Immunogenicity of Type v Capsule of Streptococcus Agalactiae with Diphtheria Toxoid Conjugate in Model Mouse, as a Vaccine Candidate

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**ABSTRACT**

Streptococcus agalactiae is a diplococci gram-positive bacterium without acid-fast property, which do not forms spore, is non-motile and has no catalase activity. The bacteria is in a doubled form with spore short chains and also has got group B Lancefield antigen. Group B of Streptococcus is the major cause of sepsis and meningitis in newborns and also is common in pregnant and non-pregnant women. Objective: The aim of this study was to evaluate the immunogenicity of type V Streptococcus agalactiae capsule polysaccharide bound to diphtheria toxoid as a conjugated vaccine, in mice. Methods: For this purpose, first, lyophilized strain of Streptococcus agalactiae type V which V was prepared by the research Campus of Azad University of Zanjan, was cultured in the Todd-Hewitt broth medium, its capsule was obtained by precipitation in cold ethanol, then was dialyzed and also depolymerized using acetic acid. In order to improve the immunogenic properties, the purified antigen as a spacer, using ADH and EDAC as a linker were attached to diphtheria toxoid. The resulting conjugate was purified using Sepharose CL-2B gel filtration column. Pyrogenicity tests revealed that the resulting conjugate is devoid of toxicity and pyrogenicity effect. Then four groups of BALB/c mice were selected and vaccinated was performed as three intraperitoneal doses with two weeks intervals. Serum samples were collected and the antibody response against capsular antigen was measured using ELISA for IgM, IgA, IgG1, IgG2a, IgG2b, IgG3 and Totall IgG. Conclusion: The results of CPS-DT and pure CPS antigen titers resulted to rise in antibody titer, for all groups of antibody. Also the CPS-DT showed higher titers, compared to pure CPS in all groups of antibody. But after the second and third doses of CPSV_DT, a significant increase was observed in all antibody titers, against CPSV. The obtained results of anti-CPS for total IgG, IgG1, IgG2A, IgG2b, IgG3, IgM and IgA, was as CPS V-DT> CPSV> DT, respectively. Rise in IgG antibody titer against CPSV was more, that it was as IgG1> IgG3> IgG2a> IgG2b, respectively. These results suggest that type V capsular polysaccharide of S. agalactiae, in conjugated form with diphtheria toxoid antibody, causes increase in antibody titers.

**INTRODUCTION**

The Streptococcus term was first established in 1874 by Bill Roth for the cocci bacteria with chain arrangement, observed in erysipelas lesions and infected wounds. In the 1879, Pastor obtained similar organisms in the blood of patients with puerperal fever. The first species of this genus, Streptococcus pyogenes, was described by pasteur and Rozenbach. Flysen in 1882, induced erysipelias in volunteers by experimentally inoculating of Streptococcus. In 1923, Dick induced scarlet fever in volunteers by hemolytic streptococcus. In 1932, Todd, indicated anti streptolysin O in streptococcal infections [1,2,3,4,5,6].

Streptococcus agalactiae first was isolated by Lancefield and Harr, from vaginal culture of asymptomatic women and located in Lancefield group B. Streptococcus agalactiae is colonized in the vagina of 15 to 40 percent of pregnant women and is the main cause of sepsis and meningitis in infants, and also will lead to loss of the placenta, preterm delivery and Corioamnionitis in pregnant women. Since the sensitivity of infants to Group B of Streptococcus, is due to immature neonatal phagocytic system, and humoral immunity, some efforts is ongoing, to develop vaccines against the bacteria and vaccinating of pregnant women to induce the production
of antibodies in the mother’s body and then transfer it through the placenta, to the baby, before birth and through breast milk to the infant and thereby its immunization against these bacteria.

All strains of group B Streptococcus have polysaccharide capsule on their surface, which is considered as the most pathogenic factor and this component is used to manufacture of vaccines [1,2]. By 2 to 4-fold increase in infections caused by Group B Streptococcus in nonpregnant adults, especially the elderly over 65 years, it has created a major concern. So the use of vaccines for the immunization of this age group, could play an important role in prevention of the infections caused by these bacteria play[2].

A, B, C, D, and G leaders of, are the most common leaders which are found in association with human infections. These bacteria are saprophytes which are also found in milk and milk products [1-2]. Group B streptococci are shaped like other hemolytic streptococci but unlike Group A, C & G, which usually create a long chain in liquid medium, would be seen in doubled form or by short chain [1,7].

C carbohydrates which are often teichoic acid, are identified by type-specific antibodies. About 21 groups of C carbohydrates were identified which are indicated as A to U. According to Lancefield Classification, most human streptococcal disease are caused primarily by beta-hemolytic streptococci group A [Streptococcus Pyogenes], secondarily by streptococcus group B [Agalactiae], and finally by organisms of Group D [enterococci, especially Fecalis] [3, 8,9]. Streptococci are aerobic and optimal anaerobic. Their metabolism is by fermentation and its main product is Lactic acid. Their growth is in a N environment is slow, but if some of serum or blood is added to the culture medium, and be exposed to 10% carbon dioxide, they will grow better [7]. The Streptococci which are human pathogens, need many different kinds of growing factors. Most Streptococci for optimum growth need to temperature of 37 °C, but enterococci grow well, at temperatures between 15 and 45 °C. In addition, the enterococci in a medium with a high concentration of sodium chloride (6.5%) or 0.1% of Methylen blue or Bile Esculin Agar, would grow well, that this feature is used to differentiate enterococci from other streptococci [2]. Group B of Streptococci, are morphologically quite like any other streptococci. Their colony might be a bit larger than S. pyogenes colonies and produce orange or reddish brown pigment [in anaerobic conditions]. On blood agar, they form opaque, gray to white colonies, covered a thin zone of beta hemolysis. Approximately 5-10% of strains are Non-hemolytic. Group B streptococci hydrolyze Sodium hippurate and has got positive CAMP test. They are also resistant to bacitracin disk and also are tolerated to 6.5 percent of salt; that by this specification could be differentiated from other streptococci [1,7,9].

CAMP Factor [Christie, atkins and munch- Peterson factor]  
This compound is an extracellular proteins and, is also secreted by C, F, G, P and V groups, in addition to B Streptococci. CAMP factor causes membrane lysis of S.aureus Beta-hemolysin [Betatoxin] treated erythrocyte. In order to test the above phenomenon on blood agar, culture Strep B perpendicular to the Staphylococcus aureus, so that they do not intersect each other [4,5]. Capsule of group B Streptococcus is the type-specific antigen and is made of Polysaccharide, which is immunogenic and one of the vaccine candidates. Capsule of B Streptococcus is the most important indicator of the virulence of the bacteria and serologically is divided to nine types: Ia, Ib and II to VIII . At last, the C capsule type like Ib c were also considered, but as mentioned in the C protein issue, the compound is not a polysaccharide and is a protein, so it has no relation with the structure of the bacterial capsule [4,5].

CPS of types Ia, Ib and III, has go five repeated sugar units which including: galactose, glucose and N-acetyl glucosamine and Sialic acid in the ratio of 1: 1:1:2 respectively, while type V, has seven repeating sugar units including D-glucose, D-galactose, two acetamids, 2-D-glucose and Sialic acid in the M ratio of 1: 2: 3: 1. It's why the V capsular type contains more complex capsular antigens, than other types.

In addition, types IV and VIII contain six repeated sugar units and type VIII polysaccharide contains four repeated sugar units. No immunological cross-reaction were observed between type V with types Ia , Ib, II, III and IV. In the diffusion test on Ouchterlony agar the precipitation line could be observed between type V antiserum and Type V polysaccharide that the precipitation line were not observed between type V antiserum with other Ia, Ib II, III and IV polysaccharides. Natural strains react with C3bi and bind to it, while the mutant strains which are lacking capsule or salicylic acid in their structure react with active form of C3, that is the C3b [4,5,10].

In all capsular types except type V, production of antibodies, would be dose-dependent. GBS conjugated vaccines compared to injection of pure capsular polysaccharide, creates better immunogenicity. Vaccine responses in 80-93% of recipients, have reached to its maximum peak, 4 to 8 weeks after immunization and then decreases; after one year, usually the antibody titer decreases to 50% of their initial rate. Produced antibodies against capsule V type in the CPS V-DT conjugate, causes to be killed in vitro, mediated by opsonophagocytic activity. Capsular type V, is unique in antigenic and immunological structure than other types, and had been the most important pathogen in newborn babies and pregnant women in the past decade [11]. One way to prevent GBS infection in mothers and infants is that women before or during pregnancy, use the polyvalent protein-polysaccharide conjugate, containing main serotypes associated with human infections[12]. Newborns, especially premature infants, are incapable of producing immunoglobulins to protect against infections caused by encapsulated bacteria such as GBS [13].
Group B streptococci are commonly found in the N flora of the throat, gastrointestinal tract and vagina. Approximately 15-20% of pregnant women, might be carriers of the organism in vagina. Transmission of group B streptococci in newborn infants is variable, but in infants who are born from vector mothers, would increase to about 50-60%. The disease prevalence in infants who have acquired infection, is low, but might have dangerous results [1,14].

Incidence of neonatal group B streptococcus infection in 1970, increase and signs of both early and late clinical infant syndrome, were studied. Group B streptococcus infection in the first six days of birth is called Early infection, that this type of infection often occurs in the first 12 hours of the infant’s life. Infants born at less than 37 weeks of gestation, compared to infants who were born on time, are more susceptible to bacterial infection. The main clinical protests of the infection include, bacteremia or septicemia, pneumonia and meningitis [15, 16]. Group B Streptococcus late neonatal infection is called to infections between days 7 up to 89 of life, and the infection, on average would happen on the day 36; and 50% of these babies, are premature. This infection has a mortality rate of 3-5%. Clinical protests such as osteomyelitis, septic arthritis, and cellulitis may also be observed. From nonspecific symptoms, lethargy, fever and shock could be noted [17,18].

Group B Streptococcus Childhood infection, is told to the infection after 3 months, which consists about 15-10% of all cases. Often this type of infection would occur in low weight children, or children who are hospitalized. Bacteremia is the most common clinical protest.

When group B streptococci disease was diagnosed in an infant, the HIV infection and congenital heart disease should be evaluated in these newborns [19,20]. Group B Streptococcus infection causes illness and deaths among adults. In one study, during 1975-1984, the incidence of Group B Streptococcus bacteremia, was 0.2 cases per 1,000 hospital admissions and 53% of Group B Streptococcal blood culture isolates, were adults [19]. Among other complications of the bacteria, female genital tract infections, pneumonia, endocarditis, arthritis, osteomyelitis, skin and soft tissue infections and meningitis, could be noted [4,5,21,22,23].

Objective and Methods:

The aim of this study was the covalent binding of Streptococcus agalactiae CPS to Diptheria toxoid, in order to raise the safety level, established by the polysaccharides of bacteria and to study the rate of stimulating production of IgG1, IgG2a, IgG2b, IgG3, Total IgG, IgM and IgA antibodies, by CPS-DT conjugate and CPS. In order to prepare Streptococcus agalactiae with Diphtheria toxoid conjugate vaccine, lyophilized strains were obtained from the Biology Research Center of Zanjan. For this purpose, first lyophilized strains of Streptococcus were cultured on blood agar and incubated for 24 h at 37 °C [1,2-3-4]. Then, presence of the bacteria in form of dense glossy gray colonies with a transparent halo around them which is due to the positive hemolysis of these bacteria, indicates the correct culturing.

But we did not satisfy with the macroscopic features, in addition by Gram stain of bacteria, observing the bacteria under a microscope as spherical and chain and proving a negative catalase test, we made sure about the presence of streptococcus agalactiae to continue the testing process [1 3-4].

Streptococcus agalactiae has the highest capsular polysaccharide growth in the Todd- Hewitt broth medium. After autoclaving of 5 flasks containing Todd- Hewitt broth medium and become isotherm with laboratory temperature, were inoculated in each flask from the blood agar plate containing Streptococcus agalactiae strain, next to the torch and then flasks were placed in CO2 incubator. After 72 hours, the culture medium became turbid and a gray colloidal layer is made at the bottom of the flask which indicated the growth of bacteria with the highest rate of capsule production [69]. After culturing the bacteria in the artificial Todd- Hewitt broth medium, the medium poured into centrifuge tubes and centrifuged for 50 min, at 2500 rpm, to separate the precipitate from the supernatant [24]. The previous step sediments collected, and one NaOH added to that as much as four folds the amount of the final sediment and then incubated for 12 h at 37 °C. The tube containing NaOH and sediment was centrifuged for 20 min and rpm2500 for debrises precipitation [24]. The supernatant obtained in the previous step, was then neutralized by 10 N HCL, then cold ethanol was added to three folds the supernatant volume, and left for one day in the fridge, then the precipitate from this step containing bacterial capsular polysaccharide were collected by repeating the centrifuge at 2500rpm, for 60 minutes.

Then 0.1 M trace Buffer which its pH was adjusted to about 7.5, was added to the obtained precipitate, as 5 ml by 5 ml till the sediment becomes completely transparent, and eventually the CPS precipitate became completely clear with about 40 cc of trace buffer, then we calculated 0.5% of SDS [sodium dodecyl sulfate] and 10 mM of CaCl2 [sodium chloride] in 40 ml of buffer volume size and added to the above solution [25]. At this stage, to the 3-fold volume of cold ethanol was added to the supernatant containing capsular polysaccharide, after 24 hours the resulting precipitate was centrifuged for 30 minutes at 2000rpm then the supernatant and final precipitant containing pure cps were stored in the refrigerator, for next steps. It should be noted that the extracted antigen should not be kept in the refrigerator, because mold does antigen because it becomes moldy [26]. After consecutive steps, in order to extracting the capsular polysaccharide of Streptococcus agalactiae, as the antigen used in the preparation of the CPS-TT conjugate vaccine, due to low amount of CPS, the above steps were repeated three times. For polymerization of capsular polysaccharide to prepare for conjugation stages,
initially 30 mg of CPS was dissolved in about 8 ml of 0.5 N NaOH, of course the amount of used NaOH must be enough that precipitate containing CPS becomes entirely clear [27]. Centrifuge tubes containing CPS sediment, dissolved in NaOH, was placed in the 70 °C bain marie for 1 hour. Then the tube at room temperature, was entered into a beaker containing ice for induction of thermal shock to the precipitate containing CPS and the solution remained in this condition for 10 minutes. For each mL of used NaOH, to dissolve CPS, 125 μl of glacial acetic acid were added to the sample. Of course it is added to the sample to the extent that pH is adjusted to 4, thus gradually CPS would be N-acetylated [see 73], and per each mL of NaOH used in CPS solution the, 200 ml of NaN2O is added with 5% w/v and sample was shaken for 2 h at 4 °C. At the end of the above steps the capsular polysaccharide was depolymerized and prepared for Conjugation [27].

The solution containing depolymerized capsule was diluted in 5 mL of deionized water, then dialyzed against distilled water exchange 3 times a day, by a 10 kDa Kataf Dialysis bag. After dialysis, the sample size was approximately 12 cc, that in order to precipitate depolymerized capsules, cold ethanol was added as 3 folds the sample volume and the sample was placed in in the fridge for a day. Final concentrating of the sample was done by centrifugation for 50 min at 2500 rpm, until the sample size containing depolymerized capsule, is reached to 0.5 ml. In polysaccharide conjugation of Streptococcus agalactiae with diphtheria toxoid by Amidation method, at first 0.5 ml of depolymerized CPS, in a minimum volume of distilled water [approximately 1 mL], was diluted, then we set the pH on 10.5 with 1 N NaOH. In this step we dissolve 0.1 g of cyanogen bromide in 0.5 ml of acetonitrile solution, then add 150 μL ml of the above solution to the sample, pH was also adjusted to 10.5.

Then, the samples was shaken for 10 min at 4 °C and sodium bicarbonate [NaHCO3] and ADH with a final concentration of 0.5 M was added, pH was adjusted to 8.5, then the samples was kept in the refrigerator for 18 hours. The sample was dialyzed against distilled water exchange 3 times a day, by a 10000 kDa Kataf Dialysis bag. After dialysis, the sample size was reached 20 cc, and since the water in the sample reduces conjugation efficiency, the sample was ultra-filtered at 2500 rpm; and finally the sample size was reached to 1.5 ml.

**Lyophilized diphtheria toxoid:**

Lyophilized diphtheria toxoid powder was diluted carefully with 4 mL of distilled water and because the sample volume was 1.5 mL, proportional to the antigen, 1500 μL Diphtheria toxoid was added and the reaction mixture was cooled on ice, then the sample pH was set to 5.8 by 0.1 N HCl. EDAC powder, with a final concentration of 0.1 N, was calculated in the final volume of the sample, which was about 3 cc, then added to the sample and stirred for 4 h on ice, and then was placed for a day in the fridge. Solution containing CPS-DT was dialyzed against non-pyrogenic distilled water in 4 °C, by a 10000 kDa Kataf Dialysis bag.

For purification of conjugated molecules from non-conjugated molecules, prepared CPS-DT conjugated sample, with a volume of 3 mL, was passed from gel filtration column containing CL-4B sepharose, with a working volume of 2 × 60 cm, balanced by 2.0 M sodium chloride solution. In the process of extraction buffer flow rate of 40 ml per hour should be set up and continues until a bed volume of fluid pass through the column. Fractions were collected in 200 tubes and were interpreted at two 210 and 280 nm wavelengths. The tubes with the highest absorbance at both wavelengths were collected and merged, as fractions containing conjugated molecules. The prepared conjugates concentrated by ultrafiltration method, and was passed by adding Merthiolate with a final concentration of 1 in 1000 from a 0.22 micron Millipore filter, divided in sterile vial and was stored at -20 °C [28].

To prepare the protein standard solution with a concentration of 100 μg/ml, first 10 mg of bovine serum albumin [BSA] was dissolved in 10 mL of distilled water and a standard 1 mL storage was made. Then 1 mL of that was diluted with 9 mL of water to prepare 100 μg/ml diluted standard to draw the curve. To measure the protein concentration by this method, the standard curve should be plotted first. The necessary standard solutions is provided according to the following table [29].

| Table 3.2: The standard curve plotting and measurement of protein concentration. |
|-------------------------------------|-------|------|------|------|------|
|                                    | Blank | 1    | 2    | 3    | 4    | 5    |
| BSA Standard solution [100mg / ml] in microliter | -     | 20   | 40   | 60   | 80   | 100  |
| Distilled water[μL]                | 100   | 80   | 60   | 40   | 20   | -    |

The Bradford reagent was added to all the tubes [5 mL] and were mixed immediately then after 10 min their absorbance was read at 595 nm. After measuring the absorbance, by insertion the concentrations of the standard sample, standard curve was plotted and after reading the absorption rate of unknown samples in the spectrophotometer, the concentration can be calculated using the standard curve. 100 μL of each unknown sample was poured into a tube, then added 5 mL of reagent and mixed. After 10 minutes, absorbance at 595 nm was read. By comparing samples absorbance with the standard curve, the protein concentration of the samples were obtained [30].
60 BALB / C female mice was bought from Razi research Institute in Karaj. The mice were divided into four groups of 15 members. The first group by CPS DT vaccin, second group by CPS vaccine, third group with DT vaccine, with a dose of 2.5 mg each, and the fourth group were vaccinated with normal saline as a control group. Vaccination was performed with three doses [each time 0.5 cc], intraperitoneally, with two-week intervals, and sampling was done two weeks after each injection, from 5 mice each group. Each time, blood serum isolated by centrifugation [3000rpm for 5 minutes], and was maintained in -20 °C freezer until ELISA testing.

In order to gain appropriate serum dilutions, cross-titration table method [Plate Checker board] was used. In this test for IgG3, IgG2a, IgG2b, IgG1, IgA and IgM antibodies titer determination, the Antigen Mediated Elisa method and for total IgG titer determination the Indirect Elisa method were used. ELISA method was performed by Triplicate method.

One-way ANOVA tukey’s test was conducted with P< 0.01 , using SPSS software with variance statistical test.

Discussion:
The results showed that the ratio of polysaccharide to protein, in CPS-DT conjugated molecule is 0.35 and efficiency of Conjugation methods based on the initial amount of CPS, is 0.41. Based on the results obtained through the Tukey’s test with P < 0.01, titer difference of all injected antigens [CPS, CPS-DT], two weeks after the first, second and third injection, compared with the control group, was significant [table 1-4]. Two weeks after the third injected dose , significant increase observed in antibody titer, against CPS in mice immunized with CPS-DT compared to CPS-GBS.

Since the sensitivity of infants to Group B Streptococcus, is due to the immaturity of the neonatal phagocytic system and humoral immunity, trying to develop a vaccine against this strain, and vaccination of women during pregnancy, resulting in the production of antibodies in the mother’s body and their transfer through the placenta to the baby, either before birth or through breast milk and baby’s immunization against the bacteria, is ongoing [31,32].

For the first time, in 1970, studies was carried out by Kasper and Baker, about the antibodies, which have protective role against the capsular polysaccharide of type III Streptococcus agalactiae. Based on their researches, it was found that the production of antibodies against the type III of the bacterial CPS, in the early stages of bacterial invasion, is very low, and a certain concentration of CPS, causes stimulating the production of IgG and in vitro initiation of opsonization and phagocytosis [33].

Study on the first vaccine against Streptococcus agalactiae, in 1978, was conducted by Kasper and Baker, that only pure CPS extracted from the types Ia, II and III of bacterial capsule, were used and the results from antibody titer, were not satisfactory. It is worth noting, however, that compared to other types of capsular types, type III, had better antibody titer and totally these vaccines did not presented significant protective efficacy[33].

In this study, in order to achieve this task , first the capsular polysaccharide from lyophilized strains of Streptococcus agalactiae was extracted and conjugated with Diphtheria toxoid as the carrier protein; and immunogenicity of the prepared vaccine with toxoid was studied in female BALB / C mice. Conjugation of purified CPS with diphtheria toxoid was performed using Amidation method, which is one of the most stable types of conjugation.

All the conjugate and CPS-GBS vaccine recipient groups, compared to the control group, showed significant difference, in all three injection doses [two weeks after the first second and third injection], in terms of the serum antibody amount, against the capsular polysaccharide of Streptococcus agalactiae. Protein-polysaccharide conjugate vaccines, are mainly designed to induce protection against pathogens carrying the polysaccharide part [segment].

Kasper and colleagues, in 1990, concluded that the binding power of a protein carrier, during the Conjugation process, depends on the polysaccharide chain length of the capsule. Namely, in the case of oligosaccharides, Conjugation would be with higher efficiency, about 3.4%, comparing when bacterial capsule is polysaccharide [33].

Most studies on the conjugate vaccine against Streptococcus agalactiae, have been as depolymerized CPS Conjugation with Tetanus toxoid, and their main difference is about how the CPS is extracted and depolymerized. Among these studies, there is a study by Fernandes JC et al [1995] that the CPS bacteria depolymerized using sodium acetate ,and without activating the depolymerized capsule by cyanogen bromide, it was conjugated with Tetany toxoid attached to the spacing molecule.

But by measurement of IgG subclasses titer, they concluded that Conjugation without the use of cyanogen bromide, has got lower efficacy, about 1.5%, compared when the depolymerized capsule is activated by cyanogen bromide [31].

Streptococcal infections, Group B, in newborns occur in the first days of their lives, because the infant is not able to generate an effective immune response against microbes and until several months after birth, the main mechanism of defense against infection, are the anti-bodies that have been transferred inactively from...
mother to the infant, maternal IgG molecules by crossing the placenta and also IgG and IgA present in breast milk by feeding, reach the baby. IgG antibodies in breast milk can pass through the intestinal epithelial cells, enter the blood stream, resulting in the infant to have similar IgG antibodies to maternal antibodies. After vaccination of pregnant women with GBS in the vaginal canal, with GBS- pro carrier conjugate vaccines during pregnancy, both causes develop of passive immunity in infant, as well as eliminating and destroying the GBS in the vaginal canal, through stimulation of mother’s mucosal immune system [34].

In another study conducted by Claudia Gravekamp and colleagues in 1999, streptocococcus agalactiae surface Alpha C protein was used as a carrier protein in Conjugation process. The results indicated that produced antibody titers of both conjugates against capsular polysaccharide is more than the time, when only polysaccharide is injected and of IgG antibodies and IgG1, IgG3, IgG2b, IgG2a sub-classes titer against the capsular polysaccharide of Streptococcus agalactiae by CPSIII-α2r conjugate was more than induced antibodies titer by CPS III-TT conjugate.

In our study we observed proper increase in antibody titer against CPS-GBS. In our study, in addition to measuring the amount of IgG, various IgG sub-classes were also examined.

Some IgG sub-classes, by binding to Fc receptors, facilitates phagocytosis of particles bound to antibody. The IgG and its Subclass titer, demonstrates significant increase after the third injection dose, in the groups vaccinated with CPS-DT, compared with pure CPS-GBS and especially in comparison with the control group. The greatest increase in production of antibodies associated with IgG sub-classes was about IgG3, IgG1 subclasses, respectively.

During similar research which has been conducted over the past years, by various researchers, it has been found that, among the various candidates in the carrier proteins for Conjugation with polysaccharides, Pseudomonas aeruginosa A exotoxin conjugate, creates the best antibody titer against polysaccharide. This issue have been expressed by John and et al. in 2004, Kassaczka et al. in 1999, Line et al in 2001, and Mai in 2003.

Conclusion:
The results of this study suggest that conjugated molecules, has got improved potency than the pure CPS-GBS and could stimulate cell immunity, which has got great importance in vaccinating pregnant women in order not to transfer GBS to the infant and transmission of anti-CP antibodies to the newborn, through the placenta and milk.

REFERENCES