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Protein-Calorie Malnutrition (PCM) in Egypt*)
Immunological changes of salivary protein in PCM

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With 1 figure and 3 tables

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During the last few years, important strides have been made in the separation and identification of the complex mixture of saliva proteins. Despite the occurrence of PCM all over the world and its prevalence in under-developed areas, it is surprising how little is known concerning variations in the saliva proteins in the disease. On revising the available literature published during the last decade, it was realized that the data are few. The saliva can be easily collected without pain or harm to the patients and at the same time changes in its constituents might reflect changes in both function of salivary glands as well as might reflect changes in the internal environment. Protein secretion and catabolism seemed to be regulated by the submaxillary and sublingual glands (13). The protein synthetic capability of the submandibular gland in rat is partially controlled by diet (14).

Studies using free electrophoresis have demonstrated that human salivary secretion contains a complex mixture of protein (17) (7).

Immunochemical experiments have established that at least 7 blood proteins including albumin and gammaglobulins are present or have their counterparts in normal saliva (4) (20). *Strober* et al. (21) found that the albumin specific activity in serum and saliva was equal, whereas the salivary IgG specific activity was approximately 45 % of that in serum, indicating that all the salivary albumin and about half of the salivary IgG originate from the plasma. The specific activity of IgA in saliva was only 1 to 8 % of that of serum at the corresponding time. Therefore at least 96 % of the salivary IgA is synthesized at local sites. Thus one may arrive to the conclusion that the major salivary glands are not always the main contributors of the proteins.

The present work was performed to study the changes of salivary protein components in order to realize the state of salivary glands in different grades of our malnourished cases.

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Table 1. Values for saliva total proteins and their separated protein

Disease	Tot. prot.	Pre-albumin	Albumin	α_1-Anti-trypsin	α_2-Macro-globulin
Control	15 — 101		0.00 — 29.00	0.00 — 8.00	
(15)	67 ± 6		19.04 ± 2.25	4.21 ± 0.76	
2nd Mars.	27 — 170	0.00 — 5.30	3.29 — 46.12	0.00 — 9.64	0.00 — 3.49
(22)	65 ± 7	1.16 ± 0.34	17.79 ± 2.20	4.52 ± 0.69	0.16 ± 0.16
p^c	0.1	0.001	0.1	0.1	0.1
3rd Mars.	24 — 170	0.00 — 4.00	4.33 — 53.46	0.00 — 19.00	
(15)	80 ± 11	1.19 ± 0.40	22.48 ± 4.02	4.64 ± 1.31	
p^c	0.1	0.01	0.1	0.1	
p^d	0.1	0.1	0.1	0.1	
Mod. Kwo.	62 — 227	0.00 — 6.74	20.16 — 57.06	0.00 — 14.74	0.00 — 6.40
(9)	133 ± 20	2.16 ± 0.93	35.48 ± 4.56	7.75 ± 1.83	1.56 ± 0.74
p^c	0.1	0.05	0.01	0.1	0.05
Sev. Kwo.	75 — 501	0.00 — 10.87	12.26 — 191.0	3.85 — 18.78	0.0 — 25.04
(19)	243 ± 53	3.41 ± 1.54	57.52 ± 18.70	10.62 ± 1.75	5.83 ± 2.80
p^c	0.001	0.1	0.05	0.001	0.05
p^d	0.1	0.1	0.1	0.1	0.1
Mars. Kwo.	30 — 341	0.00 — 8.28	2.79 — 92.51	0.00 — 45.32	0.00 — 8.48
(15)	125 ± 27	1.64 ± 0.78	32.35 ± 9.90	10.22 ± 3.31	0.56 ± 0.20
p^c	0.05	0.05	0.1	0.05	0.01

p^c when compared to control
p^d when compared to disease
0.1 not significant

Material and methods

A number of 70 PCM cases admitted to the Mounira Children Hospital, University of Cairo, form the material of this study. Their age ranged from 6 to 36 months. Cases were selected free from infection or any associated complication. On clinical reasons and as shown previously (18), cases were categorized into:

37 maramus (22 2nd grade, 15 3rd grade)
18 kwashiorkor (9 moderate, 9 severe)
15 marasmic kwashiorkor

15 healthy infants and young children of similar age range and socio-economic standard were included to serve as controls. A fasting morning whole saliva sample was collected from each subject during an optimum interval of 36 minutes. After cleaning the buccal cavity, sterilized cotton plugs were placed in the mouth using sterile forceps. Samples collected during the first 5 minutes were rejected. These cotton plugs newly administered after being soaked were placed in a plastic syringe, compressed, and the contents were received in a Wassermann tube.

Up to 4–5 ml of saliva from each patient were collected in this way. Samples were then centrifuged for 15 minutes at 3000 rpm. and the supernatant was separated in another tube. Saliva was then analysed for total proteins by the technique, described by *Pesce* and *Strande* (16). Electrophoretic separation of

components mg/100 ml in controls and malnourished cases.

Hapto-globin	Trans-ferrin	β_2-Lipo-protein	IgA	IgM	IgG
0.00 — 13.13	0.00 — 17.88	0.00 — 6.00	5.43 — 40.25	0.00 — 4.60	0.00 — 15.00
6.63 ± 1.33	7.64 ± 1.55	1.25 ± 0.51	20.96 ± 3.02	1.18 ± 0.46	5.32 ± 0.94
0.00 — 19.07	0.00 — 16.12	0.00 — 4.00	0.00 — 41.23	0.00 — 5.55	0.00 — 25.24
6.99 ± 1.15	7.70 ± 1.08	0.82 ± 0.33	14.07 ± 1.79	2.02 ± 0.42	7.54 ± 1.33
0.1	0.1	0.1	0.1	0.1	0.1
0.00 — 18.00	0.00 — 21.00	0.00 — 5.55	3.61 — 44.56	0.00 — 7.00	0.00 — 30.00
7.21 ± 1.23	8.22 ± 1.75	1.37 ± 0.55	18.73 ± 3.26	2.01 ± 0.71	12.25 ± 1.94
0.1	0.1	0.1	0.1	0.1	0.01
0.1	0.1	0.1	0.1	0.1	0.1
2.77 — 23.00	2.24 — 24.00	0.00 — 5.27	3.56 — 46.00	0.00 — 10.00	12.32 — 59.0
11.36 ± 2.60	13.13 ± 2.60	1.93 ± 0.79	23.22 ± 4.04	4.64 ± 1.31	30.43 ± 5.70
0.1	0.1	0.1	0.1	0.5	0.001
2.75 — 46.9	7.36 — 58.43	0.00 — 10.00	10.3 — 110.0	0.00 — 20.14	9.81 — 130.0
19.70 ± 4.13	23.8 ± 6.56	4.42 ± 1.48	49.8 ± 10.90	8.56 ± 2.61	61.51 ± 15.10
0.01	0.05	0.1	0.05	0.01	0.001
0.05	0.1	0.1	0.05	0.1	0.1
0.00 — 54.28	0.00 — 33.10	0.00 — 24.80	0.00 — 48.71	0.00 — 18.52	6.83 — 95.42
13.47 ± 3.54	9.13 ± 2.13	3.02 ± 1.68	21.51 ± 3.63	3.87 ± 1.59	28.08 ± 6.98
0.1	0.1	0.1	0.1	0.1	0.001

0.05 significant
0.01 moderately significant
0.001 highly significant

saliva total proteins was as given by *Johanson* (9) and quantitated (3) by the addition of 2 ml. N/50 NaOH. Antisera to IgA immunoglobulin (anti α-chain) was produced by the method described by *Brandtzaeg* (1). Estimation of IgA component as carried out by the rocket immunoelectrophoresis (11).

Results and discussion

Values of saliva total proteins and their separated components from 15 healthy control children and 70 malnourished cases are shown in table 1. Table 2 shows the concentration of immunoglobulins and the ratios of IgG to IgA and IgM in unstimulated whole saliva of control group and malnourished cases. The ratios of IgG/IgA and IgG/IgM were high in non-oedematous and more higher than normal in oedematous cases. Table 3 shows values for IgA immunoglobulin, derived from salivary glands and from blood serum in 10 healthy control children and 48 cases of different grades of PCM. The data obtained are represented in figure 1.

From our biochemical studies on PCM, it seems necessary to analyze whole saliva proteins, since the blood plasma and tissue biopsy cannot be easily handled from a great number of young severe malnourished children and to compare with another group of controls. Also, it is valuable to

Table 2. Concentration of immunoglobulins (mg/100 ml) in whole saliva of control and malnourished cases.

Classification groups	No. of cases	IgA average	IgA range	IgM average	IgM range	IgG average	IgG range	Concentration IgG/IgA	Ratio IgG/IgM
Control	15	20.96	5.43 — 40.25	1.18	0.00 — 4.60	5.32	0.00 — 15.00	0.25	4.51
Marasmus									
2nd grade	22	14.07	0.00 — 41.23	2.02	0.00 — 5.55	7.54	0.00 — 25.24	0.54	3.70
3rd grade	15	18.73	3.61 — 44.56	2.01	0.00 — 7.00	12.24	0.00 — 30.00	0.65	6.09
Kwashiorkor									
moderate	9	23.22	3.56 — 46.00	4.64	0.00 — 10.00	30.43	12.32 — 59.00	1.31	6.56
severe	9	49.80	10.30 — 110.00	8.56	0.00 — 20.14	61.51	9.81 — 130.00	1.24	7.19
Marasmic kwashiorkor	15	21.51	0.00 — 48.71	3.87	0.00 — 18.52	28.08	6.83 — 95.42	1.30	7.25

Table 3. IgA immunoglobulin (mg/100 ml) derived from blood serum and salivary glands in control and malnourished cases.

Classification groups	No. of cases	IgA immunoglobulin derived from: blood serum	salivary glands
Control	10	1.50 — 5.50	2.00 — 28.60
		3.72 ± 0.41	12.00 ± 2.56
Marasmus, 2nd grade	10	1.80 — 10.00	4.20 — 31.23
		5.03 ± 0.98	12.12 ± 2.50
		0.10	0.10
3rd grade	10	2.10 — 11.00	3.81 — 23.33
		5.58 ± 0.97	11.22 ± 2.12
		0.10	0.10
Kwashiorkor, moderate	9	2.00 — 27.00	1.56 — 19.00
		13.00 ± 2.50	10.22 ± 1.84
		0.001	0.10
severe	9	7.00 — 95.00	3.30 — 15.00
		41.88 ± 9.80	7.92 ± 1.18
		0.001	0.10
Marasmic kwashiorkor	10	2.60 — 23.30	2.00 — 15.31
		12.06 ± 2.26	8.32 ± 1.46
		0.001	0.10

study the effect of protein deficiency on the actual state of salivary glands on malnourished children. Salivary constituents do not attain a substantial rate of secretion before 8 minutes (15). Our collection of saliva was performed at 30 minutes. The utilization of different stimulators sometimes for saliva secretion used by some authors may contribute to the variation of immunoglobin levels (1). As a result of the different studies previously done (6, 10, 12, 22, 23), it is now accepted that 13 protein components (prealbumin, albumin, α_1-antitrypsin, haptoglobin, α_2-macroglobulin, transferrin, lipoprotein, IgA, IgM, IgG, α_1-acidic glycoprotein, α_1-lipoprotein and caeruloplasmin), occur in human saliva. In our control group only 8 protein components could be detected. In agreement with similar findings (6, 20, 23), albumin and IgA were the components most excreted in large amounts in saliva of our controls, followed by transferrin, haptoglobin. IgG, α_1-antitrypsin, β_2-lipoprotein and IgM in decreasing sequence.

In our marasmic cases, insignificant changes of saliva protein was obtained (slightly diminished in 2nd marasmus and slightly increased in 3rd grade as compared to control). IgA immunoglobulin was below normal in both grades of the disease. In edematous cases, a marked increase amount of saliva protein content was associated with high level of some protein components. Similar finding is supported by *Menaker* and *Navia* (15), reporting that protein concentration in saliva, as monitored on days 16, 24, 35, was always greater in severe malnourished rats when compared to control group.

PCM particularly kwashiorkor is characterized by a state of hypoaminoacidaemia (19). The later state of deficiency is believed to affect the

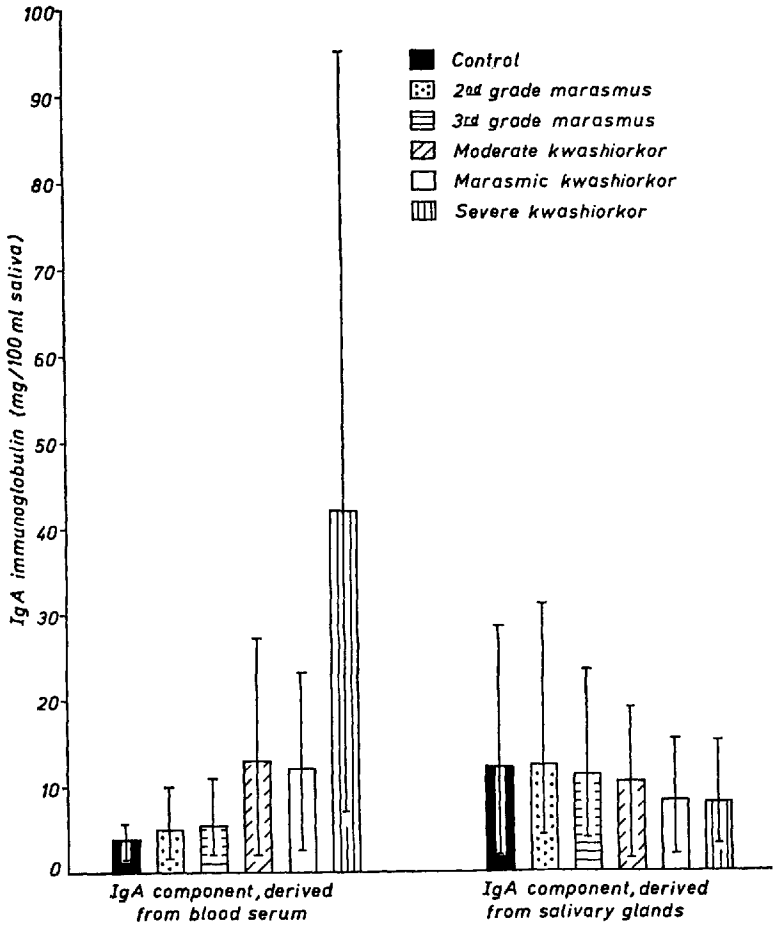

Fig. 1. IgA immunoglobulin (mg/100 ml), derived from blood serum and salivary glands in (10) healthy controls and (48) malnourished cases.

rate of protein synthesis in the different organs. According to this interpretation, one would expect that the salivary protein output is decreased as a result of restricted synthesis. However, our data showed high saliva protein content (signs of inflammatory reaction, α_1-antitrypsin, haptoglobin and transferrin) in kwashiorkor cases. *Enwonwu* (5) has confirmed atrophic edematous submandibular gland in rats with PCM. *Herschel* and *Tsuneo* (8) also reported atrophy with pathological lesion of the parotid gland in rats with amino acid deficiencies. This may explain the abnormal secretion of protein in saliva of our severe cases as one reflection of the decreased functional capacity of the gland (15). The significant rise of saliva protein content in our edematous cases was not found in the nonedematous ones. It seems that adaptability of marasmic children to the meager diet helped them to escape from complication.

Salivary IgA was decreased while IgM and IgG immunoglobulins were increased in marasmic cases. High IgG level was resulted in ratios of IgG/IgA and IgG/IgM higher than normal (table 2). Accumulation of IgG in this cases may explain many of the bactericidal effects produced by saliva, since this globulin is the antibody containing fraction of serum (22). The most striking feature, however, was a high concentrating of IgM and IgG in all of IgA deficient whole saliva of marasmic cases. Most of IgG in saliva of IgA deficient patients is derived from serum (2).

In edematous cases, all immunoglobulins were increased and the ratios of IgG/IgA and IgG/IgM were markedly high as compared to controls. The reason for this concentration of immunoglobulins in whole saliva of such cases is that the surface epithelium may act as active molecular sieve favouring transmission of IgG from blood serum. Salivary IgA was increased, but not to the extent of other immunglobulins. Such increase is not due enhanced synthesis rate. If this is the case, one would expect markedly high level of IgA where it is the most component synthesized by the salivary glands. For this reason, IgA derived separately from serum and from its local sites was estimated to realize the actual state of salivary glands in our PCM cases. IgA immunoglobulin in unstimulated whole saliva is known to have two components (2), one is antigenic to rabbit antisera and the other is non (secretory IgA molecule).

In order to verify the identity of IgA present in unstimulated whole saliva, the antigenic component derived from serum was estimated by the electroimmunoassay (11) and after subtracting this component of each case from the total salivary IgA, the amount obtained is represent the secretory IgA synthesized from the salivary glands (table 3). Regarding this secretory IgA molecule, it was drastically reduced in our advanced cases particularly those of kwashiorkor. The latter finding has also been supported by other investigators (15).

From our present studies, although protein deficiency enhanced a diminished synthetic capacity of the salivary glands, a great transudation of blood serum protein into whole saliva was detected. This may be due to salivary glandular tissue affection or cellular changes which is more marked in edematous than nonedematous cases, where in third marasmus it is partially manifested.

Summary

Whole saliva protein as well as the separated protein components were estimated in normal and malnourished Egyptian infants and young children. In normal, 8 protein components (Albumin, α_1-antitrypsin, haptoglobin, β_2-lipoprotein, transferrin, IgA, IgM and IgG) were detected, while in PCM cases two more components (prealbumin and α_2-macroglobulin) were found.

The results also showed that the level of salivary protein components are markedly increased in edematous cases. In non-edeomatous ones, the level of these constituents are slightly increased in 3rd marasmus, but diminished in 2nd grade.

It is concluded that the elevation of protein components in saliva of edematous cases could be a result of severe glandular tissue involvement as compared to controls and non-edematous cases. The value of IgA immunoglobulin as specific antibody originated from blood plasma and/or salivary glands

may be used to reflect the extent of tissue affection in salivary glands of malnourished cases.

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