

The experience of leishmanization in the Islamic Republic of Iran

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تجربة طريقة اللشمنة في جمهورية إيران الإسلامية

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خلاصة : تصف هذه المقالة برامج اللشمنة المطبقة في منطقة عالية التوطن (أصفهان) وجماعة عالية التعرض للخطر (من المجندين وأفراد الحرس الثوري) ، كما تناقش مدى فعاليتها ومضاعفاتها . وتلخص المقالة تجربة على لقاح خام مقتول . وقد تم تطعيم أكثر من مليوني شخص بطريقة اللشمنة ، وتبين أن هذه الطريقة تخفض معدل حدوث المرض بما بين سدس وثمان المئدة الأصلي . ويوصى بتطبيق هذه الطريقة حيثما يتعرض الناس بدرجة مفرطة للإصابة بهذا المرض .

ABSTRACT Leishmanization programmes in a hyperendemic area (Isfahan) and a high-risk group (army recruits and the Revolutionary Guard) are described and their effectiveness and complications are discussed. A trial of a non-living crude vaccine is outlined. More than two million people underwent leishmanization and it was found to reduce the incidence of the disease between one-sixth and one-eighth of its original level. The procedure is recommended whenever people are at a very high risk of contracting the disease.

Expérience de leishmanisation en République Islamique d'Iran

RESUME Les programmes de leishmanisation dans une zone d'hyperendémicité (Isfahan) et dans un groupe à risque (conscrits et Gardiens de la Révolution) sont décrits dans cet article et leur efficacité ainsi que les complications y sont examinées. Un essai relatif à un vaccin brut à base de promastigotes tués est mentionné. Plus de deux millions de personnes ont subi une leishmanisation et on a constaté une réduction de l'incidence de la maladie comprise entre un sixième et un huitième de son niveau d'origine. Cette procédure est recommandée chaque fois que des populations ont un risque élevé de contracter la maladie.

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Introduction

Both zoonotic cutaneous leishmaniasis (ZCL) and anthroponotic cutaneous leishmaniasis (ACL) are found in scattered foci in various parts of the Islamic Republic of Iran. In recent years, due to the increase in population, increase in areas under irrigation and many other known and unknown factors, the incidence of both types of leishmaniasis is increasing and new areas are being invaded. Very active foci are found in areas on the southern slopes of the Zagros mountains such as Khonj and Gerash in Lar district, many areas in Darab district and in Fars province. Previously the disease was rare in these areas.

Since the beginning of the studies on cutaneous leishmaniasis at the Institute of Public Health Research, one of our aims has been to control ZCL in independent foci of the disease. In the past, various control measures, carried out mainly in the Isfahan area, failed to control the disease or to permanently decrease the number of cases. Therefore, it was decided to try the method of leishmanization. This is the intradermal inoculation of virulent strains of promastigotes of *Leishmania major* in a covered area of the skin, usually on the upper part of the arm.

First, a field trial of the method was carried out in a town north of Isfahan [1]. In this trial, more than 250 children under 10 years of age without a history of ZCL were leishmanized. The incubation period in the "takes" group was from 1 to 14 months (90% were between 2 and 4 months). The two-year incidence of natural infection in this hyperendemic town was 0.8% for takes, 10.5% for "non-takes" and 40% for controls (nonvaccinated children of the same age group in the town). The duration of the lesions was from 3½ to 13½ months, mainly between 8 and 11 months. This was

significantly longer than the duration of natural infection observed and measured in the same area which is between 4 and 8 months. On the basis of this field trial, it was recommended that this method be used in the hyperendemic areas of Isfahan province for the control of ZCL.

When war broke out between the Islamic Republic of Iran and Iraq, hundreds of thousands of soldiers and paramilitary men were sent to the war front in the south-west, living for months in the middle of areas heavily infected with ZCL. Thousands of cases appeared in the first two years, and spraying of foxholes and distribution of repellents were ineffective in decreasing the number of cases. Therefore, it was decided to start leishmanization in newly recruited soldiers in the first week of their three-month training, so that after this period they would become immune for the rest of their 24-month service. The same programme was agreed for members of the Revolutionary Guard.

Materials and methods

Parasite inoculum

This was prepared by culturing an *L. major* strain isolated from the great gerbil *Rhombomys opimus* in the Isfahan area in 1964 by the senior author and maintained thereafter in the central laboratories of the Institute of Public Health Research in Teheran by regular passage in outbred laboratory mice.

For each round of vaccination, parasites were cultured from infected mice in NNN medium with saline. After several passages in new culture tubes with 10–15 days interval, the fifth passage was made in 500 large screw-top containers. The liquid phase was tested for contamination (bacteria or fungi) before distribution for use. All contaminat-

ed containers were discarded. Promastigotes from the uncontaminated liquid phase were used for inoculation when the culture was 10–15 days old. The liquid phase contained $2-3 \times 10^5$ promastigotes in 0.1 ml which was inoculated intradermally by staff experienced in BCG vaccination programmes.

Programme implementation

On the morning of the day of vaccination, the liquid phase was again tested microscopically to confirm the parasites were alive and that there was no bacterial or fungal contamination; then it was transferred into 20 ml vials, which were given to the vaccinators. At the end of the working day, the used vials were brought back to the laboratory and were again tested microscopically to make sure that the parasites had remained viable.

In Isfahan, the month of February was selected for vaccination of eligible children because the transmission season is July to mid-September in this area. The programme was implemented from 1982 to 1986. It was stopped in 1986 in the hope that the non-living crude vaccine would have better results and fewer complications. Altogether, more than 160 000 children were inoculated during the above-mentioned period.

For the army and Revolutionary Guard, parasite inoculum was prepared by exactly the same method used in Isfahan. However, in this case, each round had to be repeated once a month (in Isfahan it was once a year). Technicians were sent each month to training centres to vaccinate recruits with the help of the health services staff of the province. In addition, small specialized teams were sent to the front to vaccinate those volunteers who had started their service before the programme or had missed the vaccination. This programme continued

until after the cease-fire in 1989 and was then discontinued. Altogether, 1 800 000 military personnel and soldiers, plus 6000 war refugees were inoculated.

Results

As previously mentioned, around two million people were leishmanized in total: 160 000 in the Isfahan area, 1 800 000 military personnel, 6000 refugees and a few thousand in other groups.

The rate of takes was not exactly the same in each round. In some, there were about 90% takes, in others, between 45% and 60%. In the last round of leishmanization in Isfahan before stopping the programme in the area in 1986, it was found that the rate of takes was very low (less than 15%). After careful examination, it was found that the liquid phase had been used for leishmanization on the 6th to 8th day after culturing, i.e. during the logarithmic phase of growth not at the stationary phase. This indicated that to have higher rates of takes, cultures should be used at least two weeks after culturing, not earlier.

Effectiveness of leishmanization

Isfahan area

During 1983 and 1984, a total of about 8000 cases of disease were reported in the Isfahan area. Fewer than one hundred of these were among inoculated persons, although the population of the vaccinated group exposed to sandfly bites had been much greater than the nonvaccinated group [2].

In March 1984, 12 villages in rural areas were selected to assess the effectiveness of the programme (15 were initially selected but 3 were discarded because of a large turnover in the population) [3]. The

Table 1 Comparison of the incidence of ZCL among children in vaccinated and nonvaccinated groups in the Isfahan area

Group	Total	Cases of ZCL No.	%
Vaccinated			
Takes	671	3	0.4
Non-takes	290	13	4.5
Nonvaccinated (controls)	1716	250	14.6

Table 2 Results of challenge with leishmanization in subjects receiving non-living crude vaccine (NLCV) of *L. major*

Group	No.	%
<i>Vaccinated with NLCV and BCG (n=12)</i>		
Takes	6	50
Non-takes	6	50
<i>Vaccinated with NLCV alone (n=15)</i>		
Takes	11	73
Non-takes	4	27
<i>Control (n=30)</i>		
Takes	27	90
Non-takes	3	10

incidence of the disease in children under 5 years of age is shown in Table 1.

Table 1 shows that the incidence of ZCL in the vaccinated group is one-seventh to one-eighth of the control group and it was mostly in non-takes. Furthermore, progress of the sores in the vaccinated group was rapid, i.e. small sores lasting only 1–3 months, while in the nonvaccinated group the disease ran its natural course, i.e. larger sores of 4–8 months duration.

In 1992, 516 children who had undergone leishmanization in 1982 in five villages in the Isfahan area were examined. At

the time of vaccination, they were 6 months to 5 years of age. These children had been visited 12 months after leishmanization. At that time, 155 of them (30.0%) had active sores, 307 had scars (59.5%) and 54 were non-takes (10.5%). This showed that the rate of takes had been 89.5%. During the ten-year period, 26 of them got natural sores (5.0%), 16 of whom were in the non-take group and 10 in those with scars of leishmanization (29.6% and 2.2% respectively). The sores appeared 2–8 years after leishmanization, mostly on the legs or face. Some children had 2–3 sores. If these groups had not been vaccinated, at least 75% of them would by this time have had the disease. These figures show clearly how effective leishmanization is if the rate of takes is very high.

Army and Revolutionary Guard

As the army recruits were leaving the army at the end of their service, it was not possible to evaluate the effectiveness in that group. However, members of the Revolutionary Guard are permanent members of the armed forces, so an evaluation was carried out in this group [4].

The evaluation was carried out in 1987. Altogether, 418 leishmanized cases and 675 controls were studied. The average age in the former group was 20 years and in the latter it was 21 years. The study was carried out 15 months after leishmanization. The control group had been in the endemic area for more than 18 months. Of the 418 men vaccinated, 237 (56.7%) were takes and 181 (43.3%) were non-takes. Almost two-thirds of the takes had been in the form of a nodule and one-third had ulcers; only four cases lasted for more than one year. It is possible that the rate of takes was higher than the figure shown because it is based on the answers of the soldiers and not direct observation. In these cases, many of them

may not have noticed a nodule because it has neither pain nor pruritis. The infection rate among the vaccinated group was one-sixth that in the nonvaccinated (control) group.

*Challenge of the trial of non-living crude vaccine (NLCV) of *L. major**

At the end of the first phase of a trial on 30 volunteers in Yazd province, central Islamic Republic of Iran, with a vaccine containing killed promastigotes of *L. major* (the same strain used for leishmanization) [5], 27 of these subjects plus 30 controls were leishmanized to compare the response of vaccinated and nonvaccinated groups. The results are shown in Table 2.

There is a statistically significant difference in the rate of takes between those who received NLCV + BCG and the control group. The sores started as early as 3 weeks after leishmanization. In 10 cases, the sores were big and had to be treated (6 in the vaccinated and 4 in the control groups). In 6 cases, lesions lasted more than 12 months; all recovered either without treatment or after some treatment.

Untoward effects and complications of leishmanization

- a) There can be an immediate type of hypersensitivity reaction lasting a few hours. This is very rare and only occasionally requires treatment.
- b) About half to two-thirds of the takes will ulcerate depending on the lots used for leishmanization. The sores at the site of inoculation, even when they are small (5–10 mm in diameter), can cause problems for the vaccinated person because they last about 8–9 months, some of them even longer. About 5% of takes develop large sores (greater than 20 mm in diameter), with heavy secondary infection. This secondary infection is not

a result of contamination of the inoculum. These cases should be treated with Glucantime plus an antibiotic (we usually recommend erythromycin).

- c) In 0.5%–1% of takes, there is a cheloid formation at the site of inoculation, without any sore. This may be due to the unpurified liquid phase which contains part of the medium, dead parasites and other materials. During the war, the facilities to wash and purify promastigotes were not available.
- d) In 2%–3% of cases, the disease can last for more than one year and some need to be treated.
- e) The most important complication are non-healing cases that occur in about 1–2 per 10 000 inoculations. Even now (in 1996), there are people who still have active sores with parasites; this is almost 12 years after their leishmanization. It seems that there is some defect in their immune response as regards leishmaniasis and some of them have responded to immunotherapy.
- f) In one case there was a medical problem; this was a woman of 19 years of age who voluntarily applied for leishmanization in Isfahan. She suffered from a chronic muscle disease for which she had been under corticosteroid therapy for several years but she did not mention it at the time of leishmanization. She developed a very large deep sore lasting for more than one year; then small lesions (containing amastigotes) appeared on her arms, legs and body. She was asked to stop taking corticosteroid medication for a few months, with the permission of her physician, and was successfully treated with Glucantime.

Leishmanin skin test after leishmanization

We have deliberately avoided this aspect because different types of antigen have been used in different studies. Even at the present time, conflicting results are being reported from various studies in the Islamic Republic of Iran. The percentage of people in various endemic foci of the country who have a positive reaction to this test without having a history of the disease is not known. The first field trial showed that two years after inoculation there were 96% positive skin tests in the takes and 76% positives in the non-takes. No figures are available for controls. In the study on the Revolutionary Guard, a high percentage of controls were also skin-test positive. Further studies are needed, using a standard leishmanin, to identify the exact effect of leishmanization on the leishmanin skin test response.

Conclusions

All the studies mentioned above show that leishmanization reduced the incidence of the disease in hyperendemic areas and in high-risk groups between one-sixth and

one-eighth of its original level. Despite its complications, it should be recommended wherever people are at very high risk of contracting the disease. Although it produces lesions lasting several months, this is in a covered area and it prevents disfiguring sores on the face and also multiple sores. Non-healing cases, although relatively rare, cause some problems (we never saw such cases in leishmanized children) and in any decision about leishmanization, this should be taken into consideration. In small scale trials on any type of vaccine against leishmaniasis, a challenge at the end of the vaccination programme would give quicker and more clear-cut results than waiting to compare natural incidence in one to two years, especially in areas with low endemicity.

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