean of C3 concentration for control group at each year.

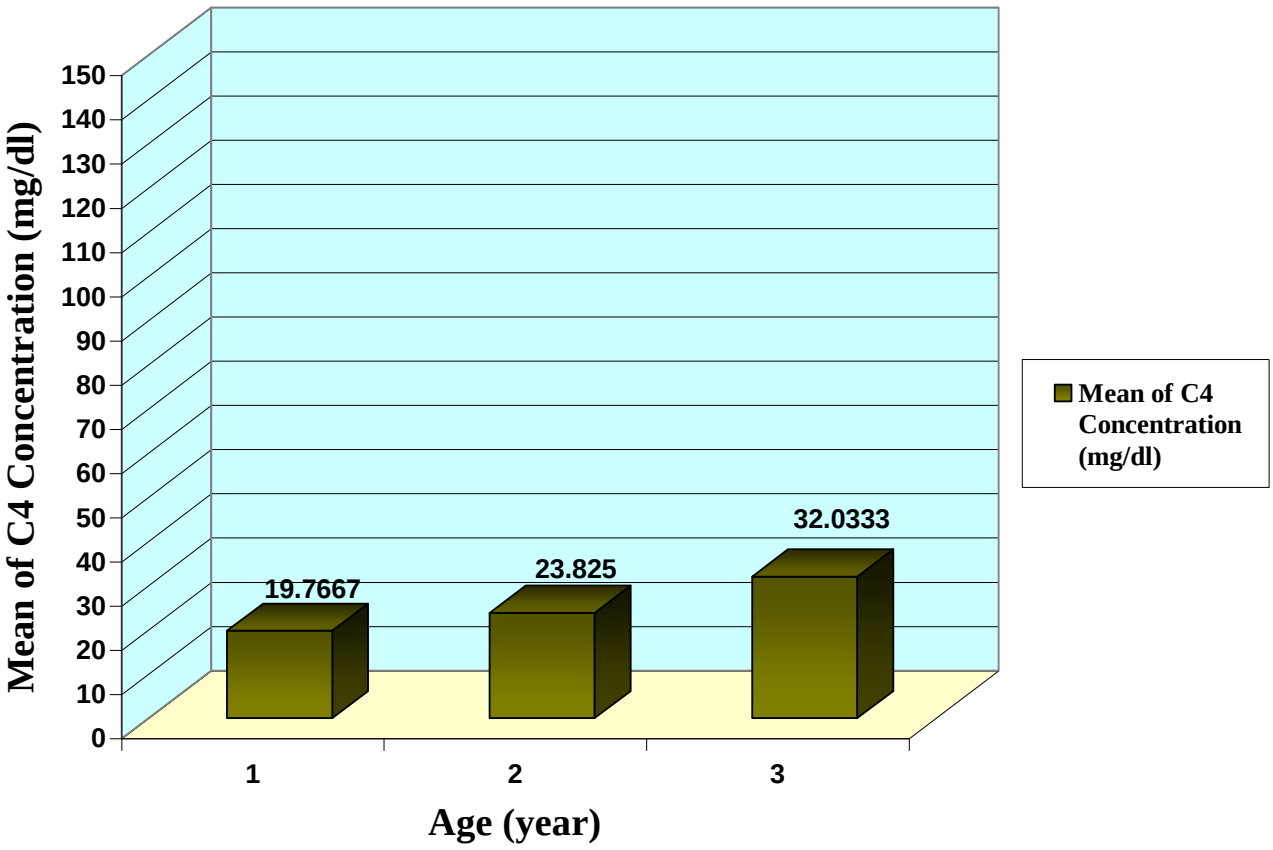

Figure (4-21): The mean of C4 concentration for control group at each year.

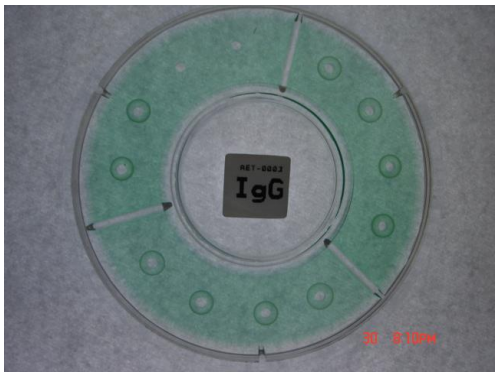

Figure (4-22): SRID test for IgG.

Figure (4-23): SRID test for IgM.

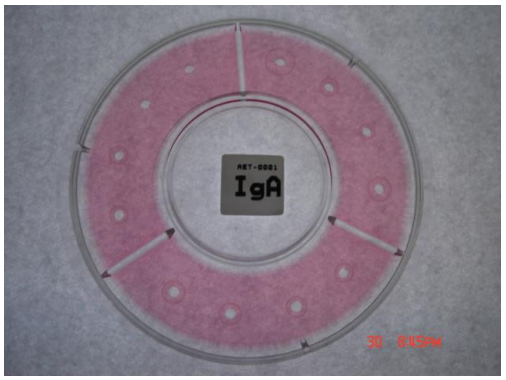

Figure (4-24): SRID test for IgA.

Figure (4-25 a): SRID test for C3.

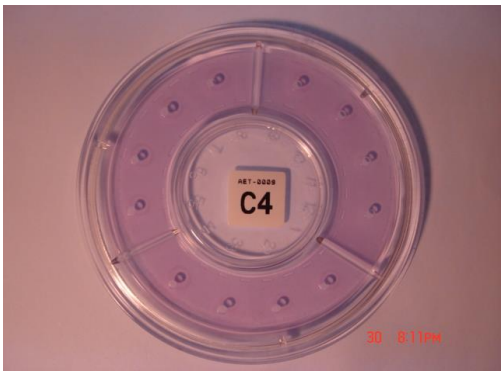

Figure (4-25 b): SRID test for C4.

Chapter Five

Discussion

5.1: Discussion

5.1.1: Anti-Measles IgG Titer and Age

In this study it was found that the IgG titer was decreasing with increasing of age. In age group A, which is the smallest age group; the total mean of IgG titers (234.82) was the highest from the other groups. The highest IgG titer (1024) was detected in 9% of sera samples of six years individuals included in this group who had got the booster dose of MMR vaccine (Schwarz strain); booster dose represents the secondary immune response (anamnestic response) characterized by greater level of antibodies due to the present primarily sensitized and memory cells (Parslow *et al.*, 2001), while the highest mode of IgG titer was (128) which was detected in 25% of group A samples.

In age group B, the pooled mean of IgG titer was (48), whereas two different IgG titer ranges were found in this age group. In 6 (9.4%) serum samples the titer (128) was the highest titer found, while the highest mode of AMAb titer was (≤64) which was found in 58 (90.6%) serum samples.

These results were nearly the same as that of (Al-Khafaji, 2006); study done in Hilla city, who found that the highest IgG titer (4096) was detected in six years children who had got the booster dose of MMR vaccine also there were different levels of antibody titer among vaccinated children (1-12 years).

Another study by (Bautista-López *et al.*, 2001) was done on 55 Peruvian children received Schwarz measles vaccine (about 10^3

plaque forming units) at about 9 months of age. They measured the AMAb titer by plaque reduction neutralization test and concluded that the children had excellent antibody responses after measles vaccination, but only 23% (8 out of 35) generated detectable lymphoproliferative responses to measles antigens (compared with 55-67% in children in the industrialized world). This difference may contribute to the less than uniform success of measles vaccination programs in the developing world.

The present work agreed with another study done in USA by (Hutchins *et al.*, 2001) on preschool-aged children (1-6 years), who found that the vaccine effectiveness was comparably high where the early two dose measles vaccination is associated with improved coverage and comparably high level of humoral immunity and clinical protection as a single dose at age ≥ 12 months. On the same manner Wong-Chew *et al.* (2003) found that high measles antibody titers interfere with the humoral response in subjects who received a booster immunization.

In age group C, the total mean of IgG titers was (26.3178), also all the tested sera of this age group revealed AMAb titer of (≤32). This age group, AMAb titer was gradually reduced when compare to the age groups A and B. This reduction may be due to the loss of antigenic stimulation.

The total AMAb titer means of the age groups (B, C, D, and E) were under the protective level (64) in vaccinated individuals (Rose *et al.*, 1986), whereas groups D and E were showed very

low IgG titer mean and they are nearly to the AMAb IgG titer mean of the control group (5.05). Therefore this study suggests that the vaccinated individuals who have been received a booster dose of measles vaccine and their AMAb (IgG) titers lower than (64) are considered to be non immune and may be susceptible to infection with measles. This result differs with (Loo *et al.*, 2003) study carried out in Iran on healthy individuals 6 months to 16 years old, residing in the town of Khodabandeh and they found that from 354 subjects studied, 310 (87.6%) had neutralizing anti-measles antibody titer of 1:8 or higher and were considered to be immune and 44 (12.4%) had lower antibody titers and were considered to be non immune.

This difference may attribute to the use of different techniques to evaluate the humoral anti-measles immune response in this study and the present work which are neutralization and ELISA respectively, in addition to difference in other environmental conditions.

While Helfand *et al.* (1998) found that mild or asymptomatic measles infections are probably very common among measles-immune persons exposed to measles cases and may be the most common manifestation of measles during outbreaks in highly immune populations.

Another study done in Taiwan by (Lee *et al.*, 2000) who suggested that measles neutralizing (NT) titer >1000 mIU/ml may prevent measles infection and NT titers >500 mIU/ ml may

prevent symptomatic infection, but vaccinees with undetectable or low NT titers may be susceptible to symptomatic infection.

Therefore, the present work may suggest that more than one booster dose of measles vaccine is necessary for the sustained control of measles and could help in measles eradication. This agreed with (Dilraj *et al.*, 2000), study done on South African schoolchildren, who found that more than one dose of measles vaccine is necessary for measles eradication also the aerosol route is thought to be more immunogenic for booster doses than traditional subcutaneous injections. Also, this suggestion of the present work agreed with (Mendelson *et al.*, 1996); study done in Palestine on young adult population who were vaccinated during an outbreak in 1991. Most vaccinees, age 18-25 years, had apparently been immunized once before as infants. Also, they found that the vaccination campaign prevented further measles cases, apparently by increasing the population's immunity, particularly in individuals with very low titers or without measles antibodies.

Another study done in Hungary by (Nagy *et al.*, 1984) found that following a period of 6 years of low measles incidence, an epidemic occurred in Hungary with more than 11,000 reported cases between September 1980 and August 1981. About 60% of the cases had a documented history of previous measles vaccination. Therefore, secondary vaccine failure due to waning immunity account for only 6.2% of previously vaccinated

patients, whereas in 93.8% of patients, including the majority of those with secondary antibody response, a primary failure of vaccination due to unsuccessful immunization was incriminated.

The present study found that the total mean of AMAb (IgG) titer of control group was (5.05) because the maternal antibodies are gradually decreased at 9 to 12 months in infants, which is nearly the same as that of (Altintas *et al.*, 1996); study done on Turkish infants, who found that the very low passive antibody at nine months of age may suggest the measles vaccination could be carried out earlier than just before the critical age of antibody level and his results agree with (Linder *et al.*, 2004), who suggested new measles vaccination recommendations for preterm infants.

5.1.2: Anti-Measles IgG Titer and Residency

In the present study, it was found that the residency plays an important role in the immune response and there were differences in immune response between rural and urban individuals i.e. the most urban age groups had AMAb IgG titer mean higher than the rural age groups. Results were so near to (Rager-Zisman *et al.*, 2004) who found out that there were differences in underlying immunologic parameters and in response to measles component of vaccine between Bedouin and Jewish children and these differences may be genetic or environmental including residency.

5.1.3: Anti-Measles IgG Titer and Sex

The present study revealed that the sex plays very important role in the immune response and there were differences in immune response between males and females according to their ages. This result is nearly the same as that proved by (Atabani *et al.*, 2000) who found that there were differences in the immune response to measles vaccines underlie the excessive female mortality by measuring the MV-specific antibody-dependant cellular cytotoxicity (ADCC) antibody response in 65 3-year-old Gambian children immunized with Edmonston-Zagreb medium-titer (EZ) or Schwarz standard vaccines during infancy.

Another study by (Green, *et al.*, 1994) on males and females aged 18-20 years, found that females exhibit a stronger humoral immune response to measles vaccine, which is nearly the same as the present work found.

5.1.4: IgG, IgM, IgA and Complement Concentrations

When compare the levels of IgG concentrations in control group with the nearest age group which is group A, the IgG concentrations are higher in group A than in control group, this may attribute to the fact that SRID measures the total IgG concentration including the concentration of anti-measles antibodies, which is present at high level in group A; which is one group of vaccinated individuals, than in control group (unvaccinated individuals).

IgG plays a direct role as neutralizing antibodies in the secondary and chronic stage of measles infection (Rose *et al.*, 1984; Abbas and Lichtman, 2006).

By comparing the levels of IgM concentrations in control group with the nearest age group which is group A, the IgM concentrations are higher in group A than in control group, this may explain depending on the fact that SRID determines the total IgM concentration including the concentration of anti-measles antibodies in group A which is one group of vaccinated individuals, than in control group (unvaccinated individuals).

IgM appears in early acute stage of measles infection and has a direct role by preventing the attachment of virus to the target cells (Rose *et al.*, 1984).

Also as the others, the levels of IgA concentrations in control group were lower than in the age group A, this may be due to the presence of anti-measles antibodies which is present at high level in group A which is one group of vaccinated individuals, than in control group (unvaccinated individuals). The main role of IgA against viral infection is their ability to act on mucosal surfaces to prevent viral attachment to target cells (Rich *et al.*, 2002).

The C3 concentrations in the first, second and third years of the age group A were 90.1333 mg/dl, 120.05 mg/dl and 90.05 mg/dl respectively, whereas in the control group, the lowest C3 concentration (70.9667 mg/dl) appeared at the first year. In the

second year, the concentration increased to (88.675 mg/dl), and to (93.0333 mg/dl) was appeared at the third year.

It appeared that C3 plays an important role in the immune response against MV by acting with antibody as opsonin to prevent viral attachment to target cells in classical pathway, as well as C3a act as anaphylotoxin to attract the immune cell to the site of viral infection (Parslow *et al.*, 2001).

By comparing the concentrations of C4 in group A (vaccinated group) and their compatible age in control group (unvaccinated group), there were slightly increasing levels in vaccinated group than controls.

C4 acts as one components of classical pathway of complement and acts as opsonin to destruct the virally infected cells (Rich *et al.*, 2002). Also, complement system aids in the elimination of Ab-viral antigen complexes (Abbas and Lichtman, 2006).

5.2: Conclusions

1. The results of ELISA test revealed that the immune response (AMAb IgG titer) decreases reversely with the age increase after the 6^{th} year.
2. The AMAb IgG titers of the vaccinated individuals in our community lower than the protective level (64) may predispose to measles infection.
3. The booster dose of MMR vaccine gave good immune response in children till the first year of the primary school.
4. The inactive immune response in the vaccinated children that we have found in this study remains to be answered.
5. The age, sex and residency play an important role in the immune response.
6. Total concentrations of IgG, IgM, IgA, C3 and C4 were higher in vaccinated individuals than controls.

5.3: Recommendations:

1. The first dose of measles vaccine should be given at the 9 months of age, while more than one booster dose of measles vaccine are required at 1.5, 3, 6, and 14 years. Also any age that shows AMAb IgG titer lower than the protective level, should be included in new vaccination campaigns that could help measles eradication.
2. Implementation of special control immunization campaigns and, once a suspected or confirmed case will be detected; all contacts may be vaccinated against measles.
3. Among the further plans to strengthen measles vaccination program in Iraq is to include the vaccination certificate requirement by children at school entry.
4. An extended study of AMAb IgG is to fully study vaccination status (both pre vaccination and post vaccination).

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List of Abbreviations

AAP	American Academy of Pediatrics
ACIP	Advisory Committee on Immunization Practices
ADCC	Antibody-Dependant Cellular Cytotoxicity
AIDS	Acquired Immunodeficiency Syndrome
AMAb	Anti-Measles Antibody
AMI	Atypical Measles Infection
CAM	Chick Chorioallantoic Membrane
C3	Complement Component 3
C4	Complement Component 4
$CCID_{50}$	Cell Culture Infectious Dose 50%
CDC	Center for Diseases Control
$CD4^{+}$	Cluster of Differentiation 4
$CD8^{+}$	Cluster of Differentiation 8
CDV	Canine Distemper Virus
CFT	Complement Fixation Test
CMI	Cell Mediated Immunity
DMSO	Dimethylsulphoxide
DNA	Deoxyribonucleic Acid
ELISA	Enzyme Linked Immunosorbent Assay
EPI	Expanded Programme of Immunisation
EZ	Edmonston-Zagreb
FO Protein	Fusion Protein Precursor

F Protein	Fusion Protein
HAIT	Haemagglutinalion Inhibition Test
HC	Health Canada
H Protein	Hemagglutinin Protein
HR	High Responder
IFAT	Indirect Fluorescent Antibody Technique
IFN-γ	Interferon-Gamma
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-2	Interleukin-2
IL-4	Interleukin-4
IL-12	Interleukin-12
IVIG	Intravenous Immunoglobulin
KMV	Killed Measles Vaccine
L Protein	Large Protein
LR	Low Responder
MM	Modified Measles
MMR	Measles Mump and Rubella
MMWR	Morbidity and Mortality Weekly Report
M Protein	Matrix Protein
MV	Measles Virus
NAP	National Academy Press

N protein	Nucleoprotein
NT	Neutralizing Titer
NVAC	National Vaccine Advisory Committee
N-V	Non-Vaccinated
PCR	Polymerase Chain Reaction
PHAT	Passive Haemagglutination Test
P protein	Phosphoprotein
PRNT	Plaque Reduction Neutralization Test
R	Rural
RIA	Radioimmunoassay
RNA	Ribonucleic Acid
RNP	Ribonucleoprotein
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SD	Standard Deviation
SDI	Samara Drug Industries
SRID	Single Radial Immunodiffusion
SSPE	Subacute Sclerosing Pan Encephalitis
$TCID_{50}$	Tissue Culture Infectious Dose 50%
Th	Helper T Lymphocyte
TMB	Tetramethylbenzidine
U	Urban
V	Vaccinated
WHO	World Health Organization

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Appendix (1)

Evaluation of Anti-Measles IgG Activity in Group A

Sample No.	Age (y)	Sex (M , F)	Residency (R , U)	¤ELISA IgG titer
1	1	M	U	256
2	1	F	U	256
3	1	M	U	512
4	1	M	R	256
5	1	M	U	256
6	1	M	R	16
7	1	M	U	256
8	1	M	U	512
9	1	F	R	16
10	1	M	R	256
11	1	F	R	4
12	1	M	R	256
13	1	F	R	512
14	1	F	U	256
15	1	F	U	256
16	1	F	U	16
17	2	M	U	128
18	2	F	U	256
19	2	F	U	128
20	2	M	U	128
21	2	F	U	128
22	2	M	U	128
23	2	F	U	256
24	2	F	U	128
25	2	F	U	256
26	2	F	R	128
27	2	F	R	256
28	2	M	U	256
29	2	F	R	128
30	2	M	R	4

Sample No.	Age (y)	Sex (M , F)	Residency (R , U)	¤ELISA IgG titer
31	2	F	R	16
32	2	M	R	16
33	2	M	U	32
34	2	F	U	128
35	2	M	U	64
36	3	M	U	128
37	3	F	U	128
38	3	M	U	64
39	3	F	U	64
40	3	F	U	128
41	3	F	R	64
42	3	F	R	128
43	3	M	U	64
44	3	M	U	128
45	3	M	U	128
46	3	M	R	4
47	3	M	R	16
48	3	F	R	2
49	3	F	R	4
50	3	M	U	128
51	3	M	U	32
52	3	F	R	512
53	3	M	U	128
54	4	M	U	128
55	4	F	U	64
56	4	F	R	64
57	4	M	U	16
58	4	F	U	64
59	4	M	U	64
60	4	F	U	32
61	4	F	U	64
62	4	M	U	128
63	4	F	U	128

Sample No.	Age (y)	Sex (M , F)	Residency (R , U)	¤ELISA IgG titer
64	4	F	U	256
65	4	M	R	16
66	4	F	R	4
67	4	M	R	64
68	5	M	U	128
69	5	F	U	256
70	5	M	U	64
71	5	F	U	64
72	5	F	U	128
73	5	F	R	64
74	5	M	R	128
75	5	F	U	128
76	6	M	U	4
77	6	F	U	32
78	6	F	R	64
79	6	F	U	32
80	6	F	U	64
81	6	F	U	256
82	6	M	U	256
83	6	F	R	512
84	6	F	U	1024
85	6	F	U	1024
86	6	M	R	1024
87	6	F	R	128
88	6	F	U	256
89	6	F	U	1024
90	6	M	R	1024
91	6	F	U	512
92	6	M	U	512
93	6	F	U	256
94	6	M	R	1024
95	6	M	U	256
96	6	F	R	256

Sample No.	Age (y)	Sex (M , F)	Residency (R , U)	¤ELISA IgG titer
97	6	F	U	1024
98	6	M	U	1024
99	6	M	R	1024
100	6	M	U	512

y = year, M = Male, F = Female, R = Rural, U = Urban

¤ELISA (1-6 y) Age Group (A) Mean = 234.82

Appendix (2)

Evaluation of Anti-Measles IgG Activity in Group B

Sample No.	Age (y)	Sex (M , F)	Residency (R , U)	¤ELISA IgG titer
101	7	M	U	64
102	7	F	U	128
103	7	M	U	64
104	7	M	U	64
105	7	M	U	128
106	7	F	U	64
107	7	F	U	128
108	7	M	U	64
109	7	F	R	64
110	7	M	R	64
111	8	F	U	32
112	8	M	U	32
113	8	F	U	32
114	8	F	U	64
115	8	M	U	16
116	8	F	R	16
117	8	M	R	64
118	8	M	U	32
119	8	F	U	32
120	8	M	U	32
121	9	F	R	32
122	9	M	R	64
123	9	M	U	64
124	9	F	U	32
125	9	F	U	32
126	9	M	R	16
127	9	M	U	32
128	9	F	U	16
129	9	F	R	16
130	9	M	R	64

Sample No.	Age (y)	Sex (M , F)	Residency (R , U)	¤ELISA IgG titer
131	10	M	U	128
132	10	F	U	32
133	10	M	U	64
134	10	M	U	128
135	10	F	U	32
136	10	F	R	16
137	10	M	R	16
138	10	M	R	32
139	10	F	R	64
140	10	M	R	128
141	10	F	R	64
142	11	F	U	64
143	11	F	U	32
144	11	M	U	32
145	11	M	U	64
146	11	M	U	32
147	11	M	R	64
148	11	F	R	16
149	11	M	R	16
150	11	M	R	64
151	12	M	U	16
152	12	M	U	16
153	12	M	U	64
154	12	F	U	16
155	12	M	R	32
156	12	M	R	16
157	12	F	R	8
158	12	M	U	8
159	12	M	R	64
160	12	M	R	32
161	12	M	R	32

Sample No.	Age (y)	Sex (M , F)	Residency (R , U)	¤ELISA IgG titer
162	12	F	R	64
163	12	F	R	32
164	12	F	R	32

¤ELISA (7-12 y) Age Group (B) Mean = 48

Appendix (3)

Evaluation of Anti-Measles IgG Activity in Group C

Sample No.	Age (y)	Sex (M , F)	Residency (R , U)	¤ELISA IgG titer
165	13	M	U	32
166	13	M	U	32
167	13	M	U	32
168	13	M	U	32
169	13	M	U	32
170	13	M	U	32
171	13	M	U	32
172	13	M	U	32
173	13	F	U	16
174	13	F	U	32
175	13	F	U	32
176	13	F	U	32
177	13	F	U	32
178	13	M	U	32
179	13	M	R	16
180	13	M	R	32
181	13	M	R	32
182	13	F	R	32
183	13	F	R	32
184	13	M	R	16
185	13	M	R	16
186	14	M	U	32
187	14	M	U	32
188	14	M	U	32
189	14	M	U	16
190	14	M	U	32
191	14	M	U	32
192	14	M	U	32
193	14	M	U	16
194	14	F	U	32

Sample No.	Age (y)	Sex (M , F)	Residency (R , U)	¤ELISA IgG titer
195	14	F	U	16
196	14	F	U	32
197	14	M	R	32
198	14	M	R	32
199	14	M	R	32
200	14	M	R	16
201	14	M	R	16
202	14	M	R	32
203	14	M	R	32
204	14	F	R	32
205	14	F	R	32
206	14	F	R	32
207	14	F	R	32
208	14	M	R	32
209	14	M	R	32
210	14	F	R	16
211	15	M	U	32
212	15	F	U	32
213	15	F	U	32
214	15	M	R	32
215	15	F	R	32
216	15	F	R	32
217	15	M	R	32
218	15	M	R	32
219	15	M	R	16
220	15	F	U	16
221	15	F	U	16
222	15	F	U	16
223	16	M	U	32
224	16	M	U	32
225	16	M	U	16
226	16	M	U	16

Sample No.	Age (y)	Sex (M , F)	Residency (R , U)	¤ELISA IgG titer
227	16	F	U	32
228	16	F	U	32
229	16	M	R	32
230	16	M	R	16
231	16	M	R	32
232	16	F	R	16
233	16	F	U	16
234	16	F	U	16
235	16	F	R	16
236	17	M	U	32
237	17	M	U	16
238	17	M	U	32
239	17	M	U	32
240	17	M	U	16
241	17	M	U	32
242	17	F	U	32
243	17	F	U	32
244	17	F	U	32
245	17	F	U	16
246	17	F	U	16
247	17	M	R	32
248	17	M	R	16
249	17	M	R	32
250	17	M	R	32
251	17	M	R	32
252	17	M	R	32
253	17	M	R	32
254	17	M	R	16
255	17	M	R	16
256	17	F	U	16
257	18	M	U	32
258	18	M	U	32

Sample No.	Age (y)	Sex (M , F)	Residency (R , U)	¤ELISA IgG titer
259	18	M	U	16
260	18	F	U	32
261	18	F	U	32
262	18	F	R	32
263	18	M	R	16
264	18	F	R	16
265	18	F	R	32
266	18	M	U	8
267	18	F	U	32
268	18	M	U	16
269	18	F	R	8
270	18	M	U	16
271	18	F	R	16

¤ELISA (13-18 y) Age Group (C) Mean = 26.3178

Appendix (4)

Evaluation of Anti-Measles IgG Activity in Group D

Sample No.	Age (y)	Sex (M , F)	Residency (R , U)	¤ELISA IgG titer
272	19	F	U	8
273	19	F	U	8
274	19	M	U	8
275	19	M	U	8
276	19	F	U	8
277	19	F	R	16
278	19	F	U	16
279	19	M	R	8
280	19	F	U	16
281	20	M	R	8
282	20	F	R	16
283	20	F	U	8
284	20	F	U	8
285	20	F	U	8
286	20	M	R	8
287	20	F	U	8
288	20	F	U	8
289	20	F	U	8
290	21	F	U	8
291	21	F	R	8
292	21	F	U	8
293	22	F	U	8
294	22	F	U	8
295	22	F	U	8
296	22	M	R	8
297	22	F	R	8
298	22	F	U	4
299	22	M	U	4
300	22	M	R	8
301	22	M	U	8

Sample No.	Age (y)	Sex (M , F)	Residency (R , U)	¤ELISA IgG titer
302	22	M	U	4
303	22	F	U	8
304	23	M	U	8
305	23	M	U	8
306	24	M	R	4
307	24	M	U	4
308	24	M	R	4
309	24	M	U	8
310	24	F	U	8
311	24	M	R	8
312	24	M	U	8

¤ELISA (19-24 y) Age Group (D) Mean = 8.1951

Appendix (5)

Evaluation of Anti-Measles IgG Activity in Group E

Sample No.	Age (y)	Sex (M , F)	Residency (R , U)	¤ELISA IgG titer
313	25	F	U	8
314	25	M	U	4
315	25	M	U	8
316	26	M	R	8
317	26	M	U	8
318	26	M	U	8
319	26	F	U	8
320	26	F	U	8
321	26	M	U	4
322	27	M	U	4
323	27	M	U	8
324	27	M	U	8
325	27	M	R	4
326	27	M	U	8
327	28	M	U	8
328	28	F	U	8
329	28	M	U	4
330	28	M	R	4
331	29	F	U	4
332	29	M	U	4
333	29	F	R	8
334	30	F	R	8
335	30	M	U	8

¤ELISA (25-30 y) Age Group (E) Mean = 6.6087

Appendix (6)

Evaluation Anti-Measles IgG Activity in Control Group

Sample No.	Age (y)	Sex (M , F)	Residency (R , U)	¤ELISA IgG titer
336	1	M	R	4
337	1	M	R	2
338	1	F	U	8
339	1	M	R	4
340	1	M	U	16
341	1	F	R	8
342	1	F	U	4
343	1	M	R	4
344	1	M	R	8
345	1	M	U	16
346	1	F	U	4
347	1	F	U	4
348	1	M	U	2
349	1	M	R	-
350	1	F	U	2
351	1	M	U	-
352	2	F	R	2
353	2	M	U	4
354	2	F	R	8
355	2	M	U	2
356	2	M	R	16
357	2	F	R	8
358	2	M	U	2
359	2	F	R	4
360	2	M	R	-
361	2	F	U	4
362	2	M	U	2
363	2	M	R	4
364	3	F	U	2
365	3	F	R	4

Sample No.	Age (y)	Sex (M , F)	Residency (R , U)	¤ELISA IgG titer
366	3	F	R	2
367	3	M	U	4
368	3	M	R	8
369	3	F	U	4
370	3	M	R	2
371	3	M	U	4
372	3	F	R	8
373	3	F	U	2
374	3	M	U	16
375	3	F	U	4

¤ELISA (1-3y) age group mean = 5.05