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Letter to the Editor

Determination of Human Chorionic Gonadotrophin (Beta Subunit) in Saliva and Its Role in Trophoblastic Diseases*

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HUMAN chorionic gonadotrophin (HCG) is a glycoprotein secreted by the trophoblast of the developing blastocyst and later by the chorion and placenta of the pregnant woman, and determination of the level of the hormone in urine or plasma has been found to be a most reliable means of detecting early pregnancy. Furthermore, HCG is found to be elevated in patients with neoplasms of trophoblastic origin such as hydatidiform mole and choriocarcinoma and in some patients with carcinoma of the breast or testis [1, 2]. As a consequence of earlier studies from these laboratories which clearly demonstrated the value of assays for steroids in saliva [3–6], with the obvious advantages of ease and the non-invasive nature of the sample collection, a sensitive radioimmunoassay was established to determine the concentration of the beta subunit of HCG (β-HCG) in saliva.

The assay employed an antiserum, raised in rabbits against β-HCG, kindly provided by Prof. W. R. Butt of the Birmingham and Midland Hospital for Women, Birmingham, U.K. Purified β-HCG (CR 115B), a generous donation from the National Institute for Arthritis, Metabolism and Digestive Diseases (NIAMDD), Bethesda, MD, U.S.A. was iodinated with ^{125}I using chloramine T, following the method of Greenwood *et al.* [7]. The precipitating second antibody was raised in sheep against rabbit gammaglobulins. Samples of saliva (200 μl) and diluted (1:50–1:500) plasma (200 μl) were incubated with antiserum and [^{125}I]-β-HCG overnight at 4°C. Following incubation, precipitating serum was added and the tubes were further incubated for 2 hr at room temperature, or overnight at 4°C. The tubes were then centrifuged (1200 *g*; 30 min at 4°C) and radioactivity associated with the pellet was measured on an NE 1600 γ-counter.

The dose–response curve covered the range 0–320 IU/l (Ist International Reference Preparation, 75/537). The lower limit of detection at the

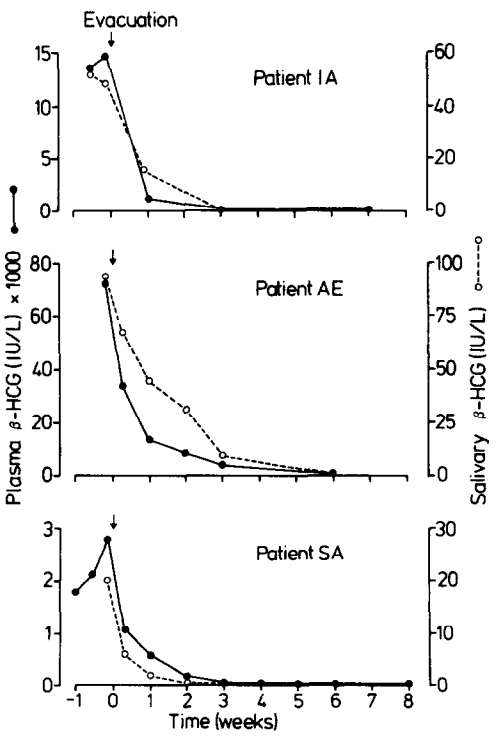

Fig. 1. Plasma and salivary β-HCG concentrations in patients with hydatidiform mole.

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95% confidence level was 0.5 IU/l. Estimated intra- and inter-assay coefficients of variation over the working range of the curve were 5.2–7.8% and 6.8–11.2% respectively. The antiserum had negligible cross-reactions with the pituitary gonadotrophins and there was no significant deviation from parallelism in the dose–response curves obtained using standard hormone in buffer and diluted saliva samples. The assay has been shown to conform to accepted validation criteria and was subsequently used to determine β-HCG concentrations in saliva and plasma samples from subjects admitted to hospital with suspected trophoblastic diseases.

Matched samples of plasma and saliva were collected before and after treatment of patients diagnosed as having hydatidiform mole. All samples were stored below −20°C until assayed. Saliva samples were centrifuged prior to assay to exclude particulate matter. Data obtained from the determination of β-HCG in samples from patients with hydatidiform mole showed considerable variations, with plasma and salivary β-HCG concentrations ranging from 2850 to 72200 and 20 to 95 IU/l respectively prior to the evacuation of the mole. Examples of the results obtained are shown in Fig. 1. The β-HCG concentration was found to decline within two days after the mole had been evacuated by suction dilation and curettage. β-HCG could not be detected in the subsequent plasma and saliva samples, collected 3–6 weeks after evacuation to monitor the remission of the disease.

The results demonstrate that the immunoreactive β-HCG may be detected in saliva using suitably sensitive radioimmunoassay techniques. Although concentrations are low, less than 1% of the circulating plasma β-HCG levels, they clearly indicate similar changes to those found in plasma. The determination of β-HCG in saliva could well be of major advantage in the clinical assessment of patients with hydatidiform mole, including choriocarcinoma, where frequent monitoring is important.

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