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EFFICACY OF MEASLES, MUMPS, RUBELLA AND PERTUSSIS VACCINATION IN CHILDREN FROM GAZA, PALESTINE

فعالية التطعيم ضد الحصبة والنكاف والحصبة الألمانية والسعال الديكي في أطفال غزة، فِلسطـــين

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DECLARATION

“I hereby declare that this manuscript is my own work and that, to the best of my knowledge and belief, it contains no material previously published, or written by another person, nor material which to a substantial extent has been accepted for the award of any other degree of the university or other institute, except where
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EFFICACY OF MEASLES, MUMPS, RUBELLA AND PERTUSIS VACCINATION IN CHILDREN FROM GAZA, Palestine

Abstract

Vaccination is a preventive strategy in fight against some infectious diseases and it is one of the most effective weapons of health protection of the modern medicine. Hence, it is necessary to continuously monitor the efficacy of vaccination programs.

Objective: This study focused on the evaluation of effectiveness and usefulness of vaccination against measles, mumps, rubella and pertussis in different age groups in Gaza Strip.

Method: Blood samples were collected from 184 children below 13 years of age, 91 males and 93 females, children were classified into 4 age groups, (2-4y), (5-7y), (8-10y) and (11-13y).

Measles, Mumps, Rubella and Pertussis antibodies were measured in serum samples using Enzyme Linked Immunosorbent Assay (ELISA).

A permission from the local Helsinki committe was obtained to ensure compliance with Ethical guidelins**.**

Data were analyzed using SPSS version 17.0.The results were significant if P value was <0.05.

Result: The study showed that the efficacy of Measles, Mumps, Rubella and Pertussis vaccination among children below 13 years in Gaza was 70.5%, 68.2%, 96.1% and 66.9% respectively, with a significant difference in efficacy among age groups except pertussis.

Conclusion: There was no significant difference between male and female in vaccine efficacy. This study indicates that vaccination is highly effective for rubella while it is less effective for measles, mumps and pertussis , which means that vaccine gives a short term protection, antibody level and vaccine efficacy decline overtime and there is a need for booster dose.

Key words: measles, mumps, rubella, pertussis, vaccination, Gaza-Palestine.

فعالية التطعيم ضد الحصبة والنكاف والحصبة الألمانية والسعال الديكي في أطفال غزة، فِلسطـــين

الخلاصة

يعتبر التطعيم من الإجراءات الوقائية للحماية من الأمراض المعدية كما يعتبرمن أكثر الوسائل نجاعة في الحفاظ على الصحة لذلك يجب تقييم كفاءة جداول التطعيم بشكل مستمر.

تهدف هذه الدراسة إلى تقييم كفاءة برنامج تطعيم الأطفال للوقاية من أمراض الحصبة والنكاف والحصبة الألمانية والسعال الديكي في قطاع غزة.

تم جمع عينات الدم من 184 طفل دون سن 13 سنة ، 91 ذكور ، 94 إناث صنفت في أربعة مجموعات عمرية مختلفة وهي (2-4) سنة و (5-7) سنة و(8-10) سنة و(11-13) سنة وقد فحصت أمصال العينات التي أخذت من كل طفل لتحديد تركيز الأجسام المضادة لكل من الحصبة والنكاف والحصبة الألمانية والسعال الديكي وذلك باستخدام تقنية (Enzyme Linked Immunosorbent Assay (ELISA).

وقد تم تحليل النتائج باستخدام البرنامج الأحصائي SPSS

أظهرت الدراسة أن كفاءة التطعيم كانت بنسبة 70.5% للحصبة وبنسبة 68.2 % للنكاف وبنسبة 96.1% للحصبة الألمانية وبنسبة 66.9% للسعال الديكي.

كما أشارت الدراسة إلى وجود فروقات ذات دلالة إحصائية في كفاءة التطعيم بين الأربع فئات العمرية ما عدا تطعيم السعال الديكي ، وعدم وجود فروقات ذات دلالة إحصائية في كفاءة التطعيم بين كلا الجنسين .

نستنتج من هذه الدراسة أن التطعيم للوقاية من الحصبة الالمانية ذو كفاءة عالية ، بينما تقل هذه الكفاءة في الحصبة والنكاف والسعال الديكي ، وهذا يعني أن التطعيم لا يزود الأطفال بمناعة طويلة الأجل ، كما أن معدل تركيز الأجسام المضادة يقل مع تقدم العمر وهناك حاجة لجرعة مقويّة أخرى.

الكلمات المفتاحية : الحصبة ، النكاف ، الحصبة الألمانية ،السعال الديكي ، التطعيم ،غزة-فلسطين .

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Dedication

الإهـــــــــــــــــداء

إلى والدي الحبيب الذي لم يبخل علي يوماً في أن يبث الأمـــل بداخلي .. صاحب القلب الحنون " م. محـــمود السيد القطـاوي.

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الى كل أحبتي وصديقاتي وزملائي الاعزاء داخل حدود الوطن وخارجه ولكل من منحني مساحة بيضاء تركت بداخلي أثراً طيباً

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LIST OF ABBREVIATIONS

BCG: Bacillus Calmette-Guerin

DPT: Diphtheria-pertussis-tetanus vaccine

DTaP: Diphtheria tetanus acellular pertussis

Td : Preparation of diphtheria and tetanus toxoids with a low amount of diphtheria toxoid, for adolescents and adults

ELISA: Enzyme Linked Immunosorbent Assay

EMR: Eastern Mediterranean Region

EPI: Expanded Program for Immunization

HAI: Haemoagglutination inhibition test

HB: Hepatitis B vaccine

HBsAb: Hepatitis B surface antibody

IPV: Intramuscular Polio Vaccine

MHC: Major histocompatibility complex

MMR: Measles Mumps Rubella vaccine

MR: Measles Rubella vaccine

FHA: Filamintous hemagglutinin

BA: Bacterial agglutination

LEIA: latex enzyme immunoassay

LA: Latex Agglutination TEST

EIA: Enzyme Immunoassay assay

PRN: Plaque reduction neutralization assay

$TCID_{50}$: median tissue culture infective dose that amount of a pathogenic agent that will produce infection in 50 per cent of cell cultures inoculated.

Pn: pertactin (membrane protein)

Fim: fimbriae (membrane protein)

NT: Neutralization test

IgG: immunoglobulin G

IgA: immunoglobulin A

PT: Pertussis toxin

OPV: Oral polio vaccine

RIA: Radioimmunoassay

TMB: Tetramethylbenzidine

WHO: World Health Organization

Chapter 1: Introduction

1.1 Background

1.1.1 MEASLES

Virology

Measles is a ubiquitous, highly infectious disease affecting nearly every person in a given population in the absence of immunization programmes [1].

Measles is a paramyxovirus, genus *Morbillivirus* [2].It is spherical, enveloped single stranded RNA viruses [1].It is an RNA virus with 6 structural proteins, 3complexed to the RNA and three associated with the viral envelope [1,2].Two of the envelope proteins, the F (fusion) protein and the H (haemagglutinin) protein are the most important in pathogenesis. Measles virus has a short survival time (<2 hours) in air, and is rapidly inactivated by heat, light and acidic pH [2].

Clinical features

Measles is a highly infectious, acute viral illness which is spread by respiratory droplets [3]. Measles is often a severe disease, frequently complicated by otitis media (7%) and bronchopneumonia (6%) [2].a generalized, reddish (erythematous), blotchy (maculopapular) rash ;a history of fever usually above 38˚C (if not measured, then "hot" to touch); and at least one of the following-cough, runny nose (coryza), or red eyes (conjunctivitis).

In addition, children with measles frequently exhibit a dislike of bright light (photophobia), and often have a sore red mouth (stomatisis).

There are many other childhood infectious diseases that also present with a measles-like rash, such as rubella (German measles) and scarlet fever. However in these diseases cough, coryza or conjunctivitis are usually not present [4].

Epidemiology

In the 24 years from 1976 to 2000, measles caused 98 deaths in Australia; this number exceeded those caused by diphtheria (4), tetanus (53), pertussis (29) and poliomyelitis (4) combined.

Although vaccination rates have improved, the uptake of measles vaccine in Australia has not yet reached optimal levels.

In 2001, the Australian Childhood Immunization Register recorded that 91% of children aged 2 years had been vaccinated for measles, but this is considered to be an underestimate of vaccine coverage. Following the Measles Control Campaign (which took place in 1998 and resulted in 1.7million primary school children being vaccinated), a national sero survey in the first quarter of 1999showed that 89% of children aged 2 to 6 years, 94% of those aged 6 to 11 years, and 91% of those aged12 to 18 years, were immune to measles. However, persons born between 1966 and 1980 are unlikely to have received 2 doses of measles containing vaccine and may remain non-immune [2].

The effectiveness of measles vaccine has been established in the United States. In 1963, before the vaccine was registered, there were 400 000 cases reported each year. In 1994 the countries of the WHO Region of the Americas established the goal of eliminating measles in the Region by the year 2000 [2].

However, in 1997 there was a resurgence, especially in Brazil (20 000 confirmed cases). In the USA there were only 86 confirmed cases in 2000, and there is continued progress towards elimination in the Region. The Eastern Mediterranean Region has established a goal for measles elimination by 2010, and the European Regional Office is also planning elimination. Measles remains a major cause of morbidity and mortality in the southeast Asian Region, where there are plans for strengthened control by the year 2003[2].

Vaccines

Two measles-mumps-rubella (MMR) vaccines are available. A monovalent vaccine is available for rubella, but no longer for measles or mumps. Vaccination with MMR results in serocon version to all 3 viruses in over 95% of recipients. Following a second dose of MMR vaccine, approximately 99% of subjects will be immune to measles. Since

the MMR vaccine viruses are not transmissible, there is no risk of infection from vaccines [2].

1.1.2 MUMPS

Virology

Mumps is an acute infectious disease caused by a paramyxovirus [5,3].closely related to parainfluenza virus [5]. with a single stranded RNA genome. It is rapidly inactivated by heat, formalin and ultraviolet light [2].

Clinical features

The classic symptom of mumps is parotitis (i.e., acute onset of unilateral or bilateral tender [2,6]. self-limited swelling of the parotid or other salivary glands [6]. lasting at least two days, but may persist longer than ten days. The mumps incubation period ranges from 12–25 days [7]. but parotitis typically develops 16 to 18 days after exposure to mumps virus[8].The rash follows, typically beginning on the face and upper neck, and then becoming generalised. Nonspecific prodromal symptoms may precede parotitis by several days, including low-grade fever which may last three to four days, myalgia, anorexia, malaise, and headache. However, mumps infection may present only with nonspecific or primarily respiratory symptoms or may be a subclinical infection [9].

Epidemiology

Mumps is reported worldwide, and is a human disease with transmission by the airborne route or direct contact [7]. It is primarily a disease of children, with a peak incidence in the group aged 5 to 9 years [10].

Eighty percent of adults in urban areas have serological evidence of immunity. A study of mumps in Alberta, Canada, confirmed the benign outcome in most cases, but indicated the potential of mumps vaccination for reducing hospital admissions for aseptic meningitis [11]. Vaccination with the live attenuated vaccine has proved

successful in the United States, with a 98% reduction in the number of reported cases between 1967 (when the vaccine was introduced) and 1985.

In Australia, there have been 10 reported deaths from mumps between 1978 and 1997. In 2000, mumps was recorded as the underlying cause of death in 2 adults, both over 80 years of age [2].

1.1.3 RUBELLA

Virology

Rubella, also known as German measles, was first described by two German physicians in the mid-18th century[12].

Rubella is an enveloped togavirus [2,13].with an RNA genome. It is related to group Aarboviruses, but does not cross-react with other members of the togavirus group. It is relatively unstable, and is inactivated by extremes of heat and pH, amantadine and UV light [2].

Clinical features

Rubella is generally a mild infectious disease [2].Among children, constitutional features are mild or absent but adults might develop fever and malaise associated with viraemia before the development of rash. The rash disappears as humoral immune responses develop, and at this stage viraemia is terminated [12].There may be a mild prodromalillness involving a low-grade fever, malaise, coryza and mild conjunctivitis.

Lymphadenopathy involving post-auricular and sub-occipital glands may precede the rash [13].It causes a transient erythematous rash, lymphadenopathy involving post-auricular and sub-occipital glands and, occasionally, arthritis and arthralgia. Other complications, such as neurological disorders and thrombocytopenia, may occur but are rare. Clinical diagnosis is unreliable since the symptoms are often fleeting and can be caused by other viruses; in particular, the rash is not diagnostic of rubella. A history of rubella should therefore not be accepted without serological evidence of previous infection .The incubation period is 14 to 23days, and the period of infectivity is from one week before until 4 days after the onset of the rash [2].

Epidemiology

Rubella occurs worldwide and is spread from person to person by airborne transmission of respiratory droplets [14].In temperate climates, the incidence is highest in late winter and early spring. Rubella incidence has fallen rapidly since vaccine licensure, and there has been a shift in the age distribution of cases, with comparatively more cases seen in older age groups. Rubella is more common in males than females, as selective vaccination for females preceded universal childhood vaccination [2]. In 1992 and1993, rubella epidemics were reported in those States where rubella was notifiable [15]. Over 3000 cases were reported again in 1993, 1994, and 1995. In 1997 this fell to 1446 cases (notification rate of7.8/100 000), and over the 2 years 1999–2000, this fell further to 697 cases (notification rate of1.8/100 000). This low notification rate probably reflects the high vaccine coverage achieved in the Measles Control Campaign in late 1998. There were no deaths with rubella reported as the underlying cause during 1998–2000 [16].

The rubella virus was isolated in cell culture in 1962 [14]. Vaccines are prepared from strains of attenuated virus and have been approved for use in Australia since 1970. Mass vaccination of school girls commenced in 1971. Non-pregnant, seronegative adult women were also vaccinated. These programs were successful and there was a significant reduction in the incidence of congenital rubella from1977. There has also been a significant increase in the percentage of pregnant women immune to rubella (in New South Wales from 82% in 1971 to 96% in 1983) [2].

Many adolescent and young adult males are non-immune to rubella because they did not receive MMR vaccine [17].The MMR vaccination program for all adolescents replaced the rubella program for girls in1993/94 [2]. A recent serosurvey by the National Centre for Immunization Research & Surveillance of Vaccine Preventable Diseases showed that only 84% of males aged 14 to 18 years (compared to 95% of females) and 89% of males aged 19 to 49 years (compared to 98% of females) were immune to rubella [17]. For this reason, adolescent and young adult males should receive MMR vaccine both for their own protection and to prevent transmission of the infection in the community [2].

1.1.4 PERTUSSIS

Bacteriology

Pertussis (whooping cough) is a highly contagious acute bacterial disease involving the respiratory tract and is caused mainly by Bordetella pertussis [18]. a fastidious, Gram-negative, pleomorphic bacillus. There are other organisms (such as Bordetella para pertussis, Mycoplasma pneumonia and Chlamydia pneumonia) which can cause a pertussis-like syndrome [2].

Clinical features

Pertussis is an epidemic bacterial respiratory infection [2]. The first phase of pertussis infection is characterized by attachment of B. pertussis to the ciliated epithelium of the respiratory tract. The second phase of infection is thought to be the result of toxin(s) secreted by the organism [19].

B. pertussis is highly infectious, spreading by respiratory droplets to 70 to 100% of susceptible household contacts and 50 to 80% of susceptible school contacts. Not all school-aged children and few adults with pertussis have the characteristic paroxysmal cough with inspiratory whoop. The cough may persist for up to 3 months and is often associated with vomiting [2].

Although pertussis can occur at any age, most serious cases and fatalities are observed in early infancy and mainly in developing countries. Major complications include pneumonia, encephalitis and malnutrition (due to repeated vomiting) [20].

The overall mortality from pertussis is 0.03% but the mortality in hospitalised babies under 6 months of age is substantially higher (3.5%). Both hospitalization and deaths are likely to be underestimated as infants, particularly if preterm, may either present without characteristic symptoms or be misclassified as sudden infant death syndrome. Pertussis causes hypoxic encephalopathy, which can result in brain damage and death. The most common cause of death in pertussis infection is pertussis pneumonia, sometimes complicated by seizures and encephalopathy [2].

The wracking coughs characteristic of this disease are sometimes so intense, the victims, usually infants, vomit or turn blue from lack of air [21].

Epidemiology

Epidemics occur every 3 to 4 years. In unvaccinated populations, these outbreaks can be very large. Invaccinated populations, smaller outbreaks with greatly reduced mortality and morbidity continue to occur every 3 to 4 years. Maternal antibody does not give adequate protection against pertussis, so babies can be infected before they are old enough to be vaccinated. In recent years among highly immunised communities, many cases of pertussis have been recognised in adults and adolescents, due to waning immunity and the increased availability of serological testing. These individuals are a significant reservoir of infection [2].

Occurrence Worldwide, B. pertussis causes at least 20 million cases of pertussis, 90% of which occur in developing countries, with an estimated 200,000 to 300,000 fatalities each year [20].

Pertussis kills about 250 000 children worldwide each year. Many children are left with brain damage from pertussis infection. From 1993 to 2001, 3 epidemics of pertussis ocurred in Australia. More cases were reported than for any time since the 1960s, with a total of over 34 000 cases between 1997 and 2001. This increase in reporting may largely relate to increased serological diagnosis in older persons [2].

Between 1993 and 1997 there were 9 deaths attributed to pertussis, all occurring in infants aged under12 months.

Introduction of a fifth dose of diphtheria, tetanus and pertussis vaccine (DTP) for 4 to 5 year old children in August 1994 has been associated with a subsequent reduction in notification rates among 5to 9 year old children despite an overall increase in rates. The pattern of decrease in notifications was consistent with a vaccine effect, occurring first among children aged 5 and 6 years old, followed by those in the 7 to 9 year old age group [2].

Currently in Australia, over 60% of pertussis notifications occur in persons over 10 years of age. This supports the need for booster doses in individuals over the age of 10 years both to reduce morbidity in them, and to reduce transmission to those most at risk

(infants <6 months of age). Immunization of adolescents, who have a high risk of pertussis infection, and adults in contact with very young infants would be expected to result in the greatest health benefits. An adult/adolescent formulation acellular pertussis-containing vaccine (dTpa) is now available for use in Australia for booster vaccination of persons aged 8 years and over [2].

Before scientists created a vaccine against the bacterium, 115,000 to 270,000 people suffered from whooping cough each year in the United States; 5,000 to 10,000 of those died from it. After the vaccine was introduced in the United States in the 1940s, the number of pertussis cases declined dramatically, hitting a low of about 1,000 in 1976. More recently, the annual number of reported cases of pertussis in the United States has been rising from 9,771 in 2002 to 25,616 in 2005. The reasons for the increase are complex. The disease strikes in cycles, and the immunity provided by the vaccine wanes over time, leaving some people susceptible in their teen years and as adults [21].

Recent studies on the immunochemistry of B. pertussis have resulted in the isolation and characterization of several biologically active substances which are important in understanding of the pathogenesis of pertussis and the determinants of immunity after disease and vaccination. This knowledge has contributed to the development of acellular pertussis vaccines and to the improvement of the serological diagnosis of pertussis [19].

1.2Vaccination schedule

Measles, mumps, and rubella (MMR) vaccine. (Minimum age: 12 months)

One dose administered at age 12-15 months.

Data indicate that the favorable benefit/cost ratio for routine measles, rubella, and mumps vaccination is even greater when the vaccines are administered as combined MMR vaccine [22].

Diphtheria and tetanus toxoids and pertussis (DPT) vaccine.

(Minimum age: 6 weeks)

The fourth dose may be administered as early as age 12 months, provided at least 6 months have elapsed since the third dose [23].

In developing countries which have already achieved high coverage with three doses of DPT vaccine in children under one year of age, the policy of using a booster dose of DPT vaccine at the end of the second year of age [24].

In developed countries, primary immunization usually-consists of three doses of DPT vaccine, given at intervals of 4 or more weeks, beginning at 2 or 3months of age, and reinforced by a fourth dose given in the second year of life. The policy of using booster doses of vaccines containing diphtheria (and tetanus) toxoid varies considerably. In some countries, booster doses of DPT vaccine are given above the age of 3 years (Hungary, and United States) [24].

Immunization schedule in Gaza Strip

In Gaza Strip the approved vaccination schedule which is applied in the primary health care centers is the expanded program for immunization (EPI) of WHO, this program is shown in table (1)

Table 1 : Vaccination schedule in Gaza Strip(24)

Age / Vaccine	HB	BCG	DPT	OPV	IPV	Td	MMR	DT	Measles	Rubella
1 day	■	■								
1 month	■				■					
2 months			■	■	■					
4 months			■	■						
6 months	■		■	■						
9 months									■	
12 months			■	■						
15 months							■			
6 years		■		■				■		
12 years										**Girls** Only
15 years						■				

HB : Hepatitis B virus vaccine

BCG: Bacillus Calmette-Guerin

DPT: Diphtheria-Pertussis-Tetanus vaccine

OPV: Oral Polio vaccine

IPV: Injectable Polio Vaccine

Td: Tetanus-diphtheria booster shots given every ten years to maintain immunity for children nineteen years of age to adults who are sixty-five years of age

MMR: Measles-Mumps-Rubella vaccine.

DT: Another option for infants is DT which is a vaccine that is a combination of diphtheria and tetanus vaccines. This is given as an alternative to infants who have conflicts with the DTaP vaccine

1.3 Aim the Study

This study aims at assessing the efficacy of MMR and pertussis vaccines in children after the four essential doses of DPT, at evaluate the immunogenicity of this dose and to follow up the duration of immunity by measuring the titer.

1.4 Specific objectives

- Measurement of measles, mumps, rubella and pertussis antibody titers.
- Duration of immunity to measles, mumps, rubella and pertussis .
- Compare between male and female sexes.
- Find antibody titers less than the protective levels.

1.5 Significance

- Measurement of measles, mumps, rubella and pertussis antibody titers among MMR and DPT vaccinated children which will enable us to evaluate the efficacy of such vaccines
- The immunogenity and effectiveness of MMR and DPT vaccine

Chapter 2: Literature Review

2.1. Historical background

The concept of immunization was based on the observation that those who survived certain diseases often failed to develop the disease a second time.

The practice of inoculating material from smallpox pustules for the prevention of the disease (variolation) was practiced in China, India and Presia long before it was introduced into Europe. Lady Mary Wortely Montague (1689-1762), wife of the British Ambassador at Constantiople, saw variolation carried out by Turkish women, and credited with its popularization in England [25]. Edward Jenner at (1798) showed that inoculating people with fluid obtained from the skin lesions of cows who where infected with cowpox virus protected them from the highly infectious and fatal disease, smallpox. This process came to be called vaccination . Jenners theory was advanced by the work of other scientist, including Louis Pasteur, Benjamin Waterhouse and Thomas Cimsdale. Pasteur who defined the concept of virulence, showed that virulence may be altered during growth in suboptimal conditions or in unrelated host, and the organism become attenuated, but retain the capacity to stimulate the immune system [26], this concept led to the use of attenuation as a mean of vaccination [27].

In 1888, Roux and Yersin noted that the diphtheria bacillus produced anexotoxin in liquid culture, and showed that the disease could be reproduced by injection of the bacteria free medium of a diphtheria culture and two years later, Von Behring and Kitasato developed a diphtheria antitoxin in horses and demonstrated that serum from an individual exposed to the toxin protected against exposure to the same toxin [28].

As shown small pox vaccine was the first vaccine used at1798 followed by other vaccines against several diseases such as rabies, plague, diphtheria, pertussis, BCG (tuberculosis), tetanus, yellow fever, polio, measles, mumps, rubella and hepatitis B virus [24].

Table 2 shows the date of introduction the first generation of vaccines for use in humans.

Table 2: The date of introduction of the first generation of vaccines for use in humans [29].

1798	Smallpox	1935	Yellow Fever
1885	Rabies	1955	Injectable Polio Vaccine (IPV)
1897	Plague	1962	Oral Polio vaccine (OPV)
1923	Diphtheria	1964	Measles
1926	Pertussis	1967	Mumps
1927	BCG (tuberculosis)	1970	Rubella
1927	Tetanus	1981	Hepatitis B virus

2.2. Types of immunization

There are two major types of immunization

2.2.1. Active immunization

Active immunity refers to the process of exposing the body to an antigen to generate an adaptive immune response: the response takes days/weeks to develop but may be long lasting—even lifelong. Active immunity is usually classified as natural or acquired [30]. Usually, both humoral and cell mediated responses are evoked, and the immunogen is recognized and eliminated. Active immunization can be induced by natural or artificial means. Natural active immunization occurs when a host is exposed to a pathogen, and

develops immunity against it. This immunity gives protection when re-exposed to the same pathogen. By contrast, artificial active immunization involves administration of a vaccine that contains a killed or a virulent form or an immunogenic component of a pathogen, designed to elicit protective immunity. On subsequent exposure to the infectious pathogen, it is recognized and eliminated, thereby affording protection against the disease [25].

2.2.1.1 Types of active immunization

2.2.1.1.1. Inactivated whole organism vaccines

Most bacterial vaccines are of this type. They include pertussis, cholera, typhoid and plague vaccines which are produced by killing the microorganisms with heat or by treatment with chemicals such as formaldehyde or phenol .

Non-living virus vaccines are available for the prevention of diseases caused by influenza, poliomyelitis (Salk) and rabies. These vaccines are prepared from viruses that grow in eggs; a continuous monkey kidney cell line, or human diploid fibroblasts, virus is then inactivated with formalin . Although the infectivity of the pathogen is destroyed by these treatments, much of their antigenic integrity remains [24].

2.2.1.1.2. Whole organism attenuated vaccine

Live, attenuated vaccines contain a version of the living microbe that has been weakened in the lab so it can't cause disease. Because a live, attenuated vaccine is the closest thing to a natural infection, these vaccines are good "teachers" of the immune system[31].

Although a number of viral vaccines are attenuated, such as, measles, mumps, rubella, vaccinia, varicella, yellow fever, influenza and oral polio. The only bacterial vaccines in this category are the bacillus Calmette-Guerin (BCG) strain of *Mycobacteriumbovis*, used to vaccinate against tuberculosis and oral typhoid vaccine [32].

A relatively small dose of virus or bacteria is given, which replicates in the body and creates enough virus or bacteria to stimulate an immune response which resembles the natural infection including humoral and cell mediated immunity, while inactivated

vaccines are not alive and can't replicate, they induce only humoral immunity and the protective immune response develops after the second or third dose [24].

2.2.1.1.3 Toxoids

For bacteria that secrete toxins, or harmful chemicals, a toxoid vaccine might be the answer [31].

Toxins can be inactivated to make harmless toxoids which are used for vaccination [33].It is the powerful toxins they produce that can cause illness [33].Administration of toxoids prepared from inactivated tetanus, botulism or diphtheria toxins elicit antibody response that neutralizes infection.

The toxicity is removed by the treatment with formalin, and the inactivated toxin always adsorbed to alum. Adjuvant which evokes high titers of antitoxic IgG antibodies toxoids are effective despite the fact that natural infection dose not always confer long-lasting immunity, presumably because the amount of toxin produced in infection may not be sufficient to elicit a strong immune response [24]. When the immune system receives a vaccine containing a harmless toxoid, it learns how to fight off the natural toxin. The immune system produces antibodies that lock onto and block the toxin [31].

2.2.1.1.4. Polysaccharide vaccines

The bacteria that cause some diseases, such as pneumococcal pneumonia and certain types of meningitis, have special outer coats. These coats disguise antigens so that the immature immune systems of infants and younger children are unable to recognize these harmful bacteria [34].

If a bacterium possesses an outer coating of sugar molecules called polysaccharides, as many harmful bacteria do, researchers may try making a conjugate vaccine for it [31].

Polysaccharide vaccines are a unique type of inactivated subunit vaccine

composed of long chains of polysaccharides. Pure polysaccharide vaccines are available for *Streptococcus pneumonia, Neisseria meningitides and Salmonella typhi*. Humoral immunity is important for protection against encapsulated pathogens, which are usually poorly immunogenic. Another problem with polysaccharide vaccines is that young chil-

dren tend not to mount antibody responses to polysaccharide antigens . An efficient way of overcoming this problem is to chemically conjugate bacterial polysaccharide to protein carriers, which provides immunogen that can be recognized by antigen specific cells, thus avoiding a T-cell independent response. By using this approach, various conjugate vaccines have been developed against *Haemophilus Influenzae* [24, 31]. an important cause of serious childhood chest infections and meningitis, and these are now
widely applied [24].

2.2.1.1.5. Synthetic peptide vaccines

Synthetic peptide vaccines are designed using the knowledge of the amino acid sequence of the protein antigen that elicits a protective immune response. In theory, synthetic peptide vaccines have the advantage that highly purified peptides may be made in large quantities and their simpler antigenic composition may afford protection with fewer side effects [33]. A problem in this type of vaccine is that peptides are not strongly immunogenic and it is particularly difficult to generate MHC class I- specific responses by *in vivo* immunization with peptides. One approach to solve the problem is to integrate peptides by genetic engineering into carrier proteins with a viral vector, such as hepatitis B core antigen, which are then processed *in vivo* through natural antigen- processing pathway . A second possible technique is the use of immune stimulatory complexes which are lipid carriers that act as adjuvants but have minimal toxicity. They seem to load peptides and proteins into the cell cytoplasm allowing MHC class-I restricted T-cell responses to peptides to develop , or by use of multiple repeating peptides to enhance immunogenicity [24].

Another disadvantage of peptide as vaccines is the configuration and recognition of peptides by immune responsive cells , Peptides usually present as linear determinants and are recognized by T cells in the context of MHC molecules and B cells, but the latter also recognize conformational determinants. Thus, the generation of B cell response against a protective conformational determinant may not be possible [28].

2.2.1.1.6. Anti-idiotype antibody vaccines

An antibody (idiotype) has idiotopes which are the hypervariable domains of the antibody molecule that serve as the paratope or antigen combining site.

Therefore, the idiotopes of the antibody is the mirror image of the epitope or antigenic determinant that it binds, when an antibody (AB1) is used to generate anti-idiotype antibody(AB2) the latter will structurally mimic the epitope, this is described as internal image of the epitope likewise, when an anti-idiotype antibody (AB2) is used to generate anti-idiotopes antibody (AB3) the latter recognized the AB2 idiotope because it is the mirror image of the epitope, similar to that of AB1 since the idiotopes of anti-idiotypic antibodies could be used in vaccine preparation as a substitute for the protective epitope(s) of pathogen. Anti-idiotypic antibodies are especially useful when the epitope(s) of the pathogen is difficult to identify or synthesize [25]. Anti-idiotype antibody vaccines are safe and free from the adverse side effects associated with vaccine preparations that contain whole cell pathogen or its components. A major disadvantage of anti-idiotype antibodies in vaccine preparation is that their immunogenicity is weak. Experimental anti-idiotype vaccines have been developed for Hepatitis B [24].

2.2.1.1.7. DNA immunization

The technique that is being tested in humans involves the direct injection of plasmids loops of DNA that contain genes for proteins produced by the organism being targeted for immunity. Once injected into the host's muscle tissue, the DNA is taken up by host cells, which then start expressing the foreign protein. The protein serves as an antigen that stimulate an immune responses and protective immunological memory develops [27].

Recombinant DNA technology provides the means for expressing protein antigens in large amounts for vaccine use .The gene that encodes the protective antigen is identified,

and then the gene is introduced into a suitable vector in which the desird recombinant protein is expressed in large quantities and then purified by chemical methods [24].

Types of vectors:

1- Viral and bacterial vectors

The gene that encodes the protective antigen is inserted into the genome of the attenuated microorganism, the inserted gene together with genome of the microorganism is transcribed and translated and the desired protective antigen along with other proteins of the microorganism is expressed. These vaccines elicit humoral and cell mediated immune response . Several viruses may be used for this purpose such as vaccinia, adenovirus and bacteria such as *Salmonella typhimurium*, *Escherichia coli* and *Bacillus Calmette-Guerin*[33].

2- Plasmid vectors

Vaccination with a plasmid encoding the DNA sequence for a protective antigen linked to a strong mammalian promoter can elicit an immune response to the expressed protein [32]. The plasmid vectors are taken up, usually by muscle cells, at the site of inoculation; they remain extra chromosomal within these cells and are transcribed and translated into the desired immunogen. The proteins expressed by nucleic acid vaccination are usually processed and presented by MHC class I pathway, and elicit cytotoxic

T cell responses. Helper T cell and antigen specific humoral responses are also elicited [24].

DNA vaccines have a group of advantages such as the absence of infection risk, greater stability relative to protein vaccines and could be useful for immunizing young children who still have maternal Abs . The feasibility of DNA immunization has now been demonstrated against several viral, bacterial, and protozoal infections in laboratory animals, several DNA vaccines are undergoing testing in humans to determine their usefulness in prevention or treatment of HIV, malaria and Hepatitis B. However, no DNA vaccines are currently used in humans [33].

2.2.1.1.8. Edible vaccines

Edible vaccines contain DNA fragments from the original pathogen. These fragments code for a protein that is usually a surface protein of the pathogen. This is responsible for eliciting the body's immune response [35].

The idea of edible vaccines, resulting from the expression of antigenic proterins in transgenic plants, comes from Arntzen and colleagues. In the first instance, the gene for HBsAg was inserted into cells of tobacco plants, and these produced antigen that was immunogenic on injection. The heat-labile enterotoxin of *E.coli* has been engineered into potatoes, which accumulate 1 mg per raw potato. The virus capsid antigen of Norwalk virus had been similarly engineered, and 5-g samples were fed to mice, which produced serum and secretory antibodies. Currently, efforts are being directed at the genetic engineering of bananas. The long term hope is the possibility of a multi subunit vaccine, including an oral adjuvant, which could be eaten, and could be cheap and acceptable in a third world setting [36].

2.2.2. Passive immunization

Passive immunity refers to the process of providing IgG antibodies to protect against infection; it gives immediate, but short-lived protection—several weeks to 3 or 4 months at most. Passive immunity is usually classified as natural or acquired . The transfer of maternal tetanus antibody (mainly IgG) across the placenta provides natural passive immunity for the newborn baby for several weeks/months until such antibody is degraded and lost [30].

Passive immunization is the administration of preformed antibodies produced by another individual or animal. This method of immunization provides protection against a pathogen or toxin without the need for a course of immunization The most common reason for the passive immunization of healthy immunocompetent individuals is exposure to a toxin or poison [25]. Passive immunization can occur naturally as is the case during transfer of antibodies through the colostrum or therapeutically when

performed antibody is administered for the prophylaxis or therapy of infectious disease such as diphtheria, botulinum and tetanus toxins, after known or presumed exposure to rabies . Immunocompromized or immune deficient individuals, who cannot mount humoral response, may also be given passive immunization. Children with hypogam-maglobulinemia, individuals with AIDS, patients receiving chemotherapy, and organ transplant recipients receiving immunosuppressive therapy cannot respond appropriately to natural infection or active immunization and, therefore may require passive immunization and supportive immunoglobulin transfusion to prevent severe infection [24].

A major disadvantage of passive immunization is its brief period of effectiveness, transfused immunoglobuins have a short half-life (typically 25days for IgG) and passive immunization does not evoke immunological memory in the recipient[25].

2.3. Pertussis, Measles, Mumps and Rubella Vaccine Preparation

Measles, rubella, and mumps vaccines are available in monovalent measles , rubella, or mumps form and in combinations: measles-mumps-rubella(MMR), measles-rubella (MR) , and rubella-mumps vaccines. Each dose of the combined or monovalent vaccines contains approximately 0.3 milligrams of human albumin, 25 micrograms of neomycin, 14.5 milligrams of sorbitol, and 14.5 milligrams of hydrolyzed gelatin. Live measles vaccine and live mumps vaccine are produced in chick embryo cell culture. Live rubella vaccine is grown in human diploid cell culture [22].

2.3.1.Measles

Measles virus was first isolated by John Enders in 1954 [22]. The first measles vaccines were licensed in 1963. In that year, both an inactivated (killed) and a live attenuated vaccine (Edmonston B strain) . were licensed for use in the United States.The inactivated vaccine was withdrawn in 1967 because it did not protect against measles virus infection [22].

Measles vaccine produces an in apparent or mild, non communicable infection. Measles antibodies develop among approximately 95% of children vaccinated at age 12 months and 98% of children vaccinated at age 15 months (CDC, unpublished data).

Studies indicate that, if the first dose is administered no earlier than the first birthday, >99% of persons who receive two doses of measles vaccine develop serologic evidence of measles immunity [37].

Although vaccination produces lower antibody levels than natural disease, both serologic and epidemiologic evidence indicate that the vaccine induces long-term probably lifelong-immunity, in most persons [38].

Distribution in the United States of a live, further attenuated vaccine (Schwarz strain) first introduced in 1965 has also ceased. A live, further attenuated preparation of the Enders-Edmonston virus strain that is grown in chick embryo fibroblast cell culture, licensed in 1968, is the only measles virus vaccine now available in the United States. This further attenuated vaccine (formerly called "Moraten") causes fewer adverse reactions than the Edmonston B vaccine [22].

2.3.2. Rubella Vaccine

The live rubella virus vaccine currently distributed in the United States is prepared in human diploid cell culture. This vaccine, containing virus strain RA 27/3, was licensed in the United States in January, 1979 and replaced previous rubella vaccines (e.g., HPV77 and Cendehill) because it induced an increased and more persistent antibody response and was associated with fewer adverse events [22].

In clinical trials, □95% of susceptible persons aged □12 months who received a single dose of strain RA 27/3 rubella vaccine developed serologic evidence of immunity. Clinical efficacy and challenge studies indicate that >90% of vaccinated persons have protection against both clinical rubella and viremia for at least 15 years. Follow-up studies indicate that one dose of vaccine confers long-term-probably lifelong-protection [39].

Although antibody titers induced by the vaccine are generally lower than those stimulated by rubella infection, vaccine-induced immunity protects, in nearly all instances, against both clinical illness and viremia after natural exposure[40]. In studies that attempted artificial reinfection of persons who received RA 27/3 vaccine, resistance to reinfection was similar to the resistance that follows natural infection [41]. However, several reports indicate that viremic reinfection following exposure may occur among

vaccinated persons who have low levels of detectable antibody . The frequency and consequences of this phenomenon are unknown but it is believed to be uncommon. Clinical reinfection and fetal infection among persons who developed immunity as a consequence of infection with wild virus have been documented, but are apparently rare. Rarely, clinical reinfection and fetal infection have been reported among women with vaccine-induced immunity [22].

Rare cases of Congenital Rubella Syndrom CRS have occurred among infants born to mothers who had documented serologic evidence of rubella immunity before they became pregnant [22].

2.3.3. Mumps Component

The only mumps vaccine now available in the United States is a live virus vaccine (Jeryl-Lynn strain) that is prepared in chick-embryo cell culture. The vaccine produces a subclinical, non communicable infection with very few side effects [22].

More than 97% of persons who are susceptible to mumps develop measurable antibody following vaccination and, in controlled clinical trials, one dose of vaccine was approximately 95% efficacious in preventing mumps disease . However, field studies have documented lower estimates of vaccine efficacy, ranging from 75% to95% . Antibody levels induced by the vaccine are lower than antibody levels resulting from natural infection [42]. The duration of vaccine-induced immunity is unknown, but serologic and epidemiologic data collected during 30 years of live vaccine use indicate both the persistence of antibody and continuing protection against infection [22].

2.3.4. Pertussis Component

Acellular Pertussis Vaccines

Acellular pertussis vaccines contain inactivated pertussis toxin (PT) and may contain one or more other bacterial components (e.g., filamentous hemagglutinin {FHA}, a 69-kilodalton outer-membrane protein -- pertactin {Pn}, and fimbriae {Fim} types 2 and 3). PT is detoxified either by treatment with a chemical (e.g., hydrogen peroxide, formalin and/or glutaraldehyde) or by using molecular genetic techniques.

Acellular pertussis vaccines contain substantially less endotoxin than whole-cell pertussis vaccines [43].

Since 1991, two acellular pertussis vaccines (Tripedia{Registered} and ACEL-IMUNE{Registered}) have been licensed for use in the United States. Until recently, both vaccines were licensed for use only as the fourth and fifth doses of the diphtheria, tetanus, and pertussis vaccination series among children aged 15 months-6 years who had received three primary doses of whole-cell DPT. This licensure was based on findings of studies conducted in Sweden and Japan. These studies did not evaluate the efficacy of acellular pertussis vaccines administered to infants on a schedule similar to the one used in the United States and did not directly compare the efficacy of DTaP vaccines with that of whole-cell DPT vaccines [43].

Four diphtheria and tetanus toxoids combined with whole-cell pertussis (DPT) vaccines are presently licensed for use in the United States. Vaccines of this type, prepared from suspensions of inactivated Bordetella pertussis bacterial cells, have been licensed for routine vaccination of infants since the mid-1940s. Based on controlled efficacy trials conducted in the 1940s and on subsequent observational efficacy studies, a primary series comprising four doses of whole-cell DPT vaccine is considered 70%-90% effective in preventing serious pertussis disease[43].

2.4. Techniques for measuring antibody response

2.4.1.Pertussis

Even though whole cell pertussis vaccine has been used successfully for several decades, there still is no reliable measure of immunity to pertussis. Although many of serological techniques have proven useful as diagnostic procedures, it is unclear whether any of them is sensitive and specific as a measure of immunity to pertussis. The bacterial agglutination test that has been used for many years does not necessarily correlate with immune status.

New assay techniques have been developed for the measurement of antibody to well-defined B. pertussis antigens that develop after immunization or natural disease. Their usefulness as a measurement of immunity is not yet proven [19].

2.4.1.1. Bacterial agglutination test

The bacterial agglutination (BA) test was the first method developed to measure pertussis antibody and it is still the most frequently used method. It uses a simple technique for measuring antibodies induced by the agglutinogens of the antigenic form of B.pertussis, designated phase I. Freshly recovered, encapsulated pertussis bacteria generally belong to phase I. Passage of pertussis bacteria in culture may result invariant forms, which are deprived of immunogenic antigens and designated as phase II, III, or IV organisms [19].

However, vaccination or recovery from pertussis does not always induce agglutinins and some individual slacking antibody are protected. The measured agglutinin titers seem to be markers of protection, rather than protective antibodies.

The BA test suffers from low sensitivity and it has not been standardized. The agglutinin titers strongly depend on the bacterial strain used in the agglutinogens. BA antibodies correlate best with IgG and IgA antibodies determined by the ELISA test [19].

2.4.1.2. Enzyme-linked immunosorbent assay

The enzyme-linked immunosorbent assay(ELISA) uses purified protein antigens of *B. pertussis* (such as FHA, PT, or AGG) to measure serum IgG, IgM, and IgA responses following disease or vaccination .The ELISA test is sensitive, specific, relatively cheap, and requires only a small amount of serum. However, the accuracy of the test depends on the purity of the antigens involved. With mixed preparations (whole bacteria, sonicate or extract of bacteria), it is not possible to identify the particular antigens to which the antibody response is directed [19].

2.4.1.3. In vitro neutralization test

The in vitro neutralization test (NT) is conducted in a microplate culture of Chinese hamster ovary (CHO) cells. PT induces a distinct cytopathogenic effect with clustering of CHO cells in the microplate culture. Only a small amount of PT (about1 ng) is needed to produce the clustering of CHO cells. This property of PT allows the in vitro neutralization test to measure antibodies neutralizing pertussis toxin .The NT is laborious, requires tissue culture facilities, and involves subjective readings. The NT is significantly less sensitive for the diagnosis of pertussis than the determination of the IgG response to PT by ELISA. Furthermore, not all patients develop measurable neutralizing antibodies after clinical and culture-confirmed whooping cough [19].

Techniques not widely used

There are other techniques to measure pertussis antibody response, but these are not widely used.

They include Passive protection of mice against *B. pertussis* infection by serum antibody. This method is expensive, requires mice, and is poorly reproducible.

An immunoblot technique in which antigens, separated electrophoretically, are allowed to react with antibodies that are then reacted with I^{125}-labelled antibody to human immunoglobulin and autoradiographed .

Indirect hemagglutination, bactericidal reaction, immune diffusion , and complement fixation [19].

2.4.2 Mumps

Plaque reduction neutralization (PRN) assay

Neutralizing anti-mumps virus antibody titers were determined by PRN assay [5]. The Plaque reduction neutralization test is used to quantify the titre of neutral lising antibody for a virus [44].

The serum sample or solution of antibody to be tested is diluted and mixed with a viral suspension. This is incubated to allow the antibody to react with the virus. This is poured over a confluent monolayer of host cells. The surface of the cell layer is covered in a layer of agar or carboxy methyl cellulose to prevent the virus from spreading indiscriminately. The concentration of plaque forming units can be estimated by the number of plaques (regions of infected cells) formed after a few days. Depending on the virus, the plaque forming units are measured by microscopic observation, fluorescent antibodies or specific dyes that react with infected cells [44].

Enzyme Immunoassay EIA assay

All sera were also tested with IBL (Hamburg, Germany) and Wampole Laboratories (Cranbury, New Jersey) mumps virus immunoglobulin G (IgG) EIA kits according to the manufacturers' instructions. Both manufacturers' assays for mumps virus IgG are based on capturing virus-specific human IgG on a preparation of purified virus antigen (derived from the mumps virus Enders strain) immobilized on plastic wells. In the Wampole assay, sera are diluted 1:21, whereas for the IBL assay, sera are diluted 1:101. For both assays, following incubation with sera, wells were washed three times in phosphate-buffered saline and incubated with anti-human IgG conjugated to horseradish peroxidase. After being washed, wells were incubated with tetramethyl benzidine substrate solution. The reaction was stopped by addition of H_2SO_4. Plates were then read on an Emax precision microplate reader (Molecular Devices, Sunnyvale, CA) at 450 nm using a reference wavelength of 650 nm. All reagents used were provided with the EIA kits. Absorbance value cutoffs and interpretation of results were carried out according to the manufacturer's instructions [5].

Additional EIA testing was carried out on a subset of 10 serum samples with neutralization dilutions greater than or equal to 1:32 that were diluted in phosphate-buffered saline to achieve PRN dilutions of 1:4 and 1:8 [5].

2.4.3 Measels

Serologic testing, most commonly by enzyme-linked immunoassay (ELISA or EIA), is widely available and may be diagnostic if done at the appropriate time. Generally, a previously susceptible person exposed to either vaccine or wild-type measles virus will first mount an IgM response and then an IgG response. The IgM response will be transient (1–2 months), and the IgG response should persist for many years. Uninfected persons should be IgM negative and will be either IgG negative or IgG positive, depending upon their previous infection history .

A variety of tests for IgG antibodies to measles are available and include ELISA, hemagglutination inhibition (HI), indirect fluorescent antibody tests, microneutralization, and plaque reduction neutralization. Complement fixation, while widely used in the past, is no longer recommended [45].

2.4.4 Rubella

Haemagglutination Inhibition (HI) TEST

The RubeHIT test is a standard haemagglutination inhibition assay .The sera were treated with kaolin to remove non specific inhibitors, and stabilised human 0erythrocyteswere used as indicator cells. Positive and negative control sera were supplied by the manufacturer[46].

Latex Agglutination (LA) TEST

The Rubalex test is an indirect latex agglutinationtest .Undiluted serum (25 Ml) was pipetted on to a test card. The same volume of a suspension containing latex particles coated with rubella virus antigen was added and mixed carefully with the serum, and the card was tilted for three minutes. The test was regarded as positive if agglutination was seen within this time [46].

ENZYME IMMUNOASSAY

The Enzygnost rubella assay is an indirect enzyme linked immunosorbent assay for detecting IgG class antibody to rubella virus.

A dilution of 1 in 40 of the serum was tested to assess the negative results obtained by the HI test and LA tests. Twenty two sera were screened and used to determine the cut-off value of LEIA [46].

LATEX ENZYME IMMUNOASSAY

The LEIA was performed with rubella virus antigen sensitised latex particles (Rubalex) as a solid phase immunoadsorbent. The principle was similar to that of a heterogeneous noncompetitive indirect immunoassay such as the ELISA [46].

Chapter 3:Materials and Methods

3.1. Study design and selection of subjects

Samples were obtained from apparently healthy children who had received the MMR vaccine, 3 primary doses of DPT vaccine and the fourth booster dose. The percent of DPT vaccine coverage in 2002 was 100% for the 3 primary doses and 99.3% for the booster dose according to the annual report of the Palestinian Ministry of Health [31]. Subjects were excluded if they had a major congenital defect or serious chronic illness, any confirmed or suspected immune suppressive or immunodeficient condition, receipt of immunoglobulin or blood product therapy.

Sample size was calculated according to the formula of :

$$ss = \frac{Z^2 * (p) * (1-p)}{c^2}$$

Z = Z value (e.g. 1.96 for 95% confidence level)

p = percentage picking a choice, expressed as decimal

(.5 used for sample size needed)

c = confidence interval, expressed as decimal

(e.g., .04 = ±4)

3.2. Ethical considerations

This study was conducted according to the good clinical practice permission and the declaration of Ministry of Education. Sample collection was conducted under the supervision of School Health Directorate and Ard El Insan Association. Before study enrollment, the purpose of the study was explained to the child's parents.

3.3. Materials

3.3.2. Reagents

There are four reagents used in the study:

1- ELISA kit for pertussis antibody IgG.

2- ELISA kit for measles antibody IgG.

3- ELISA kit for mumps antibody IgG.

4- ELISA kit for rubella antibody IgG.

3.3.3. Instruments for reading

ELISA reader (TC 89+) Teco Dignostics.

3.4. Serum samples

A volume of 3-4 ml of blood drawn by veinpuncture under aseptic conditions and serum was separated by centrifugation, each sample was distributed into 3 tubes and then stored at – 80$_{o}$C until the time of serological assay.

3.5. Assessment of pertussis, measles, mumps and rubella antibody titer

Before test performance a pilot study was made by selecting random samples from each age group and examined together with controls to master the technique.

3.5.1. Determination of Pertussis, Measles, Mumps and Rubella antibody level

The antibody titer was estimated in all serum samples by ELISA technique. The NovaTec (*Bordetella pertussis, Measles, Mumps and Rubella)* IgG-ELISA is intended for the quantitative determination of IgG class antibodies against (*Bordetella pertussis, Measles, Mumps and Rubella*) toxin in human serum. This allows the determination of the immune status of the subjects after vaccination.

3.5.1.1. Principle of the assay for (Pertussis, Measles, Mumps and Rubella)

The quantitative immunoenzymatic determination of IgG-class antibodies against *Bordetella pertussis, Measles, Mumps and Rubella* is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique. Microtiter strip wells were precoated with *Bordetella pertussis, Measles, Mumps and Rubella*/antigens to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material, horse radish peroxidase (HRP) labelled anti-human IgG conjugate was added.

This conjugate binds to the captured *Bordetella pertussis, Measles, Mumps and Rubella* antigens specific antibodies.

The immune complex formed by the bound conjugate was visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of (*Bordetella pertussis, Measles, Mumps and Rubella toxin)* specific IgG antibodies in the specimen. Sulphuric acid was added to stop the reaction. This produces a yellow endpoint color. The optical density (OD) at 450nm was read using an ELISA microwell plate reader.

3.5.1.2. Content of the kit

1- Microplate

One microplate of 12 strips of 8 breakable wells. The wells are coated with (*Bordetella pertussis, Measles, Mumps and Rubella)* antigens. Ready to use.

2- Conjugate

One vial of 20ml of solution containing peroxidase labeled rabbit antibody to human-IgG, coloured blue, ready to use.

3- Controls (Pertussis, Measles and Mumps)

The bottles labelled with positive, cut-off and negative Control contain a ready to use control solution. It contains 0.1% Kathon and has to be stored at 2-8C. After first opening stability until expiry date when stored at 2-8C.

3.1 Controls (Rubella)

The bottles labellad with Standard A, B, C and D contain a ready to use standard solution calibrated in accordance with the International Standard of the WHO. The concentration of the standards are:

Standard A : 0 IU/ml

Standard B : 10 IU/ml

Standard C : 50 IU/ml

Standard D : 100 IU/ml

4- Sample Diluent:

One vial containing 100 ml of phosphate buffer pH 7.2 ± 0.2., stabilizers, Preservatives and an inert yellow dye. It is used for the dilution of the patient specimen. Ready to use.

5- Washing Buffer 20X

One vial containing 50 ml of a 20-fold concentrated buffer, detergents and preservatives for washing the wells. pH 7.2 ± 0.2.

6- Substrate (TMB)

One vial containing 15ml 3, 3', 5, 5'tetra-methylbenzidine (TMB)/hydrogen peroxide system. Ready to use.

7- Stop Solution

The bottle contains 15 ml 0.2 M sulphuric acid solution (R 36/38). This ready to use solution has to be stored 2-8C

3.5.1.3. Assay procedure

Samples and controls were brought to room temperature (20-25°C) before starting the test run.

3.5.1.3.1. Sample dilution

Before assaying, all samples were diluted 1:100 with sample diluent. By dispensing 10µl sample and 1ml sample diluent into tubes to obtain a 1:100 dilution and thoroughly mixed with a Vortex.

3.5.1.3.2. Test preparation and procedure

A. Into the respective wells, 100 µl of each Controls and diluted samples were dispensed while one well was left for substrate blank.

B. Wells were covered with the foil supplied in the kit, and incubated for 1 hour ± 5 min at 37±1°C.

C. When incubation had been completed, the foil was removed, the content of the wells aspirated and each well was washed three times with 300µl of Washing Solution. At the end, the remaining fluid was carefully removed by tapping strips on tissue paper prior to the next step.

D. The conjugate was added in which 100µl (*Bordetella pertussis, Measles, Mumps and Rubella* anti-IgG) dispensed into all wells except for the blank.

E. Wells were covered with the foil and the plate was incubated for 30 min at room temperature.

F. Step C repeated.

G. The substrate was added (100µl TMB Substrate Solution dispensed into all wells).

H. Wells incubated for exactly 15 min at room temperature in the dark.

I. Finally, 100µl Stop Solution was dispensed into all wells in the same order and at the same rate as for the TMB Substrate Solution.

Any blue color developed during the incubation turned into yellow.

J. The OD of the specimen measured at 450/620 nm within 30 min after addition of the Stop Solution.

3.5.1.3.3. Calculation of Pertussis, Measles and Mumps antibody titers

The cut-off is the mean absorbance value of the Cut-off control determinations.

Example: Absorbance value Cut-off control 0.39 + absorbance value Cut-off control 0.37=0.76 / 2=0.38.

3.5.1.3.4. Results in NovaTec Unites

Patient (mean) absorbance value x 10 /cut-off = [NovaTec-Units=NTU]

Exampale: (1.216 x10)/0.38 = 32 NTU

Cut-off : 10 NTU

Negative = < 9 NTU

Positive = >11 NTU

3.5.4.3.3. Calculation of Rubella antibody titers

In order to obtain quantitative results in IU/ml, the (mean) absorbance values of 4 standards A, B, C and D was plotted against their corresponding concentrations (0 / 10 / 50 and 100 IU/ml) by the use of Excel software; which is a computer program that facilitates calculations, (absorbance values on the vertical y-axis, concentrations on horizontal x-axis), then results were calculated.

3.5.5.3.4. Results (Rubella).

Reactive: > 15 IU/ml

Gray zone: 10-15 IU/ml

Non reactive: <10 IU/ml

Chapter 4: Results

4. Data analysis

The present study included 184 children from age 2 to 13 years, "91 males and 93 females" (Table 3), the subjects were classified according to age into 4 age groups; (2-4) years, (5-7) years, (8-10) years and (11-13) years.

Data were coded numerically and entered on Statistical Package for Social Science (SPSS) and sorted according to sex and age. (Table 3).

Table 3. Shows the number and percentage of each category according to age and sex.

4.1 Table 3 : Age and Sex distribution of the study sample.

Age (years)	Sex		Total
	Male	Female	
2-4	27	25	52
	51.9%	48.1%	100
5-7	9	13	22
	40.9%	59.1%	100
8-10	26	19	45
	57. 8%	42.2%	100
11-13	29	36	65
	44.6%	55.4%	100
Total	91	93	184
	49.4%	50.5%	100

4.2 Antibody Level for all categories.

Results of table 4 showed that 70.5% of children bellow 13 years old were well immunized against Measles and had protective level of Measles antibody. (Table 4)

In addition, about 90.4% of children of 2-4 years age group were protected against measles and 9.6% were not protected , In 5-7 olds 66.7% were protected and 33.3% were not, in 8-10 olds 55.6% were protected and 44.4% were not, in 11-13 olds 66.2% were protected and 33.8% were not. There was a significant difference regarding measles after the MMR dose among different categories, (P=0.001)

Results showed that 68.2% of children bellow 13 years old were well immunized against Mumps and had protective level of Mumps antibody.

In table 4, about 84.6% of children of 2-4 years age group were protected against mumps and 15.4% were not protected , in 5-7 olds 76.2% were protected and 23.8% were not, in 8-10 olds 43.6% were protected and 56.4% were not, in 11-13 olds 67.2% were protected and 32.8% were not. There was a significant difference or regarding mumps after the MMR dose among different age categories, (P=0.00)

Results showed that 96.1% of children bellow 13 years old were well immunized against Rubella and had protective level of Rubella antibody .

As shown in table 4, about 100.0% of children of 2-4 years age group were protected against rubella and 0.0% were not protected , in 5-7 olds 100.0% were protected and 0.0% were not, in 8-10 olds 97.7% were protected and 2.3% were not, in 11-13 olds 90.6% were protected and 9.4% were not. There was a significant difference regarding rubella after the MMR dose among different categories, (P=0.037).

Results showed that 66.9% of children bellow 13 years old were well immunized against Pertussis and had protective level of Pertussis antibody .

As shown in table 4, about 75.0% of children of 2-4 years age group were protected against pertussis and 25.0% were not protected , in 5-7 olds 45.5% were protected and 54.5% were not, in 8-10 olds 62.5% were protected and 37.5% were not, in 11-13 olds 70.7% were protected and 29.3% were not. There was no significant difference regarding pertussis after the DPT dose among different categories, (P=0.077)

4.2.Table 4 : Antibody levels for all categories .

Age (years)	Measels Antibodies		Total	Mumps Anti-bodies		Total	Rubella Anti-bodies		Total	Pertusis Anti-bodies		Total
	P	N		P	N		P	N		P	N	
2-4	47	5	52	44.0	8.0	52.0	52.0	0.0	52.0	39.0	13.0	52.0
	90.4%	9.6%	100 %	84.6%	15.4%	100 %	100 %	0.0%	100 %	75.0%	25.0%	100 %
5-7	14	7	21	16.0	5.0	21.0	21.0	0.0	21.0	10.0	12.0	22.0
	66. 7%	33.3%	100 %	76.2%	23.8%	100 %	100 %	0.0%	100 %	45.5%	54.5%	100%
8-10	25	20	45	17.0	22.0	39.0	43.0	1.0	44.0	25.0	15.0	40.0
	55. 6%	44.4%	100 %	43.6%	56.4%	100 %	97.7%	2.3%	100 %	62.5%	37.5%	100%
11-13	43	22	65	41.0	20.0	61.0	58.0	6.0	64.0	41.0	17.0	58.0
	66.2%	33.8%	100 %	67.2%	32.8%	100 %	90.6%	9.4%	100 %	70.7%	29.3%	100%
Total	129	54	183	118.0	55.0	173.0	174.0	7.0	181.0	115.0	57.0	172.0
	70.5%	29.5%	100 %	68.2%	31.8%	100 %	96.1%	3.9%	100%	66.9%	33.1%	100%
P value	.001			.000			.037			.077		

P= Positive N=Negative Significant level P<0.05

4.3.Table 5 : Measles Antibodies levels according to age categories

Results in table 5 show that 35.9% of children from (2-13) years not protected against measles and 64.1% of children (2-13) years were protected .There was significant difference between measles antibodies levels according to age categories , (P=.000) .
Table 5 : Measles Antibodies levels according to age categories

Age (year)	Measles Ab_cat (NTU)				Total
	0-10.9	11-30	31-50	> 50	
2-4	5	13	19	15	52
	9.6%	25.0%	36.5%	28.8%	100.0%
5-7	52	12	1	0	22
	100.0%	54.5%	4.5%	.0%	100.0%
8-10	24	20	0	1	45
	53.3%	44.4%	.0%	2.2%	100.0%
11-13	28	33	3	1	65
	43.1%	50.8%	4.6%	1.5%	100.0%
Total	66	78	23	17	184
	35.9%	42.4%	12.5%	9.2%	100.0%
P value	.000				

NTU=NovaTec-Units Significant level P value <0.05 Ab=Antibody

4.4 Table 6: Mumps Antibodies levels according to age categories

Results in table 6 shows that 42.4% of children from (2-13) years not protected against mumps and 57.6% of children (2-13) years were protected .There was significant difference between mumps antibodies levels according to age categories , (P=.000) .

Table 6: Mumps Antibodies levels according to age categories

Age	Mumps Ab_cat (NTU)				Total
	0-10.9	11-20	21-30	>30	
2-4	10	19	12	11	52
	19.2%	36.5%	23.1%	21.2%	100.0%
5-7	7	7	6	2	22
	31.8%	31.8%	27.3%	9.1%	100.0%
8-10	29	11	3	2	45
	64.4%	24.4%	6.7%	4.4%	100.0%
11-13	32	23	7	3	65
	49.2%	35.4%	10.8%	4.6%	100.0%
Total	78	60	28	18	184
	42.4%	32.6%	15.2%	9.8%	100.0%
P value	.000				

NTU=NovaTec-Units Significant level P value <0.05 Ab=Antibody

4.5.Table 7 : Rubella Antibodies levels according to age categories

Results in table 7 show that 5.4% of children from (2-13) years are not protected against rubella and 94.6% of children (2-13) years were protected .There was significant difference between rubella antibodies levels according to age categories , (P=.000) .

Table 7 : Rubella Antibodies levels according to age categories

Age (year)	Rubella Ab_cat (NTU)				Total
	0-14.9	15-55	56-96	>96	
2-4	0	12	7	33	52
	.0%	23.1%	13.5%	63.5%	100.0%
5-7	1	5	6	10	22
	4.5%	22.7%	27.3%	45.5%	100.0%
8-10	2	27	7	9	45
	4.4%	60.0%	15.6%	20.0%	100.0%
11-13	7	36	8	14	65
	10.8%	55.4%	12.3%	21.5%	100.0%
Total	10	80	28	66	184
	5.4%	43.5%	15.2%	35.9%	100.0%
P value	.000				

NTU=NovaTec-Units Significant level P value <0.05 Ab=Antibody

4.6. Table 8: Pertussis Antibodies levels according to age categories

Results in table 8 show that 40.8% of children from (2-13) years are not protected against pertussis and 59.2% of children (2-13) years were protected .There was no significant difference between pertussis antibodies levels according to age categories , (P=.555)

Table 8: Pertussis Antibodies levels according to age categories

Age (year)	Pertussis Ab_cat (NTU)				Total
	0-10.9	11-40	41-70	>70	
2-4	15	26	7	4	52
	28.8%	50.0%	13.5%	7.7%	100.0%
5-7	12	7	1	2	22
	54.5%	31.8%	4.5%	9.1%	100.0%
8-10	21	18	4	2	45
	46.7%	40.0%	8.9%	4.4%	100.0%
11-13	27	30	6	2	65
	41.5%	46.2%	9.2%	3.1%	100.0%
Total	75	81	18	10	184
	40.8%	44.0%	9.8%	5.4%	100.0%
P value	.555				

NTU=NovaTec-Units Significant level P value <0.05 Ab=Antibody

4.7. Table 9 : Antibody levels against Mumps according to sex

Results show that Mumps vaccine efficacy was 63.6%in male and 72.9% in female, there was no significant difference between male and female (P=0.125)

Table 9 : Antibody levels against Mumps according to sex

Sex	Mumps_Ab		Total
	Positive	Negative	
Male	56	32	88
	63.6%	36.4%	100.0%
Female	62	23	85
	72.9%	27.1%	100.0%
Total	118	55	173
	68.2%	31.8%	100.0%
P- value	0.125		

Significant level P value <0.05

4.8. Table 10 : Antibody levels against Rubella according to sex

Results show that Rubella vaccine efficacy was 97.8%in male and 94.5% in female, there was no significant difference between male and female (P=0.227)

Table 10 : Antibody levels against Rubella according to sex

Sex	Rubells_Ab		Total
	Positive	Negative	
Male	88	2	90
	97.8%	2.2%	100.0%
Female	86	5	91
	94.5%	5.5%	100.0%
Total	174	7	181
	96.1%	3.9%	100.0%
P- value	0.227		

Significant level P value <0.05

4.9. Table 11 : Antibody levels against Measles according to sex

Results show that Measles vaccine efficacy was 67.8%in male and 73.1% in female, there was no significant difference between male and female (P=0.264)

Table 11 : Antibody levels against Measles according to sex

Sex	Measels_Ab		Total
	Positive	Negative	
Male	61	29	90
	67.8%	32.2%	100.0%
Female	68	25	93
	73.1%	26.9%	100.0%
Total	129	54	183
	70.5%	29.5%	100.0%
P- value	0.264		

Significant level P value <0.05

4.10. Table 12 : Antibody levels against Pertussis according to sex

Results show that Pertussis vaccine efficacy was 64.7%in male and 69.0% in female, there was no significant difference between male and female (P=0.333)

Table 12 : Antibody levels against Pertussis according to sex

Sex	Pertussis_Ab		Total
	Positive	Negative	
Male	55	30	85
	64.7%	35.3%	100.0%
Female	60	27	87
	69.0%	31.0%	100.0%
Total	115	57	172
	66.9%	33.1%	100.0%
P- value	0.333		

Significant level P value <0.05

Chapter 5: Discussion

This study focused on the humoral immune response to measles, Mumps, rubella (attenuated viruses) and pertussis (killed bacteria), this was accomplished by measuring antibody levels for these antigens in the sera of children younger than 13 years old, who were classified into 4 different age groups.

Since the integration of immunization programs in Gaza, the vast majority of children have been vaccinated against the major vaccine preventable diseases, including BCG, DPT and measles. The trends in mortality and morbidity from these diseases have subsequently decreased dramatically [24].

In Palestine, immunization coverage remains high. Based on the reports received from our preventive medicine department in PHC, the average coverage rates were more than (99%) for all vaccines, which directly impacts on the reduction in the incidence of vaccine preventable diseases.[47]

Palestine is considered as one of the countries which is about to achieve this goal of elimination of measles. The Incidence of measles still under control, approximately one to three cases reported yearly in the last several years.

In the year 2006, only one case was reported. While no reported cases in 2008, where one case was reported in Ramallah Governorate during 2009, and one case reported in Jerusalem during 2010, where no cases were reported during 2011[47].

World Health Organization criteria for elimination of measles have been implemented in Palestine. Since December 2003, there was a large outbreak of mumps in the Nablus Governorate that affected children, mainly below 15 years of age, of whom (72.9%) were previously immunized. In total, more than (4,000) children, both refugees and non-refugees, were affected. The outbreak reached its peak during April and May 2004, subsided there after, but spread out to other districts in the West Bank. The mumps

outbreak was attributed to possible breakdowns in the public sector cold chain system due to frequent power cuts [47].

In the year 2006, there was a notable decrease in reported cases of mumps in the West Bank, wherein (260) cases were reported with an incidence rate of (7.8 per 100,000) compared with (192.8 per 100,000) in the year 2005 [47].

5.1. MEASLES

5.1.1. Efficacy of measles vaccination

The efficacy of measles vaccination which represents the percentage of children who have antibody levels equals to or more than the protective level . About 70.5% of children in the different age groups protected and 29.5% of children are not protected, this finding is lower than the efficacy obtained by Syed M. Akramuzzaman et al [48] who evaluated the measles vaccine effectiveness in several age groups in the Dhaka, Bangladesh, which estimated at 80%.

Christopher R Sudfeld et al [49] investigated effectiveness of measles vaccination and vitamin A treatment and found that the vaccination was 85% effective in preventing measles disease.

Some researchers estimated vaccine effectiveness in group of children, it was 90% for one vaccine dose [50].

In the study of some authors measles vaccine efficacy in highly vaccination population was highly effective in preventing infection 95% [51].

Group of researchers compared between efficacy of one dose MMR at 12 months of age to monovalent measles vaccination at 9 months followed by MMR revaccination at 15 months of age [52].seroconversion and clinical protection rates were significantly higher in children who received only MMR at 12 months of age than in children revaccinated at 15 months of age. Seroconversion rate for measles was 69.9% in children who received MMR at 12 months of age and 90.3% in children revaccinated at 15 months of age (P=0.0003). While there was no measles case in children who were revaccinated, 12 (2.7%) children in the first group acquired measles during the follow-up period.

Vaccination at 12 months of age appeared to be better than the current national standard. The late elimination of maternal antibodies and the inhibitory effect of a weak antibody response after the first dose of vaccine at 9 months may explain the better immunogenicity and efficacy of the MMR vaccine given at 12 months of age.

Other studies determined the efficacy of measles vaccine in united States during 1989 and 1990 , vaccine efficacy was 95% [53]

Results of our study are lower than the efficacy obtained by other researchers who studied safety and immunogenicity of a measles-mumps-rubella-varicella vaccine given as a second dose in children up to six years of age. seropositivity rates were 96.4% for measles[54].

A group of researchers studied long- term persistence of antibodies after one or two doses of MMR vaccine for 160 students (17-23) years. The proportion of subjects with positive antibody titer was higher in those who received two vaccines against measles (77.1% versus 58.7% p=0.05) [55].

All above studies show that the efficacy of measles vaccine is higher than efficacy vaccine in our study except the last study.

The different between our results and others may be explained on the basis that nutritional status of our children is not as good as others, this may affect the immune response of our children. It is well known that malnutrition in the main cause of immunodeficiency worldwide.

5.2. MUMPS

5.2.1. Efficacy of mumps vaccination

In our study 68.2% of children in the different age groups protected and 31.8% of children are not protected, this finding is in agreement with the study of long- term persistence of antibodies after one or two doses of MMR vaccine for 160 student (17-23) years. The proportion of subjects with positive antibody titer was higher in those who received two vaccines dose mumps (67.5% versus 55.6% p=0.009)[55].

Our study is indisagreement with other study which investigated and immunogenicity of a measles-mumps-rubella-varicella vaccine given as a second dose in children up to six years of age. seropositivity rates were 94.3% for mumps, 99.5% for measles and 100% for rubella [54]

A group of researchers estimated the effectiveness of the mumps component of the measles , mumps , rubella (MMR) vaccine by using the screening method from January 2004 through March 2005,vaccine effectiveness was 88% for one dose and 95% for 2 doses. The effectiveness of 1 dose declined from 96% in 2-year-olds to 66% in 11- to 12-year-olds, and the effectiveness of 2 doses declined from 99% in 5- to 6-year-olds to 86% in 11- to 12-year-olds ($p<0.001$ for 1 or 2 doses).Waning immunity may contribute to mumps outbreaks in older vaccinated populations.[56]

In another study Two-dose vaccine effectiveness was 76–88% with no significant difference for attack rates between one and two doses. Among two-dose vaccine recipients,74% of the population (1482/2009) and 79% of the case-students (75/95) had received the second dose >10 years before.

A large mumps outbreak occurred despite high two-dose vaccination coverage in a population most of whom had received the second dose >10 years before. Two-dose vaccine effectiveness was similar to previous one-dose estimates [57].

A group of researchers who studied from October 1988 to April 1989, a large mumps outbreak occurred in Douglas County, Kansas. Of the 269 cases, 208 (77.3%) occurred among primary and secondary school students, of whom 203 (97.6%) had documentation of mumps vaccination. vaccine effectiveness among Douglas County junior high school students was estimated to be 83% [58]

The differece between this study and previous ones may be attributable to defect in cold chain which may affect the efficiency of vaccines and consequently will have negative effects on the efficacy.

5.3. RUBELLA

5.3.1. Efficacy of rubella vaccination

In our study 96.1% of children in the different age groups were protected and 3.9% of children were not protected, is lower than the study of Greaves WL et al [59] who investigated clinical efficacy of rubella vaccine in 83 cases high school students which estimate 90% and these results indicate that rubella vaccine is highly effective in preventing clinical rubella and do not support proposals for routine revaccination.

The study compared rubella antibody persistence after immunization in 16 years follow up in the Hawaiian Islands and found seropositive rates of 92.4% and 96.4% of(protective level) and (lowest detectable level) IU/ml, respectively. The seropositive rates were not related to reinfection or reimmunizations. These findings indicate that vaccine-induced rubella antibodies are detectable in almost all persons up to 16 years after successful vaccination [60].

A group of researchers investigated the persistence of measles , mumps, and rubella antibodies in an MMR-Vaccinated Cohort and found the seropositivity for rubella to be 100% [61].

Also another studies tested for rubella antibodies 13-17 years later and found that 98% were seropositive [62].

In one of studies MMR antibodies measured by ELISA for 4-6 and 11-13 children .

Rubella ELISA seropositivity was 90% in 4-6 years compared to 67% in 11-13 years old p(<0.01) which indicate waning immunity [63]

Reasearchers who estimated serum levels of rubella specific antibodies in Swedish women fillowing 3 decades of vaccination programmes,95.8% of all women had anti- rubella IgG levels > or = 10 IU/ml [64]

All previous studies are consistent with the results of this study for the efficiency of the rubella vaccine , and which shows that the efficiency of the vaccine is very high and this thing gives a good impression to avoid contracting this disease. People in our area com-mitted to vaccination and it appears that immunodeficiency among female in minimal.

5.4. PERTUSSIS

5.4.1. Efficacy of pertussis vaccination

In our study 66.9% of children in the different age groups protected and 33.1% of children are not protected, this finding is low compared with the study which found that vaccine efficacy was high but differed between epidemic (87%) and non- epidemic (93%) periods (p=0.03) [65]

a group of studies found vaccine efficacy to be 85% for children vaccinated with three doses of a whole-cell pertussis vaccine [66].

A group of researchers studied the efficacy of whole cell pertussis immunization .It was from 84-100% in six different retrospective analyses or outbreak investigations and a protective efficacy of 92% by clinical trial [67].

Some authors reported that efficacy of the vaccine was calculated fell from 100% in the first year to 46% in the seventh, being 84% in the fourth and only 52% in the fifth [68].

In other group of researchers where calculated of the effectiveness of the pertussis vaccination programe in the United States, which made by using the screening method, indicated that the overall effectiveness of 3 doses of pertussis vaccine against clinical disease was 82% [69].

Some authors reported that vaccination at 12 months of age appeared to be better than the current national standard. The late elimination of maternal antibodies and the inhibitory effect of a weak antibody response after the first dose of vaccine at 9 months may explain the better immunogenicity and efficacy of the MMR vaccine given at 12 months of age [52].

The results of this research is different from all the previous studies, where the efficiency of the pertussis vaccine in all previous studies were too high and far from the results obtained in this research, which shows us that the efficiency of the vaccine is very low and does not give immunity and protection for children under the age of 13 from a disease cough whooping dangerous.

Differences in the efficacy and mean antibody levels of our study and other studies may be due to several factors, Storage and distribution of vaccines may affect the validity of vaccine; all vaccines are sensitive biological substances and lose their potency, i.e. their ability to give protection against disease, with time. The rate of loss increases as vaccines are exposed to higher temperatures [24].

In order to maintain their efficacy, vaccines must be continuously stored at the appropriate temperature which is(2-8)OC for DPT and MMR from the time they are manufactured until the moment of use. Once potency is lost it cannot be regained or restored. Without proper care a vaccine may eventually lose its entire potency. If this occurs, the vaccine no longer provides any protection against the disease and is useless. In some cases, heat exposure leads to loss of potency [24].

The cold chain is the system for keeping and distributing vaccines in good condition.

It consists of a series of storage and transport links, all of which are designed to keep the vaccine at the correct temperature until it reaches the user [70].

BCG, measles, MR, MMR and rubella vaccines are equally sensitive to light (as well as to heat). Normally, these vaccines are supplied in vials made from dark brown glass, which gives them some protection against light damage, but care must still be taken to keep them covered and protected from strong light at all times [70].

Some vaccines are also sensitive to low temperature; freezing or exposure to temperatures below 0°C which can cause loss of potency and the vaccines become useless. Therefore, it is essential to protect them not only from heat but also from freezing [70].

DPT is considered the third most sensitive vaccine for high and low temperatures as well as light, while the other forms of vaccine (DT, TT and Td) are less sensitive [70].

Taking into consideration that the electricity in Gaza is disconnected frequently, this may explain in part the relatively low efficacy of measles, mumps and pertussis vaccine.

There are some other factors including some frequently observed program errors such as, too much or too little vaccine in one dose or immunizations given in wrong part of body and variability in vaccine synthesis and preparation [70].

5.5 Efficacy versus age groups

The subjects children were classified into 4 age groups (2-4), (5-7),(8-10) and (11-13) years old, children in this group completed the three primary doses of DTP vaccination at 2, 4, 6 months of life and received a booster dose at 12 months and first dose of MMR vaccination at 15 months of life and second dose at 6 years .

We can observe from Table (4) the efficacy of the four vaccines in all groups were gradually declining with age.

some authors reported that immunity following vaccination appears to wane over time. Waning immunity plays an important role in the occurrence of pertussis in older age group [69].

The pertussis vaccine or its schedule of use does not seem to provide sufficient herd immunity to prevent outbreaks of whooping cough. Matters might be improved if vaccination against pertussis were included in the preschool immunization programme [71].

A group of authors estimated the effectiveness of vaccination with whole-cell pertussis vaccine by age group in Poland 1996-2001and examined changes in the effectiveness of pertussis vaccination in 4 age groups during 1996- 2001, using surveillance data. He found that over that period a decrease occurred in the reported effectiveness (in children aged 2 to 5 y, from 97.3% in 1996 to 73.5% in 2001 and in 6 to 9 years olds, from 84.3%, to 68.8%) [72].

5.6 Booster dose

As shown in table (2) efficacy for measles, mumps, rubella and pertussis vaccination was (70.5%, 68.2%, 96.1% & 66.9%) respectively which represents the efficacy in all age groups, while efficacy in the last age group was (66.2%,67.2%, 90.6% &70.7%) respectively which is low.

On the other hand, these results were obtained by ELISA and may be lower when performed by other reference methods such as in vivo neutralization technique.

Moreover, efficacy gradually declines with age. Hence, there is a need to booster dose at the age of 13-14 year.

5.7. Efficacy and sex

As shown in Table (7) among all children 70.5% of subjects have protective level for mumps attenuated virus , the percentage of protected female was72.9% which is 9.3% higher than protected male(63.6%) but the difference between male and female is not significant (p=0.125). And measles antibody level was 67.8 % for male versus 73.1% female,(p value=0.264) which indicates that there is no significant difference. For rubella the percent protected female was 94.5% versus 97.8% males, (p value=.227)

actually it is difficult to determine the significance because7 cases only had antibody titers less than the protective level. And finally as shown in Table (10) percent of protected female for pertussis was 69.0% versus 64.7%, (p=0.333) .Hence, there is no significant difference in efficacy between male and female, because they receive the same vaccine and the same dose, and there is no difference between male and female in the immune response.

Chapter 6:

6.1. Conclusion

Finally we conclude that the efficacy of Rubella vaccination among children below 13 years in Gaza is very high (96.1%), while that of Measles, Mumps and Pertussis was little lower (70.5%, 68.2%,66.9%) respectively. Although, efficacy for measles, mumps and pertussis is lower but it's valuable, this indicates that vaccination against measles, mumps, rubella and pertussis has an important role in protection against diseases. On the other hand, we can recognize that vaccination efficacy and antibody levels in older children is lower than the younger ones, this could be noted clearly from the significant difference between the age groups, furthermore, relatively low antibody levels were elicited by primary immunization, which means that vaccine may not give a long term protection; hence, children over of 13 years need a booster dose.

In addition, our study shows that there is no significant difference between male and female in efficacy. This means that the effect of measles, mumps, rubella and pertussis vaccination in both sexes is the same.

6.2. Recommendations

- It's necessary to give a booster dose at the age of 13-14 years.

- There is a need for serologic monitoring of the whole vaccination program in Gaza Strip due to the instability of sociopolitical conditions which affect the validity of vaccines.

- Other studies should be conducted to cover other age groups to determine where we stand from herd immunity.

- Continuously monitor the cold chain preservation

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جمعية أرض الإنسان
ARD EL INSAN - PALESTINE 1984

السيد ولي أمر الطفل : .....

بعد التحية...

تقوم باحثة بالجامعة الإسلامية بإشراف جمعية أرض الإنسان الفلسطينية الخيرية بعمل دراسة حول كفاءة التطعيم المعطى للأطفال ضد بعض الأمراض الخطيرة وستقوم الباحثة بجمع عينات دم عشوائية لهذا الغرض لذلك نرجو التكرم من سيادتكم بالموافقة على سحب عينة من دم طفلكم (3-4) والمخصص للبحث العلمي فقط ولفائدة عموم أطفالنا .

ولكم جزيل الشكر

اسم ولي الأمر:..... رقم الهاتف أو الجوال : .....

رقم بطاقة الهوية : ..... ختم وتوقيع الجمعية

توقيع ولي أمر الطفل : .....