Isolation of cutaneous leishmania parasite by using RPMI1640 and Schneider drosophila media

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الخلاصة
أجريت هذه الدراسة في محافظة النجف الاشرف للمدة من1/9/2007 إلى 1/4/2008 باستعمال نوعين من الأوساط الزرعية و ذلك لتحديد وقت الإفاقة ألازم لعزل طفيلي اللشمانيا الجلدية.

Abstract

The study was done in Najaf Government from 1.9.2007 to1.4.2008. The aim of the
study was to isolate the *Leishmania* parasite and to determine the recovery time by using two different Medias: Schneider drosophilae and AMP 1640 with fetal calf serum, the result showed that the former medium has short recovery time than the latter medium and more rapid in isolation of the parasite.

Introduction

The *Leishmania* parasite is a protozoan which belongs to the order of Kinetoplastida and family Trypanasomatidae and the genus *Leishmania* include more than 20 species(3). The parasite exists in biphasic forms. The flagellated promastigote (15-30 p.m) in length found in vector and culture media and the non flagellated amastigote (3-5 !Am) which live in macrophage of mammalian host in which can survive and multiply with in the acidic phagosomes of the host cell (3). Culture is one of methods used in isolation of leishmaniasis causative parasite, Nicolle was the first who use Nove-MacNea-Nicolle media (NNN) for isolation of agent from orient sore in 1904 then many other Media used for isolation and cultivation of leishmania in the world. In Iraq there is few trials used only NNN media and semi-solid media for Isolation and cultivation of leishmania then a RBLM (Rasaam Basher Liquid modified)Media made by Al-Bashir (2).

Materials and methods

Sampling:

The samples of the patients were collected Randomly from Dermatology consultant unite in AL- SADER teaching hospital in AL- Najaf governorate in beginning of December 2007 to the end of April 2008. These patients were attended complained from skin lesions mostly in exposed part of the body as the face, leg, arm and clinically diagnosed as cutaneous Leishmaniasis.

Procedure of Sample Collection

The sample from the cutaneous lesion tacked by fine needle aspiration as the following steps.

1- Disinfect the lesion and skin surrounded the lesion.
2- Use sterile syringe of lml contain 0.4 ml of sterile normal saline and
3- inject the fluid intradermaly through intact skin in to the active red border of the lesion.

4- Aspirate the injected fluid as the needle drawback till the bloody stained fluid aspirate.
5- Take small amount of this aspirated fluid and stained it after fixation by absolute methanol then used Giemsa stain to see Amastigote which diagnosed as round or spherical shape with kinetoplast.
6- inoculate 0.05 ml of this aspirated fluid into five ml of media. This media incubated in 26°C then examination done every two day using 40 x power of compound microscope searching for initiation of growth.

Calculation of active promastigotes was done every day by using 40 x to confirm the diagnosis then 10 x power was used for calculation the number of active promastigotes seen in haemocytometry (WBC chamber). Calculation done by the following equation:

Total number of promastigotes in ml = the number of promastigotes in 64 small square of haemocytometry ^{x} 2 5 x dilution degree ^{x} 103 **Schneider's**

Drosophila media with Glutamine.

It was prepared by adding 500 ml of this liquid media to 150 ml (30%) of preheated fetal calf serum then the PH has been justified to 7.4 then sterilization done by Nalgen filter of 0.22 micrometer then incubated in 37°C for 24 hour to ignore the contaminated tube and the sterile one kept in 4°C till use (10)

RPM I 1640 (Roswell park medium institute)

This media was prepared by adding 10.4 gram of powder media in 900 ml of distill water and 100 ml of preheated fetal calf Serum (55°C for 50 minute) then added 1 ml of previously prepaid antibiotic solution and

the PH was justified to 7.2. Sterilization has been done by Nalgen filter of 0.22 micrometer then distributed 5 ml of media in sterile tube of 10 ml size and incubated in 37°C for 24 hour to ignore the contaminated tubes and the sterile tubes put in 4°C till use.

Result

Growth of Isolated Cutaneous *Leishmania* parasite from Patient in Schneider Drosophila Media with 30% FCS Growth curve of isolated cutaneous *Leishmania* parasite in 26C°, in which recovery time at seventh day of inoculums time. The peak reach at thirteenth day from inoculation then decline occur for six days after peak

Growth of Isolated Cutaneous *Leishmania* parasite from Patient in RPM! 1640 with 10 % FCS

Growth curve of isolated cutaneous *Leishmania* parasite in 26°C in

Which the recovery time was at eighth day from inoculums time, peak reach at thirteenth day from inoculation time then decline occurred for

seven days from the peak till reach to zero in number at twenty one day of inoculation.

Discussion

The present study concludes that Schneider Drosophila media is most Sensitive media for *Leishmania* parasite isolation and this agree with result of (10) as he concludes that Schneider media was the most sensitive media for *Leishmania* detecting from patient and This agree with result of (5) when reach that Schneider Drosophila media was the most sensitive media for isolation of post-kala—azar dermal Leishmaniasis strain.

According to figure (1), has been found that the recovery time of leishmania parasite was at seven day of inoculation time in this media and this earlier to the result of (1) when use biphasic blood agar media with 0.1 ml of Schneider Drosophila to isolate **F. *tropica*** as he found that the recovery median time was nine and half day this difference

because of additional material was used and our result was earlier than the result of (8) who found that the median of recovery time in Schneider Drosophila media with 20% fetal calf serum was nine day and this may be due to

lower fetal calf serum percentage and that result is closer to the result of (6) as he found that the recovery time in above media was at sixth day

Isolation of *Leishmania* Parasite in RPM! 1640 10% FCS

Figure (2) of this study proves that growth of isolated cutaneous *Leishmania* was at thirteen day of inoculation which is longer to the time of result of (7) who use RPMI1640 and found that the growth peak of isolated cutaneous *Leishmania* was at eight day and this difference may be due to different strain of this parasite or different culturing environment.

The same figure shows that the recovery time of *leishmania* parasite was eight day and this time is longer to the result of (4) who found isolation time in RPMI 1640with FCS was six day, this could be due to different culture method used.

In that figure promastigotes growth over three to fourteen day of culture time was lx ^{106} cells /m1 and this result is lower than the result of (9) that isolates amastigote of ***L. guyanensis*** *and* ***L. panamensis*** from infected human macrophage in RPMI 1640 who proves that the cell yield was 4-5x10^{7} cell\ml in nine to ten day of culture period and this may be due to different conditions.

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