Biochemical reference values in elderly black subjects

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Summary

Biochemical reference values for the black age group of ⩾ 65 years were determined from the black urban population of the Orange Free State. Biochemical investigations performed were those included in the Sequential Multiple Analyser Computer profile because it includes the 20 most requested clinical chemistry investigations. Most of the reference values corresponded to values for the same age groups in the Western world. There was no age-related rise in the alkaline phosphatase values, which suggested absence of occult Paget's disease. Reference values for serum total protein and globulin were found to be higher than values derived from elderly white groups.

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Reference values for the commonly performed clinical chemistry investigations for the age group ⩾ 65 years are well documented for different Western communities.[1-8] No similar set of reference values exists for blacks and it is not known if the reference values for Western populations are applicable to the blacks of South Africa. The need for such a set of values in clinical medicine is obvious, since more and more blacks are reaching ⩾ 65 years. Moreover, because of the increase in living and health care standards in South Africa it has been estimated that the number of blacks in this age group will have increased by 129% by the year 2000.[9]

A study was undertaken to establish a set of biochemical reference values for the black age group of ⩾ 65 years using the black population of the OFS. The biochemical investigations performed were those included in the Sequential Multipe Analyser Computer (SMAC) profile because it includes the 20 most requested clinical chemistry investigations.

Subjects and methods

Allowing for a highest expected prevalence of 30% for any particular variable studied and an acceptable error of 5%, the sample size, calculated for 95% probability, was 323 subjects. It was decided to admit 400 people into the study. The male:female ratio of South African blacks aged ⩾ 65 years

according to the Human Sciences Research Council's (HSRC) correction of the relevant 1985 census ratio is 1:1,15. There were 182 men and 218 women in the study, giving a male: female ratio of 1:1,20. Informed consent was obtained from all participants.

Ten towns, with a black population of 1 500 or more according to the 1985 census, were randomly selected. The selected towns constituted 64,5% of the total urbanised black population in the OFS according to HSRC estimations.

Plot numbers were randomly selected in each of the towns and participants were visited in order of selection. An elderly person was defined as someone aged $\geqslant$ 65 years. There were 25 non-responders, giving a non-response rate of 6,3%. A non-response was recorded if a selected person refused to participate in the study or if that person could not be found at home after a third visit or was out of town for the duration of the research team's visit. The non-responders were replaced by continuing with the original lists of random plot numbers. In view of the low non-response rate it was considered that this action would not seriously bias the results.

A detailed history was taken in the person's home language and a standardised clinical examination was performed on each subject. Blood specimens were obtained by venepuncture in the cubital fossa and venous stasis was, as far as possible, avoided. Specimens were taken randomly between 09h00 and 12h00 and were delivered to the laboratory within 3 hours. Biochemical tests were all determined by the Department of Chemical Pathology, University of the Orange Free State, Bloemfontein.

All laboratory estimations were performed on a continuous-flow Technicon SMAC system, which is a multi-measuring clinical chemistry analyser requiring approximately 500 μl of serum for 20 different tests. The determinations are all based on well-established technologies and methods[10] consisting of iron-selective electrodes for serum sodium and potassium, and ultraviolet multipoint enzyme determinations for serum alanine aminotransferase (ALT, EC 2.6.7.2), aspartate aminotransferase (AST, EC 2.6.1.1), lactate dehydrogenase (LD, EC 1.1.1.27) and γ-glutamyl transferase (GGT, EC 2.3.2.2). A single-point method was used for serum alkaline phosphatase (ALP, EC 3.1.3.1), total calcium, total CO_2, chloride, creatinine, inorganic phosphorus, urea and albumin. Blank corrected assays were used for serum total protein, total- and direct bilirubin. Enzymatic assays were employed for the following substrates: cholesterol, glucose, triglycerides and uric acid. Commercial quality control (QC) sera were included in each analytical run and the results of the unknown samples were accepted only if the QC values had fallen within 2 SD of the mean provided by the manufacturer. Results from an external QC program confirmed that there was no bias in any of the determinations during the period of this investigation and that the analytical process had been properly controlled.

The following statistical characteristics and measurements were determined: (*i*) the type of distributions; (*ii*) arithmetic mean $\pm$ SD on the normal (Gaussian) distributions; (*iii*) log transformation of values with log-normal distribution patterns and the calculation of 95% intervals; (*iv*) comparison between groups of men and women with Student's *t*-test (Gaussian distributions) and the Mann-Whitney *U*-test (log-normal distributions); and (*v*) the effect of age on the abovementioned biochemical values by means of simple linear regression analyses.

Results

The study group consisted of subjects from the following ethnic groups: South Sotho (50%), Tswana (16%), Xhosa (15%), Zulu (7%) and Northern Sotho (4,8%), while 7,2% belonged to other ethnic groups. The average age was 73,5 $\pm$ 7,0 years for men and 73,7 $\pm$ 6,7 years for women. There were 146 subjects (36,5%) aged $\geqslant$ 75 years which included 35,2% of the men (64 subjects) and 37,6% of the women (82).

Types of frequency distributions found are shown in Table I together with distributions found in other studies on similar constituents. Table II shows the mean $\pm$ SD of the values of the constituents with normal distributions. Results are shown for the group as a whole and for men and women separately. Serum sodium and potassium, uric acid and inorganic phosphate differed statistically significantly between men and women ($P < 0,05$).

TABLE I. COMPARISON OF FREQUENCY DISTRIBUTIONS

Constituent	Wooton and King[13]	Roberts[3]	Leask *et al.*[1]	Present study
Sodium	N	N	N	N
Potassium	N	N	N	N
Chloride	N	N	N	N
Calcium	N	N	N	N
Phosphate	N	N	N	N
Uric acid	N	N	N	N
Total protein	N	N	N	N
Albumin	N	N	N	N
Globulin	N	N	N	N
Urea	L	L	L	L
Creatinine	L	L	L	L
LDH	–	L	–	L
Bilirubin	L	L	L	L
ALP	L	L	L	L
GGT	–	–	–	L
ALT	–	–	–	L
AST	–	–	–	L

N = normal; L = log-normal; — = not done; LDH = lactate dehydrogenase; ALP = Alkaline phosphatase; GGT = γ-glutamyltransferase; AST = aspartate aminotransferase; ALT = alanine transferase.

The values with log-normal distributions are shown in Table III. Results are shown as the logarithmic means $\pm$ SD as well as the calculated 95% confidence limits. The values for men and women were compared by non-parametric methods (Mann-Whitney). Significant differences existed between serum creatinine (M > F), LD (F > M), cholesterol (F > M), ALP (F> M), GGT (M > F), AST (M > F), ALT (M > F) and bilirubin (M >F).

Table IV shows the results when regression of the values was done on age. Serum albumin, ALT and calcium (corrected for albumin) showed a significant decrease with increasing age ($P < 0,05$).

Discussion

When dealing with reference values it is important to assess the significance of findings and statistical methods. It can be assumed that a community-based population study of elderly people will be more likely to approximate the hypothetical concept of a 'normal' elderly person than a study population based on hospital patients, outpatients or blood donors. Only 5,9% of the selected group did not take part in the study. A further 2,8% were excluded because of practical problems encountered (broken blood tubes and haemolysed blood). Due to a satisfactory response rate of 91,3% it was assumed that the sample under investigation was representative of the urbanised black population in the OFS.

TABLE II. SEX DIFFERENCES IN BIOCHEMICAL MEASUREMENTS WITH GAUSSIAN DISTRIBUTORS

Constituent	SI unit	Mean M and F	Mean M	Mean F
Sodium	**mmol/l**	**139,45 ± 2,86**	**138,98 ± 2,75**	**139,82 ± 2,88***
Potassium	**mmol/l**	**4,05 ± 0,55**	**4,13 ± 0,55**	**3,99 ± 0,55 NS**
Chloride	**mmol/l**	**102,06 ± 3,23**	**101,80 ± 3,20**	**102,28 ± 3,24 NS**
Calcium	**mmol/l**	**2,34 ± 0,12**	**2,34 ± 0,12**	**2,34 ± 0,12 NS**
Phosphate	**mmol/l**	**1,04 ± 0,17**	**0,98 ± 0,17**	**1,09 ± 0,16****
Uric acid	**mmol/l**	**2,30 ± 0,09**	**2,30 ± 0,08**	**2,31 ± 0,08 NS**
Protein	**g/l**	**78,05 ± 6,73**	**77,78 ± 7,71**	**78,10 ± 5,81 NS**
Albumin	**g/l**	**41,96 ± 4,05**	**42,32 ± 4,32**	**41,65 ± 3,79 NS**
Globulin	**g/l**	**36,22 ± 5,62**	**35,96 ± 5,59**	**36,45 ± 5,70 NS**

Significance of differences between males (M) and females (F) (Student's t-test):
* $P < 0,0001$,
** $P < 0,0004$.

TABLE III. RESULTS OF CONSTITUENTS WITH LOG-NORMAL DISTRIBUTIONS

Constituent	SI unit	M and F Log-mean (95% CI)	M Log-mean (95% CI)	F Log-mean (95% CI)
Urea	**mmol/l**	**0,623 ± 0,250** **(2,13 – 8,26)**	**0,626 ± 0,143** **(2,22 - 8,06)**	**0,620 ± 0,156** **(2,06 - 8,43)**
Creatinine	**mmol/l**	**1,886 ± 0,128** **(43,16 – 137,05)**	**1,920 ± 0,104** **(52,02 - 133,00)**	**1,86 ± 0,14** **(38,51 - 136,27*)**
LDH	**IU/l**	**2,241 ± 0,118** **(2,89 – 7,81)**	**2,206 ± 0,119** **(2,66 - 7,57)**	**2,27 ± 0,109** **(3,18 - 7,83*)**
Bilirubin	**mol/l**	**0,877 ± 0,201** **(3,04 – 18,66)**	**0,926 ± 0,212** **(3,24 - 21,95)**	**0,836 ± 0,181*** **(3,03 - 15,52)**
ALP	**IU/l**	**2,012 ± 0,160** **(49,93 – 211,64)**	**1,994 ± 0,159** **(48,12 - 202,13)**	**2,026 ± 0,160**** **(51,57 - 218,57)**
GGT	**IU/l**	**1,372 ± 0,402** **(3,84 – 144,52)**	**1,473 ± 0,410** **(4,67 - 198,06)**	**1,287 ± 0,375*** **(3,56 - 105,20)**
AST	**IU/l**	**1,342 ± 0,191** **(9,28 – 52,04)**	**1,376 ± 0,176** **(10,74 - 52,60)**	**1,314 ± 0,198*** **(8,43 - 50,36)**
ALT	**IU/l**	**1,274 ± 0,283** **(4,94 – 63,34)**	**1,293 ± 0,278** **(5,60 - 68,85)**	**1,208 ± 0,282***** **(4,52 - 57,64)**

M and F values compared by Mann-Whitney *U*-test.
* $P < 0,0001$,
** $P < 0,0004$,
*** $P < 0,0009$.
CI = confidence interval.

A factor that may have affected the results was that blood samples were taken randomly and not in a fasting state. It is known that phosphate levels may be lower after a meal.[11]

Difficulty surrounds the proper statistical presentation of biochemical results[1,2,3,7,12] but it would seem to be a minimum essential to determine the type of frequency distribution for each constituent. Distributions found in this study (Table I) show excellent agreement with previous findings.[1,3,13] Those constituents with normal distribution have been presented as the arithmetic means ± SD, from which the 95% confidence interval (CI) can easily be determined. Constituents with a log-normal distribution were shown as the logarithmic mean together with the upper and lower limits of the 95% CI range — the range which can be used as reference range by clinicians.

Although the serum sodium, potassium and LDH differed statistically significantly between groups of men and women, this difference is probably not clinically significant. Chen and Millard[14] found 55% of serum sodium concentrations in old people at home to be below 137 mmol/l. Our study did not support those findings. In the study by Leask *et al.*,[1] all the people who used diuretics were excluded because of certain effects on the electrolytes. It was not possible in this study to obtain information about diuretics (mainly due to illiteracy) and therefore subjects who used diuretics may have been included. However, the values that came into question, namely serum sodium, potassium, chloride, urea, creatinine and uric acid, did not differ from the results of Leask *et al.*[1] and the serum calcium values in this study were actually lower, the opposite to what would be expected. The serum calcium values in this study were slightly lower than reported values in other studies.[1-3] Leask *et al.*[1] found a significant decrease in serum calcium with increasing age. Our results did not show any significant serum calcium decrease with increasing age, a finding which corresponded with results reported in the review article of Dykbaer *et al.*[8] Women had significantly higher serum phosphate levels than men, and there was no significant change with age. Both findings corresponded with the findings of Leask *et al.*[1]

The significant sex difference found in uric acid concentration corresponded with reports by Reed *et al.*,[7] Leask *et al.*,[1]

TABLE IV. RESULTS OF SIMPLE LINEAR REGRESSION: BIOCHEMICAL CONSTITUENTS AGAINST AGE

Constituent	Correlation	r^2	% SEE	*P*
Sodium	**0,0226**	**0,07**	**2,248**	**0,6026**
Potassium	**– 0,0920**	**0,85**	**0,546**	**0,0707**
Chloride	**0,0382**	**0,15**	**3,228**	**0,4532**
Calcium	**– 0,1275**	**1,63**	**0,204**	**0,0120**
Phosphate	**– 0,0933**	**0,87**	**0,1722**	**0,0665**
LDH	**0,0250**	**0,06**	**56,85**	**0,6172**
Bilirubin	**0,0347**	**0,12**	**7,29**	**0,4953**
ALP	**– 0,0033**	**0,00**	**57,11**	**0,9476**
GGT	**– 0,0804**	**0,65**	**65,21**	**0,1137**
AST	**– 0,0801**	**0,64**	**16,19**	**0,1151**
ALT	**– 0,1590**	**2,51**	**19,58**	**0,0017**
Urea	**0,0710**	**0,50**	**1,591**	**0,1629**
Creatinine	**0,0076**	**0,01**	**41,00**	**0,0557**
Uric acid	**– 0,0972**	**0,95**	**0,087**	**0,2929**
Total protein	**– 0,0708**	**0,50**	**6,722**	**0,1639**
Albumin	**– 0,2138**	**4,57**	**3,963**	**0,0002**
Globulin	**0,0849**	**5,610**	**5,610**	**0,0963**

and Dodge and Mikkelson,[5] but we did not find any significant age-related rise in uric acid levels as were found in the studies of Leask *et al.*,[1] and Dodge and Mikkelson.[5] In the review article by Dykbaer *et al.*[8] it was reported that the values in men stayed more or less the same and the values in women showed an age-related rise.

Although Leask *et al.*[1] found significant differences between serum urea values in men and women (this study found no significant difference), they regarded it as too small to be of any practical importance. The upper cut-off value of serum urea, calculated from the 95% CI for this population, was 8,3 mmol/l.

Serum creatinine did not show a significant trend with increasing age in this study; this does not support previous reported rises with increasing age.[3] A possible explanation for this could be the decrease of mean body mass with increasing age[15] and a simultaneous decrease of renal function,[16] which together could maintain the serum creatinine levels.

A significant sex difference in serum bilirubin values was found in other studies.[1,7] The clinical significance of this could, however, be questioned. The liver enzymes (ALP, GGT, ALT, AST) also showed a significant difference but the question still remains whether this is of clinical significance. Leask *et al.*[1] reported a rise in ALP with age, which could be attributed to an increasing prevalence of occult Paget's disease of bone in the elderly. Our findings did not support this possibility and, on the contrary, suggested absence of increased bone disease in elderly black subjects.

The total serum protein and globulin values were found to be higher than those reported for elderly populations in the Western World,[1,3] while the albumin values corresponded to those mentioned. Previous workers[17] found the same differences in serum globulin values in black and white populations. The reason for this remains unknown but genetic as well as living circumstances were mentioned as possibilities.[17] The significant decrease in serum albumin with age closely resembles findings in most other studies.[2,3,6] According to serum albumin levels it appeared that the nutritional status of black elderly subjects compared well with Western groups of the same age. The same impression was found within this study group on clinical and anthropometric evaluation.

In summary, it can be said that most reference values for elderly black groups corresponded with values for the same age group in the Western world. There was no age-related rise in the ALP values, which suggested absence of occult Paget's disease. Reference values for serum total protein and globulin were found to be higher than values derived from elderly white groups.

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