

IMMUNOLOGICAL INVESTIGATION OF MALARIA AND NEW PERSPECTIVES IN PALEOPATHOLOGICAL STUDIES

N. Cerutti, A. Marin, E. Rabino Massa, *D. Savoia

Department of Animal and Human Biology,

*Department of Clinical and Biological Sciences, University of Turin

INTRODUCTION

Today numerous new malaria diagnostic techniques are available. Some attempt (1) to improve the sensitivity and facilitate the execution of conventional light microscopy by concentrating the parasites in the blood sample, by improving their visualization and detection by means of staining with fluorescent dyes, or by a combination of these approaches i.e. the quantitative buffy coat technique and the Kamamoto fluorochrome technique (2). All these methods vary in complexity and need sophisticated equipment, but none so far have been shown to have the wide applicability of light microscopical examination of Giemsa-stained blood films for malaria diagnosis. Various other diagnostic methods have been based on the identification of parasite-specific molecules such as antigens and nucleic acids; the sensitivity in some cases may be improved by the polymerase chain reaction (PCR) technique. These assays have been designed to detect parasites with limits of detection equal to, or better than, those provided by light microscopy.

Recent advances have, however, made it possible to consider the feasibility of supplementing light microscopy with a standardized antigen detection test using high technology production methods.

Most of the new malaria diagnostic techniques could be applied to ancient remains like mummified, skeletal and dental specimens. This would lead to new and interesting perspectives in paleopathological studies.

MATERIALS AND METHODS

We studied 72 individuals coming from an Egyptian population dated about 3200 (B.C.) from the archeological site of Gebelen (Luxor area), which belongs to the Anthropological and Ethnographic Museum of Turin (Italy). The specimens were collected by Prof. Marro during the Italian Archeological Mission, coordinated by Prof. Schiaparelli, from 1911 to 1923.

In previous works (3,4) extensive anatomical and histological investigations have diagnosed severe anemia in some of these mummies, probably due to thalassemia and sicklemlia. The distribution of thalassemia and sicklemlia fits quite well with the larger pattern of Plasmodium falciparum malaria. In fact the hemoglobinopathies represent ecological mutation able to protect the individuals from some infections including malaria. Owing to these observations, we investigated the presence of malaria in the Egyptian mummies.

P. falciparum parasites synthetize several proteins containing large amounts of the aminoacid histidine. One of these proteins PfHRP-2, that has been sequenced and cloned, shows to be actively secreted as a water soluble protein from parasite-infected erythrocytes and used for a diagnostic kit.

We applied this technique based on ELISA immunoenzymatic assay for qualitative detection of the histidine-rich protein, effectuating some modifications (5) in order to apply this method to ancient remains like mummified tissues (skin, muscle), bone and dental samples.

Parallely to the immunological detection of malaria, we examined patterns of severe anemia by means of anatomical and radiological diagnosis in order to ascertain if malaria might play a role in skeletal alterations. According to Angel (6) we observed the outer lamina of the skull for the occurrence of porotic hyperostosis and cribrous areas in the orbital roofs as well as on the parietal and occipital bones.

RESULTS

The mummified remains resulted positive to the immunological test in 42%

of the cases.

This frequency of Plasmodium falciparum malaria is compatible with other observations effected on modern populations living in similar ecological conditions, i.e. marshy areas.

Extensive bone pathologies, in particular the typical patterns of severe anemia, have been observed in the skeletal remains. In fact 61% of the individuals present patterns of porotic hyperostosis and cribra, a value similar to the average of other ancient populations with analogous life habits, such as agropastoral groups.

Although there are various causes of hyperostosis and cribra orbitalia (nutritional deficiency, hemoglobinopathies, Fe deficiencies etc.), it is quite interesting to observe a very high frequency (92%) of these patterns in subjects positive for Plasmodium falciparum infection, as demonstrated in our results. This suggests that malaria was an important cause of anemia in this population.

DISCUSSION

Paleoimmunological studies have provided an important contribution to the knowledge of diseases, living conditions and evolutionary process in ancient populations. Immunological analyses have lead to the identification of several parasitic infestations like schistosomiasis (7), hydatid cysts (8), Taenia, Filaria infections, at the same time of P. falciparum (9) infection. This indicates that the immunological method is particularly suitable for interesting investigations of paleopathological problems.

The immunological test employed in this study has been shown to provide consistently reproducible results with a threshold of detection of P. falciparum parasitemia similar to that obtained by high quality malaria microscopy and with a high specificity and sensitivity of about 90%. We have found that the PfHRP-2 antigen can be detected not only in mummified tissues, but also in dental and skeletal remains, with stability, over more than 5000 years. The PfHRP-2 antigen detected in the assay supports the potential value and interest of the paleoimmunological diagnosis in investigating the distribution of different diseases, such as malaria.

This leads to the possibility of extensive investigation of the presence of malaria in ancient populations of the Mediterranean area.

We applied a paleoimmunological investigation, using an immunoenzymatic assay revealing trophozoite derived Plasmodium falciparum histidine rich protein-2 antigen (PfHRP-2). The investigation was carried out on skin, muscle and bone samples. We examined predynastic egyptian mummies (3200 B.C.) from Gebelen, belonging to the Marro's Collection of the Anthropological and Ethnographic Museum of Turin, to assay the presence of malaria.

The results obtained suggest an incidence of malaria of about 40% in the mummies of Gebelen group. Data are compatible with other observations effected on populations living in similar ecological conditions of malarial areas.

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Address reprint requests/correspondence to Prof. E. Rabino Massa, Dept. of Animal and Human Biology, University of Turin, via Accademia Albertina 17, I-10123 Torino.