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Universidad de Tarapacá
Arica, Chile

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MALARIA IN ANCIENT EGYPT: PALEOIMMUNOLOGICAL INVESTIGATION ON PREDYNASTIC MUMMIFIED REMAINS

Emma Rabino Massa*, Nicoletta Cerutti*, A. Marin D. Savoia**

We examined samples from the Marro's Collection, belonging to the Anthropological and Ethnographic Museum of Turin, to determine the presence of malaria antigens. The specimens we assessed belong to predynastic mummies (3,200 B.C.) from Gebelen. For detection of malaria, we applied a paleoimmunological investigation, using an immunoenzymatic assay (Para Sight™ - F test) revealing trophozoite derived from Plasmodium falciparum histidine rich protein-2 antigen (PfHRP-2) on skin, muscle and bone samples. Excluding only the poorly conserved mummies, we analyzed about fifty of the 85 individuals of this collection. In these Egyptian mummies we have detected the presence of malaria, according to the observation of thalassemia in the same mummies, as reported in previous paper (E. Rabino Massa 1977). In parallel, we undertook a morphological study on the skeletons to detect macroscopic signs of malaria, to confirm immunological results. The skulls exhibit the "hair-on-end" pattern, while the vertebrae often display compression of the central portions of the vertebral bodies. The results obtained suggest an incidence of malaria of about 40% in the mummies of the Gebelen group examined. These data are compatible with other observations effected on populations living in similar ecological conditions of malarial areas.

Key words: Plasmodium falciparum, paleoimmunology, ancient Egypt.

Examinamos muestras de la Colección de Marro del Museo Antropológico y Etnográfico de Turín para analizar la presencia del antigeno de la malaria. Los especímenes que analizamos pertenecen a las momias predinásticas (3,200 A.C.) de Gebelen. Para detectar malaria, hemos aplicado una investigación paleoimunológica usando un análisis inmunoenzimático para revelar trofozoitos derivados del Plasmodium falciparum, del antigeno proteína-2 rica en histidina (PfHRP-2). Excluyendo sólo las momias mal conservadas, analizamos 50 de los 85 individuos de esta colección. En estas momias egipcias hemos detectado la presencia de malaria de acuerdo con la observación de thalassemia en las mismas momias, como fue reportado en el estudio de E. Rabino Massa (1977). Paralelamente, efectuamos un estudio morfológico en los esqueletos para detectar señales macroscópicas de malaria, a fin de confirmar los resultados inmunológicos. Los cráneos exhiben el patrón de "pelo en punta", mientras las vértebras demuestran compresión de la porción central de los cuerpos vertebrales. Los resultados sugieren una incidencia de malaria de 40% en las momias del grupo Gebelen examinado. Estos datos son compatibles con otras observaciones efectuadas en poblaciones vivas en condiciones ecológicas parecidas de áreas malariales.

Palabras clave: Plasmodium falciparum, paleoimunología, antiguo Egipto.
The Egyptian collection of the Anthropological and Ethnographic Museum of Turin, Italy, was collected by Prof. Marro during the Italian Archeological Mission from 1911 to 1923. This collection is composed of about 2000 skeletal and mummified remains, of which 20 are complete mummies. All individuals come from the archeological sites of Assiut and Gebelen, located in Upper Egypt.

Extensive research on this collection has revealed the presence of severe anemia in ancient Egypt. It has been possible to observe well preserved red cells in histological preparations of mummified tissues. In sections of blood vessels of one individual, hypochromatic red cells were found (Rabino Massa, 1967). At first this hypochromacy was interpreted as the poor ability of benzidine to interact with the hemoglobin molecule. More accurate observations revealed the existence of hemolytic disorders, probably thalassemia and sicklema. In fact, "target cells" and sickle cells could be observed in histological preparations of one individual, indicating pathological variations of hemoglobin.

Figure 1 shows a "Target cells" and sickle cells together with normal red cells of a mummy.

Radiographic examination confirmed the existence of hemoglobinopathy (Massa 1977): in some mummified heads there was the classical presence of "brush skulls".

Porotic hyperostosis occurs in many other ancient populations of the Mediterranean area (Ascenzi et al. 1985). This distribution fits quite well with the larger pattern of Plasmodium falciparum malaria and the Old World occurrence of thalassemias and sicklema. The hemoglobinopathies represent ecological mutations able to protect the individual from some infections, including malaria.

In view of the observations of thalassemia and sicklema by anatomical and histological diagnosis, we investigated the presence of malaria in these mummies by means immunological methods.

Paleoimmunological studies have provided an important contribution to the knowledge of diseases, living conditions and the evolutionary process in ancient populations.
Immunological analyses on Egyptian mummies have led to the identification of several parasitic infestations like schistosomiasis (Miller et al. 1994), hydatid cysts (Lewin 1968), Taenia, P. Falciparum (Curry in David 1979) and Filaria infections.

**Material and Methods**

We used an ELISA immuno-enzymatic assay generally applied to blood samples in medical diagnosis for the qualitative detection of the histidine-rich protein (PfHRP-2), a trophozoite derived from *Plasmodium falciparum*. This antigen is a water-soluble protein released from parasitized erythrocytes. We made some modifications (Miller et al. 1994) in order to apply this method to ancient remains like mummified tissues (skin, muscle), bone and dental samples. Parallel 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 (Tayles 1996).

According to Angel (1966) 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.

We examined about 80 predynastic individuals dated 3200 (B.C.) from the archeological site of Gebelen (Luxor area). We could test 88% of them (Figure 2a): in 43% of those tested, we could investigate both the presence of *Plasmodium falciparum* infection and hyperostosis; in 33% we performed only morphological analyses because we could not find suitable mummified material for immunological tests; in contrast, in 24% we could test for malaria but could not investigate macroscopic signs of anemia at the skeletal level.
Results and Discussion

The mummified remains were positive to the immunological test for *Plasmodium falciparum* in 42% of cases (Figure 3). This frequency of malaria is compatible with other observations effected on populations living in similar ecological conditions, i.e. marshy areas. Extensive bone pathologies, indicating severe anemia, were observed in the skeletal remains: 61% of individuals (Figure 4) present the typical skeletal patterns of severe anemia (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 deficiency, etc.), it is quite interesting to observe a very high frequency (92%) of these patterns in subjects positive for *Plasmodium falciparum* infection (Figure 5). This suggests that malaria was an important cause of anemia in this population.

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* Department of Animal and Human Biology, University of Turin, Via Academia Albertina, Turin, Italy.

** Department of Clinical and Biological Sciences, University of Turin, Italy

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