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Conjugation to a Carrier Renders a Self Molecule Immunogenic besides Imparting Immuno-Prophylactic Benefit

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Abstract

Conjugation of hCGβ subunit with tetanus toxoid generates simultaneously independent set of antibodies against both hCG and tetanus. The former are competent to neutralise the bioactivity of hCG, and the latter impart immunoprophylactic benefit against tetanus.

Similarly the decapeptide hormone LHRH which is common to both males and females can be rendered immunogenic by linking it to a carrier. It is conserved across species and is common to humans, rodents and dogs. Given to male rats, it causes a drastic reduction of testosterone and atrophy of testes and ventral prostate. Monoclonal antibody against LHRH can be employed to suppress the seasonal estrus in dogs With due approval of the Regulatory Authorities and Ethics Committees, the LHRH-DT (diphtheria toxoid) vaccine has undergone clinical trial in 28 patients of Carcinoma of Prostate in India and Austria. The vaccine was effective in reduction of testosterone and PSA (prostate specific antigen). It reduced the cancerous tissues and benefited the patients clinically.

Keywords: Vaccines; hCG; LHRH; Fertility control; Carcinoma of Prostate

1. Introduction

The immune system is tolerant to self molecules and has an inbuilt mechanism for elimination of T-cells reactive with body's constituents. Most vaccines developed for preventing infections have employed attenuated or killed micro-organisms which are foreign to the body's immune system to induce immunity. To develop a vaccine against the pregnancy hormone hCG (human chorionic gonadotropin), which is a self molecule, for potential control of fertility, we had to think of a way by which hCG or its subunit could be made immunogenic in women. hCG is a product of the embryonic cells. Eggs fertilized *in vitro* start making it at the blastocyst stage [1]. It plays a crucial role in implantation of the embryo on to the endometrium [2] leading to the onset of pregnancy. The mother and the developing fetus are flooded with hCG, to which her immune system is fully tolerant. How could one render it antigenic to make antibodies competent to neutralize its biological action enabling the implantation of the embryo? It was indeed a challenge.

Realizing that the B cells of the immune system that make antibodies, require T cell help to trigger response, we considered employing 'altered' hCG β subunit carrying also a molecule foreign to human immune system that could negotiate with T cells. We thought of linking it with tetanus toxoid (TT) as carrier. TT was available in plenty at cheap rates. It is a good immunogen, and the antibodies are protective against tetanus at all stages of life. At the time that we took this decision (early nineteen seventies), tetanus used to kill a large number of women delivering their progeny in the field or at home in aseptic conditions. Thus in case TT used as carrier with hCG β raised also antibodies against tetanus (besides hCG which was not sure at that stage), at least protection against tetanus would be imparted to women immunized with the conjugate vaccine.

2. Evidence of Elicitation of Anti-hCG and Anti-tetanus Antibody Response by hCGβ-TT Vaccine in Women

Extensive toxicology studies were carried out to determine the safety of hCGβ-TT vaccine [3-8]. The vaccine was found to be fully safe. The only cross-reaction of antibodies generated by hCGβ-TT was with hLH. However, the degree of cross-reaction did not appear to interfere with continued ovulation by women, nor in disturbance of menstrual regularity. Investigators at the Population Council, New York carried out lifelong chronic toxicology studies of 5-7 years duration in rhesus monkeys to determine the consequences of hyper-immunization of the rhesus with β-oLH given along with Freund's complete and incomplete adjuvant. The antibodies were frankly cross-reactive with monkey LH and hCG. The effect on control of fertility was reversible and animals returned to normal gestation on decline of antibodies [9]. Furthermore, no ill consequences of the anti LH antibodies were seen on the pituitary [10].

With approval of the Ethics Committee of the All India Institute of Medical Sciences, four women who have had elective tubectomy after completing their families, agreed by written consent to get immunized with the hCG β-TT vaccine. They received four injections of 80 µg of the vaccine at 14 days intervals. All of them raised antibodies against both hCG and tetanus [11,12]. Figures 1 A, B show the response in two of these women Both generated antibodies against hCG as well as against tetanus The response was reversible and antibody titres declined in course of time. None of these women experienced any adverse reaction.

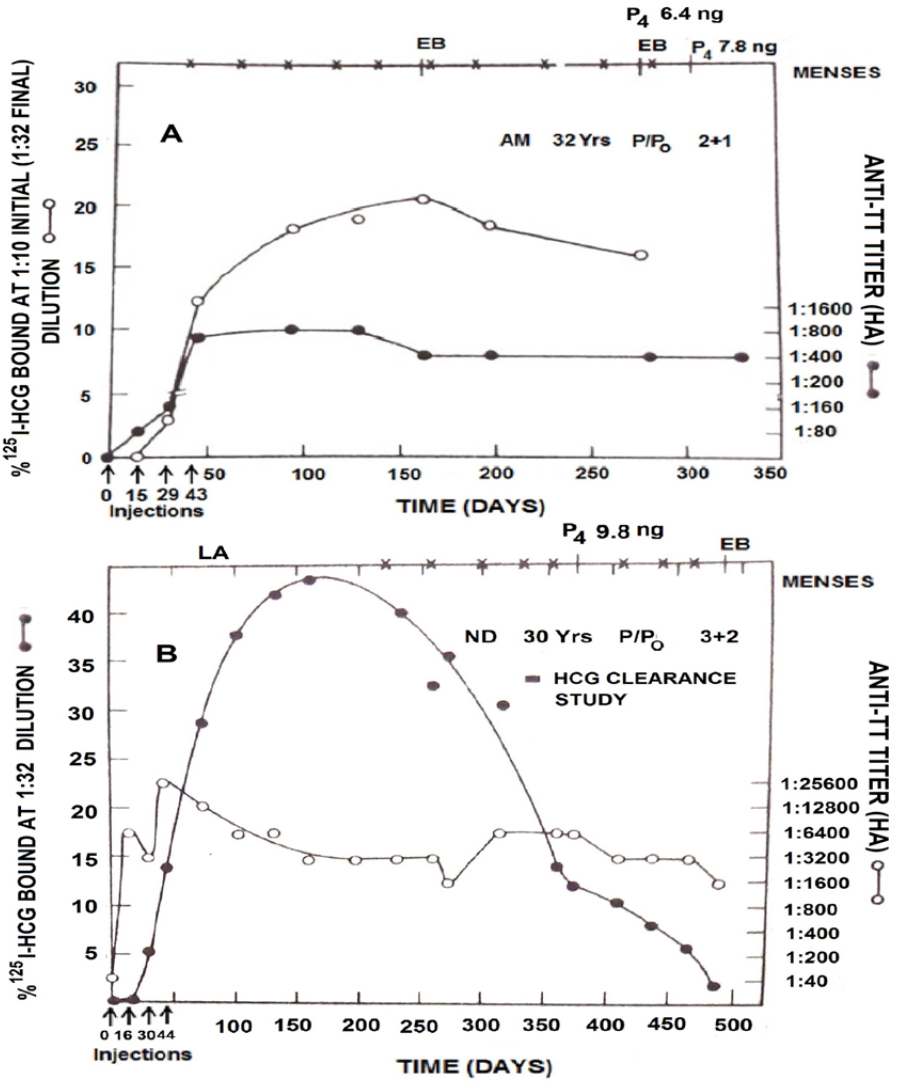

Figure 1: Anti-hCG and anti-tetanus antibody response to hCGβ-TT in 2 fertile women, AM and ND. ND had three children and two terminations of pregnancy (MTP) before tubectomy whereas, AM had two children and one MTP. Both generated antibodies reactive with hCG and tetanus [11,12].

In order to determine whether the antibodies generated against hCG recognized and bound to hCG *in vivo*, we conducted a 'challenge' test. To the immunized woman (KW) who was amongst the 4 women immunized with the hCG β-TT vaccine, a load dose of 5000 IU of hCG was given.

As shown in Figure 2 the administered hCG was 'captured' by the circulating antibodies resulting in fall of antibody titres, which however reverted to the original level in course of time. No discernible effect of administered hCG was seen on anti-tetanus antibody titres, indicating that the two types of antibodies were independent, raised against hCG and tetanus.

A similar observation was made in another woman (ND). She was given two injections of 4000 IU and 2000 IU of hCG on consecutive days hCG given in both injections was duly bound by circulating antibodies, with no effect on anti-tetanus titres. After a transient decline, the anti-hCG titres returned to pre-challenge level in course of time (Figure 3).

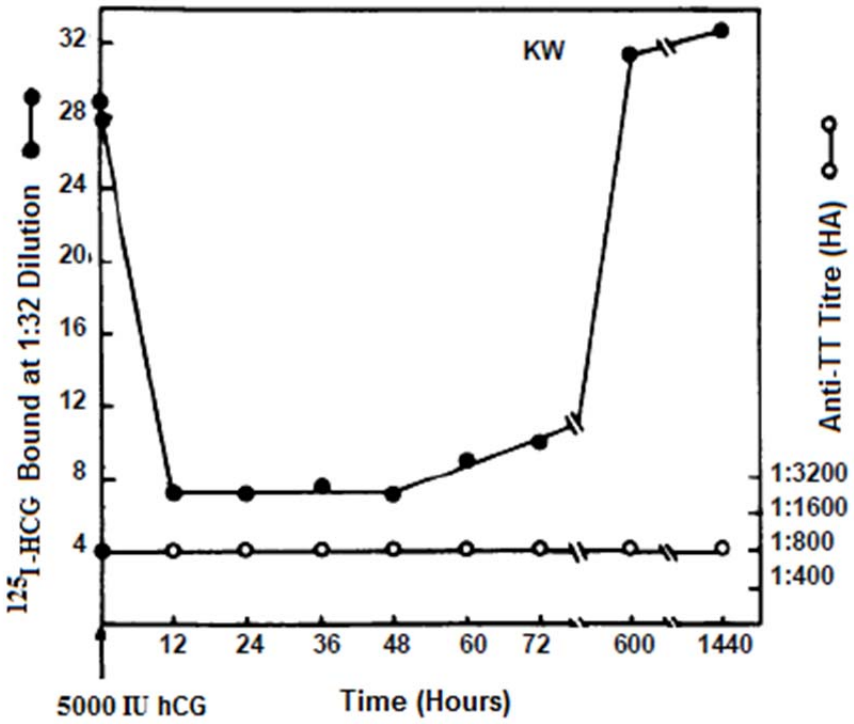

Figure 2: Injection of 5000 IU hCG caused a temporary drop in anti-hCG antibody titres in woman (KW), which returned to original level in course of time. The anti-tetanus remained at the same level indicating that the vaccine generated independent antibodies against both hCG and tetanus [11,12].

The ability of hCGβ-TT vaccine to generate antibodies against hCG and tetanus in women was confirmed by clinical trials conducted in Sweden, Finland, Chile and Brazil by the International Committee on

Contraception Research of the Population Council. These trials confirmed also the safety and reversibility of response [13].

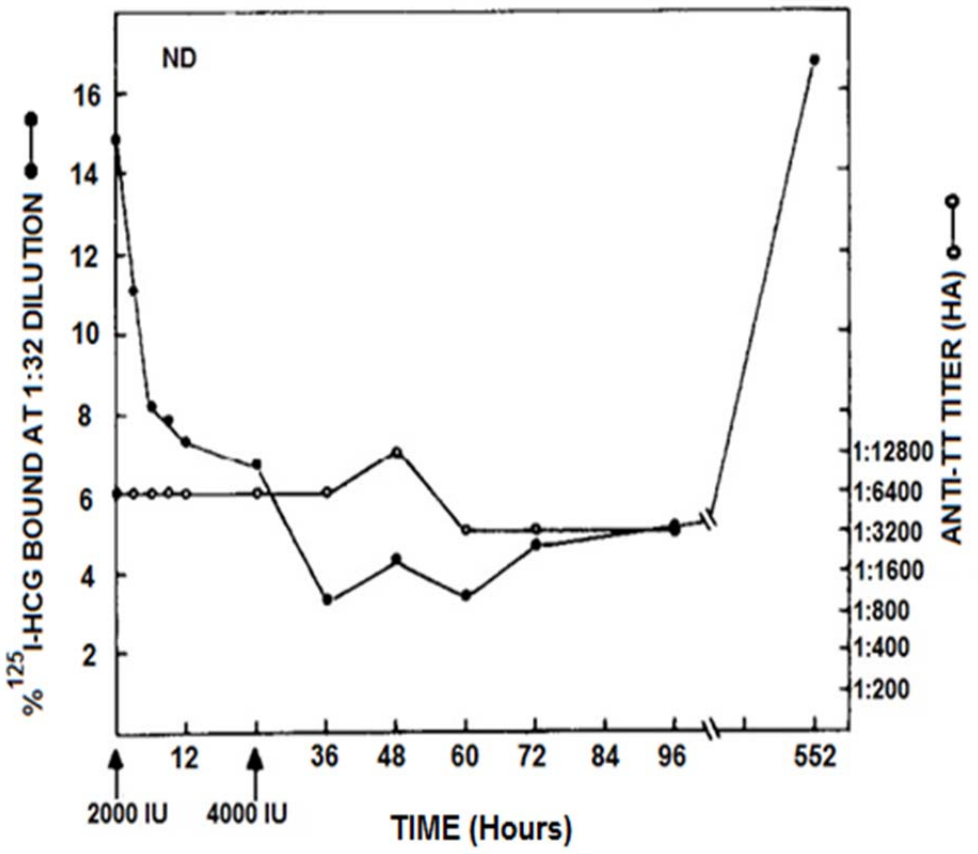

Figure3: Injection of 2 lots of hCG caused a temporary drop in anti-hCG antibody titres in woman (ND), which returned to original level in course of time The anti-tetanus remained at the same level indicating that the vaccine generated independent antibodies against both hCG and tetanus [11,12].

3. Carrier-Induced Immuno-Suppression

While linkage to carrier, such as tetanus toxoid, renders hCG β immunogenic, repeated use of the hCG β linked to tetanus toxoid can lead to shutting 'off' of the the immune response [14]. The suppression can be overcome by changing the carrier i.e linking hCG β to an alternate carrier such as Diptheria toxoid (DT) which restores the immune response to hCG β [15].

Herzenberg and Tokuhisa [16] reported the presence of epitopes in various proteins which have either helper or suppressor function. Should one not avoid suppressor epitopes, so as not to experience the problem of suppression of immune response after giving a booster?

4. Construction of a Multimer Vaccine Excluding Suppressor Epitopes

For doing this work, we thought of a vaccine against the decapeptide hormone LHRH. LHRH is made in the hypothalamus, travels through the portal circulation to the pituitary, where it stimulates the production and secretion of the gonadotropins FSH and LH, which in turn act on gonads (male or female) to generate respectively spermatozoa, testosterone (in male) and egg, estradiol and progesterone in females It is thus a master molecule controlling reproduction in both males and females. Its structure is largely conserved during evolution. Mice, rat dogs, man and women have the same LHRH.

LHRH was rendered immunogenic, by linking it to a carrier, TT or DT. Minor modifications in LHRH were done to enhance its biological life. Glycine at position 6 was replaced by lysine with an extra amino group [17]. To this was linked a spacer 6-aminohexanoic acid followed by TT or DT. The vaccine adsorbed on alum indeed generated antibodies against LHRH, which were biologically effective [17].

To evaluate the virtue of employing only immunogenic epitopes of the carrier instead of the whole carrier, we designed a multimer vaccine, where 4 or 5 units of LHRH were linked through epitopes of more than one carrier e.g Cicrumsporozoite protein (CSP) of *Plasmodium falciparum*, *Mycobacterium tuberculosis* (MTB), Tetanus toxoid (TT), Respiratory syncytial virus (RSV) and Measles. One or two glycines were used as spacer in between LHRH and the carrier epitope [18]. Our hope was that the Multimer LHRH vaccine will be more immunogenic and response will occur against more than one infections. After purification of the recombinant vaccine expressed in *E. coli* and characterization [19], the immunogenicity and efficacy of the vaccine on testosterone and ventral prostate was investigated in rats [20].

The vaccine did bring down the testosterone to literally orchiectomized level, with stark atrophy of testes and ventral prostate (Figure 4). As with the earlier semi-synthetic LHRH vaccine, these effects were reversible. It was used successfully by Lowell Miller at National Wild Life Research Centre, USA to sterilize pigs. Given along with Adjuvac, an oily adjuvant approved by United State Food and Drug Administration (USFDA), it sterilized 100% of the pigs tested [21].

5. Control of Estrus in Dogs

We developed a monoclonal antibody against LHRH [22]. It was reactive with a conformation native to the whole decapeptide, and neutralized effectively the bioactivity of LHRH. Given as a single injection to female dogs in beginning of estrus, it arrested effectively their attraction to the male dogs. Synthesis and secretion of both estradiol and progesterone was blocked (Figures 5 & 6).

The antibody given as a single injection remained in circulation over 14 days of observation period. The treated animal regained its normal biology in course of time. Her next estrus was fully normal on mating,

it conceived and gave birth to progeny confirming the full reversibility and lack of any permanent damage to its reproductive system on exposure to the anti-LHRH antibody.

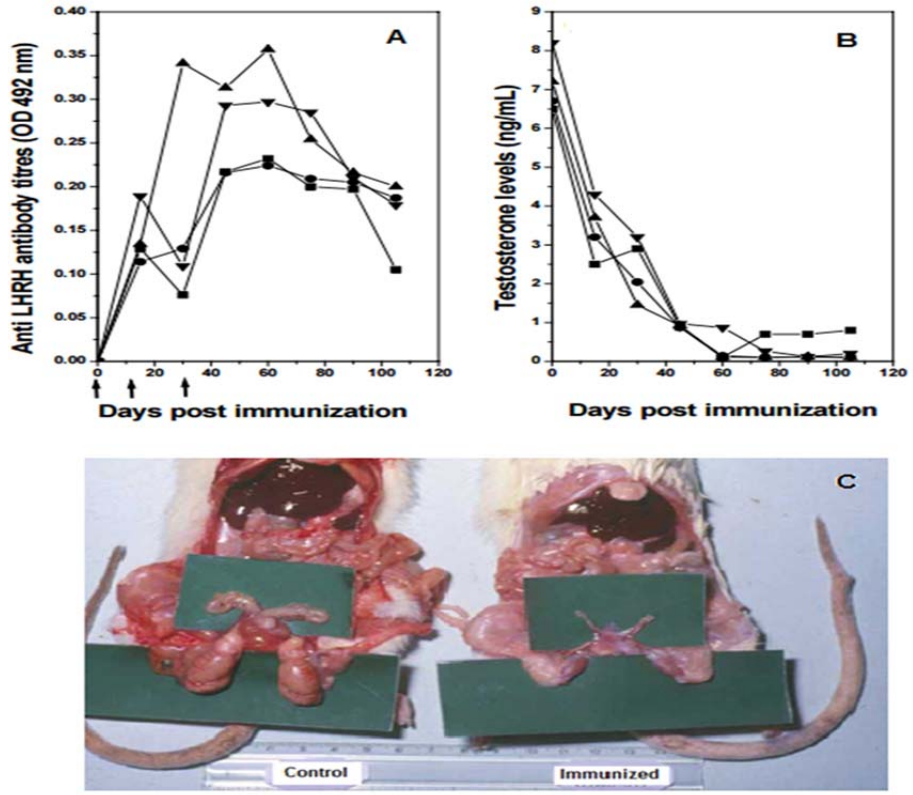

Figure 4: (A) Antibody response to recombinant multimer LHRH vaccine in rats Arrows indicate the time points at which primary immunization and boosters were given (B) Blood testosterone levels in each of the immunized rats represented by different symbols (C) Effect of anti-LHRH vaccine on rat prostate [20].

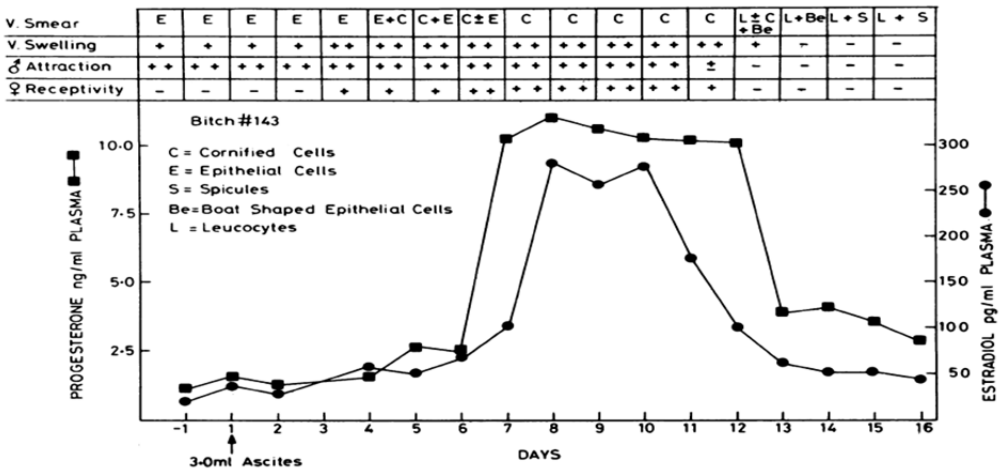

Figure 5: Normal progression of estrus in female dog treated i.v with 3 ml of the ascites fluid obtained with mouse myeloma cells (SP2/0) and devoid of anti-GnRH antibodies [22].

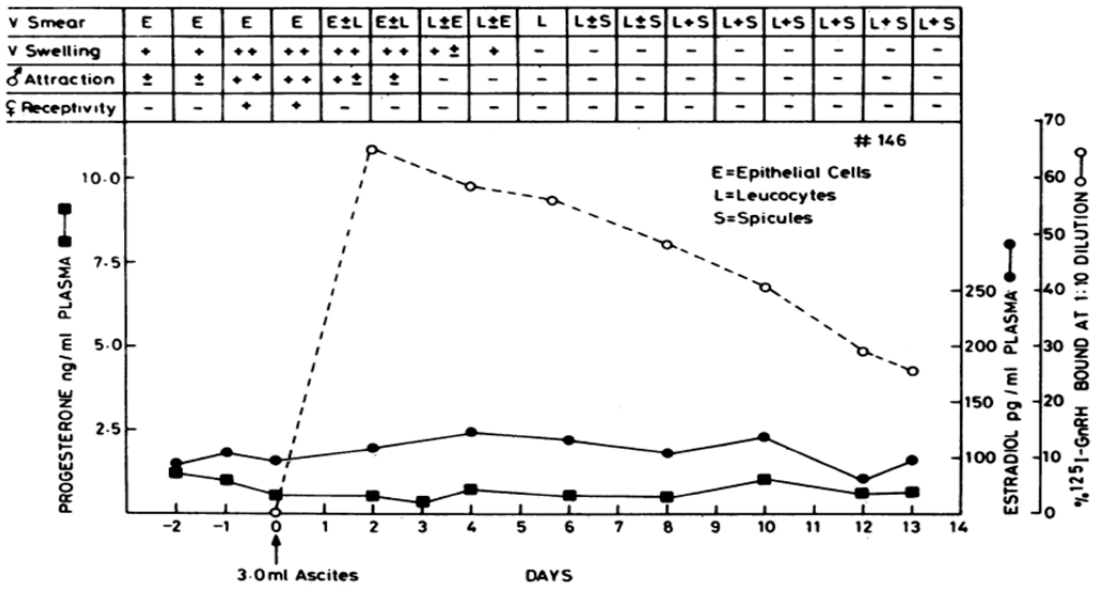

Figure 6: Typical effect of administered anti-GnRH monoclonal antibody given to female dogs in the proestrus stage. The antibody suppressed heat within 48 hours as determined by the four criteria listed in the upper part of the figure. The increase in blood progesterone and estradiol normally occurring during estrus was also prevented. Dashed line shows amount of antibody in circulation on different days after a single i.v injection of 3 ml of the ascites fluid of clone $P_8 16_{62}$ [22].

6. Phase I/II Clinical Trials on the Semi-Synthetic Anti-LHRH Vaccine in Prostate Carcinoma Patients

After obtaining Regulatory and ethics approval in India and Austria, clinical trials were conducted in 24 patients suffering from carcinoma of prostate at the All India Institute of Medical Sciences (AIIMS), New Delhi, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh and in 4 patients at the Salzburg Urologische Centrum in Austria. No ill effect of immunization with the vaccine was observed in any patient. In fact, immunization with the vaccine lowered the serum testosterone and Prostate Specific Antigen (PSA) of a patient suffering from carcinoma of protate (Figure 7).

At AIIMS 400µg dose was better than 200µg dose to cause clinical improvement of the patients (Table1). At PGIMER Chandigarh, serial nephrostograms showed notable reduction of mass of the cancerous tissue (Figure 8).

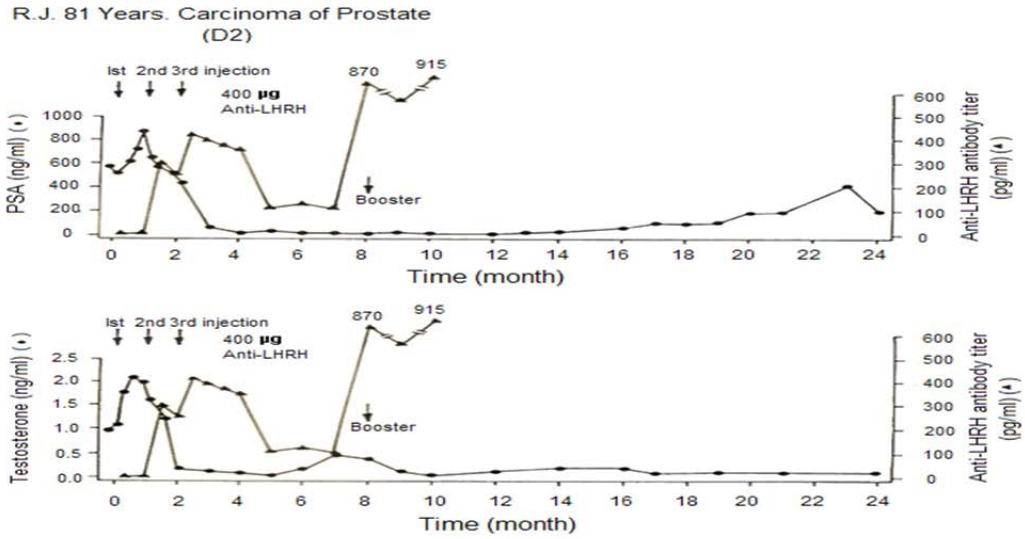

Figure 7: Effect of anti-LHRH vaccine on a patient with advanced carcinoma of prostate. With the generation of antibodies, testosterone and Prostatic Specific Antigen (PSA) levels fall and stay low for several months [23].

Table 1: Observations in clinical trials conducted at AIIMS in patients of carcinoma of prostate after immunization with either 200 µg or 400 µg of anti-LHRH vaccine. Vaccine was administered as 3 primary injections at monthly interval followed by a booster at 8th month [23].

Effect of immunization Dose Level	200 µg (n = 6)	400 µg (n = 6)
Clinically Stable/Improvement in Symptoms	4	5
Reduction in Prostatic Size/Hardness	1	3
Reduction in Acid Phosphatases	1	4

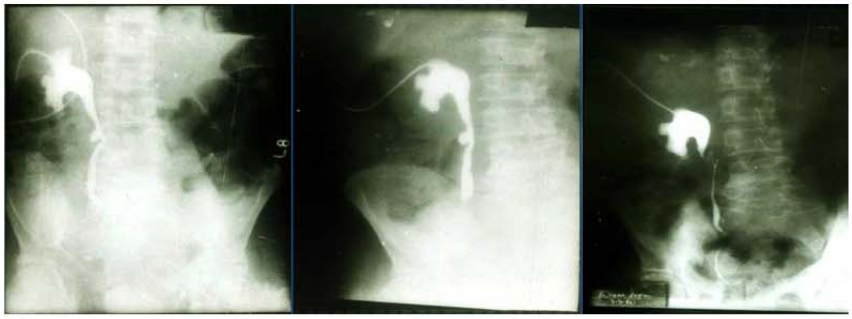

Figure 8: Nephrostograms showing the noticeable reduction of prostatic tissue mass at various stages of immunization with anti-LHRH vaccine [23].

Acknowledgements

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