The potential of salivary biomarkers of nutritional status and dietary intake: A Systematic Review


Published in:
Journal of Dentistry

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
Link to publication record in Queen's University Belfast Research Portal

Publisher rights
Copyright 2021 Elsevier. This manuscript is distributed under a Creative Commons Attribution-NonCommercial-NoDerivs License (https://creativecommons.org/licenses/by-nc-nd/4.0/), which permits distribution and reproduction for non-commercial purposes, provided the author and source are cited.

General rights
Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.

Open Access
This research has been made openly available by Queen's academics and its Open Research team. We would love to hear how access to this research benefits you. – Share your feedback with us: http://go.qub.ac.uk/oa-feedback

Download date: 15. Sep. 2023
Title: The potential of salivary biomarkers of nutritional status and dietary intake: A Systematic Review

Abstract

Objectives: To explore whether nutritional salivary biomarkers could be used to aid nutritional status assessment and/or support traditional dietary assessment methods for patients.

Data and Sources: Searches were performed using four electronic databases; MEDLINE, EMBASE, Scopus and Web of Science. Trial registers (i.e. Cochrane), grey literature and reference lists were searched.

Study Selection: Studies which measured nutritional salivary biomarkers related to nutritional status and/or dietary intake outcome were included. No restrictions on participants’ age, study design, publication date, setting or health status. Animal studies, non-English language studies, commentaries, and conference abstracts were excluded.

Results: Study titles and abstracts were screened (n=7982), full-texts assessed (n=176) and 85 studies included in a narrative synthesis. The most promising salivary biomarkers for nutritional status included: glucose, where saliva and serum levels were positively correlated in those with type 2 diabetes (T2D), higher salivary calcium levels in post-menopausal women in general and specifically those with lower bone mineral density (BMD), and salivary vitamin D to assess vitamin D status in healthy volunteers. Higher salivary total antioxidant capacity (TAC), nitrate/nitrite and fluoride were observed with increased antioxidant, nitrate/nitrite and fluoride dietary intake, respectively. A meta-analysis found significantly higher mean salivary glucose (n=12) in T2D compared with healthy controls, but there was substantial heterogeneity (I²=94%) and evidence of publication bias.

Conclusions: The most promising salivary biomarkers identified were, glucose, vitamin D, calcium, TAC, nitrate/nitrite and fluoride. However, this was based on a small number of studies
of varying quality, with many lacking a salivary assay performance assessment.

Clinical Significance: At present, nutritional salivary biomarkers cannot be used alone to assess nutritional status or dietary intake. Further research into the most promising nutritional salivary biomarkers is required.

Introduction

Nutritional status is defined as the state of an individual’s health, in terms of the intake and utilisation of nutrients [1]. The traditional assessment of nutritional status is complex, as it typically involves a combination of different measures including, anthropometry, clinical assessment of disease state, dietary intake assessment, a blood test interpreted alongside a clinical examination and environmental assessment relating to factors affecting eating behaviours [2]. Individuals with a combination of unsatisfactory assessment results such as, blood results outside of normal ranges or inadequate dietary intake, may be at risk of for example, malnutrition, iron-deficiency anaemia (IDA), osteoporosis or T2D, if nutritional intervention does not take place.

Dietary data collection as part of a nutritional status assessment and additionally the individual collection of dietary data to assess dietary intake, is not without challenge. This is because the usual methods of dietary data collection including, a food frequency questionnaire (FFQ), 24-hour recall or food diary, are subjective, at risk of recall bias and are prone to under- and over-reporting due to difficulty associated with reporting portion sizes [3,4]. Therefore, one method to help overcome these limitations, is using dietary biomarkers to objectively support traditional dietary assessment methods and aid nutritional status assessment [4]. Dietary biomarkers can more accurately associate dietary intake with disease risk and nutritional status, in comparison to self-reported methods [4]. Similarly, biomarkers can help detect, monitor and diagnose conditions [5].

Blood is generally considered the best body fluid to evaluate biomarkers of systemic health and dietary intake, however it is not without its limitations, as collection is inherently invasive, it can cause discomfort and induce patient anxiety from a fear of needles, and a risk of infection exists
for both the patient and practitioner [6]. Blood collection in older adults can also prove difficult
due to poor venous access, while it also tends to be less favoured amongst children in research [6].
Therefore, the process of assessing nutritional status is currently complex and invasive, creating a
need for a non-invasive, timely and cost-effective method to provide a biological sample which
could help assess nutritional status [7,8]. Similarly, the use of a quick method to support
traditional dietary assessment methods would be helpful in dietary research.

This is where the use of saliva has come to attention as it is often referred to as a “window on
health status” or a “mirror of the body” [9]. Saliva mainly consists of water (99%), but the
remaining 1% consists of various inorganic compounds, organic compounds and proteins/
polypeptide compounds [10]. Collection of saliva is considered the least invasive of all biological
samples [11] and may offer a credible means to evaluate health status [12]. Saliva collection is
relatively simple with specialised training not required and a biological sample produced which is
easier to ship and store, compared to serum samples, which need processed relatively immediately
after collection [7]. One previous systematic review [13] plus narrative literature reviews [9,14–
17] have suggested the possible clinical usefulness of different salivary biomarkers in the early
diagnosis and treatment of various oral or systemic disorders including, dental caries, cancer and
cardiovascular disease. Previous research also suggested that saliva may be a source of nutritional
biomarkers, as nutritional deficiencies may affect salivary function and composition [18,19].

To date, however, no systematic review has investigated the available range of potential nutritional
salivary biomarkers in relation to different nutritional states and dietary intake. The aim of this
systematic review was to explore whether nutritional salivary biomarkers in humans of any age
could aid nutritional status assessment and/or support traditional dietary assessment methods in
comparison to corresponding nutritional serum biomarkers or control groups (where applicable).

**Methods**

**Protocol and registration**

This systematic review was conducted and reported according to the Preferred Reporting Items for
Systematic Reviews and Meta-Analyses (PRISMA) guidelines [20] (Supplementary File 1). The review protocol was prospectively registered with PROSPERO: International Prospective Register of Systematic Reviews (CRD42018107667).

Eligibility criteria

Broad criteria were predefined to select papers for inclusion, using the participants, interventions, outcomes and study design reporting system (PICOS). The predefined list of inclusion and exclusion criteria used for this systematic review are detailed in Table 1.

Information sources

Four electronic bibliographic databases (MEDLINE, Web of Science, Scopus and EMBASE) were searched by the principal author (DL) to identify eligible studies. Trial registers (i.e. Cochrane) and other grey literature sources (open grey, google and google scholar) were searched using the key words, as well as relevant reference lists. The literature search was updated on January 9, 2021.

Search strategy

The literature was initially scoped in order to develop the search strategy. The search terms employed were either Medical Subject Headings (MeSH) terms or key words classified under general (all fields) category. The search terms were then combined with an “OR”, and PICO categories combined using “AND” to produce the search query. Appropriate adaptations were made to allow search strategies to be carried out on all the databases (Table 2). All searches were limited to include published, English and human studies and the library at Queen’s University Belfast retrieved any full-texts of identified articles that were not able to be accessed online.

Study selection

Study titles and abstracts were screened against the eligibility criteria. Duplicates were excluded automatically on an online programme (Covidence, Veritas Health Innovation Ltd, Australia) and manually checked. Those not meeting the inclusion criteria were removed and reasons noted. Full texts were retrieved if studies were considered potentially eligible. Studies meeting inclusion criteria were included in the narrative synthesis. If multiple studies measured the same salivary biomarker and nutritional state, with the same study design and outcome, they were considered
appropriate for a meta-analysis. Reviewer disagreements relating to study selection were resolved through discussion.

**Data collection process and data items**

Data extraction was carried out independently by two researchers (DL and SW) for those studies meeting inclusion criteria. The following data were extracted including: first author’s name, year of publication, study design, country, study population, demographics (sample size, age range and gender), salivary collection methods and analysis, primary outcome, as well as the comparator (i.e. serum) if available, covariates and main conclusions. Any disagreements between reviewers during data extraction were resolved through discussion and a consensus reached prior to finalising extracted data. Data items extracted from the included studies are detailed in Supplementary File 2.

**Missing data**

As this review included studies which either had the identification and/or level of nutritional salivary biomarkers, corresponding authors were not contacted for missing data.

**Risk of bias and quality assessment of included studies**

Risk of bias was assessed by the principal author (DL) according to individual study design. The Newcastle-Ottawa Scale (NOS) was used to assess the quality of non-randomised studies [21]. However, adaptations ensured responses were suitable in relation to non-response rate and ascertainment of exposure (Supplementary File 3). For a good quality case-control study, 7-9 stars were required, 5-7 stars for a fair quality study and anything lower suggested a poor quality study. For cross-sectional studies an existing adapted NOS version was used [22]. This was further adapted with the same changes as case-control studies. A maximum of 9 stars were given, where 0-3 was unsatisfactory, 4-5 was satisfactory, 6-7 was good and 8-9 was very good [22]. Randomised controlled trials (RCT) used the Cochrane risk of bias tool [23] and randomised cross-over trials used an existing adapted Cochrane risk of bias tool [24]. For before and after studies with no control group, the National Institute of Health quality assessment tool was used [25].

**Summary measures**

**Outcome measures**

The primary outcomes in this review were the identification and/or level of nutritional salivary
biomarkers in relation to nutritional status or dietary intake outcomes. Secondary outcomes were the comparison of the corresponding serum biomarkers to the salivary biomarkers if available.

**Synthesis of results**

Salivary glucose was the only biomarker appropriate for a meta-analysis to identify any glucose differences in those with and without T2D. This was presented as a cumulative forest plot with standardised mean differences and 95% confidence intervals (CI), using a random-effects model. Statistical heterogeneity was quantified using I-squared (I²) statistics from the RevMan meta-analysis (Version 5.3, Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). The other nutritional salivary biomarkers were not suitable for meta-analyses due to data heterogeneity, thus a narrative synthesis was carried out for these. A league table was also devised to determine which salivary biomarkers were the most promising and could be explored further in additional future research (Supplementary File 5).

**Risk of publication bias across studies and additional analyses**

Publication bias across the meta-analysis studies was assessed visually using a funnel plot of salivary glucose levels in those with and without T2D. No additional analyses was conducted.

**Results**

**Study selection**

The initial search retrieved 6585 possible studies and, following the removal of duplicates, 4836 studies were identified and screened. Of these, 134 full texts were assessed and 64 studies were eligible. The search was updated in March 2020 and this identified a further 1906 studies, of which 16 studies met the inclusion criteria after full text review. A third search update was conducted in January 2021 and a further 1240 studies were identified, of which five met the inclusion criteria after full text review, leaving a total of 85 studies for inclusion in the review. Full details of the process are presented in the PRISMA Flowchart including reasons for exclusions (Figure 1).

**Study characteristics**

**Studies included for narrative analysis**

The data from each included study (n=85) were summarised in Supplementary File 2. 63 studies
identified salivary biomarkers relating to nutritional status and 22 related to dietary intake. Of the 85 included studies, 53 were case-control studies, 20 cross-sectional, six randomised controlled crossover trials, four before and after studies (with no control group), one RCT, and one study which included aspects of a cross-sectional study and a before-after study (with no control group). Studies were conducted across different continents including; 35 in Asia, 19 in Europe, 14 in South America, eight in North America, five in Africa, three in Australia and one in Oceania. The total sample size was 16486 participants, ranging from six up to 8317 participants. Participants’ age varied from six weeks to >65 year olds. Studies were published between 1956 up until 2020.

**Studies included for meta-analysis**

Of the salivary biomarkers identified, glucose had the greatest number of studies with the same study design and outcome measure, compared to other biomarkers. A total of 12 case-control studies were identified as eligible for a salivary glucose meta-analysis [26,27,36,37,28–35]. These studies measured glucose in saliva of children and adolescents [37], adults [26–30,32,36], adolescents, adults and older adults [35], adults and older adults [31,34] and one study the age was not stated [33]. These studies ranged in sample size from 40 [30] up to 127 participants [31].

**Risk of bias within studies**

Risk of bias within each study was summarised according to study design (Supplementary File 4). The quality of case-control studies varied, ranging from one to eight and a 4.28 average out of nine (poor quality). This average is reflected by only 10 studies having a consecutive or representative set of cases [29,30,38–45]. The quality of cross-sectional studies ranged between two and five, with a 3.86 average out of nine (unsatisfactory). This average is reflected by only nine studies having a truly representative sample [73–81]. All case-control and cross-sectional studies lacked at least one or more of the ascertainment of exposure criteria. The one RCT was of fair quality due to two unclear criteria [93]. Of the six randomised controlled crossover trials, three were poor [94–96], one fair [97] and two good [98,99] quality. Of the five before and after studies, two studies achieved six [85,100], two achieved seven [101,102] and one achieved nine [103] out of 12.

**Synthesis of results**
Meta-analysis of the included studies: salivary glucose levels in all ages

A meta-analysis was performed for salivary glucose levels in those with T2D and controls [26,27,36,37,28–35]. The random-effects model using standardised mean difference revealed that the mean glucose levels were significantly higher in individuals with T2D by on average 1.26 standard deviations (SD) compared to controls (p<0.0001) (Figure 2). However, substantial heterogeneity existed as the $I^2$ statistic was 94% (Figure 2). According to the funnel plot analysis, potential publication bias was illustrated by the non-symmetrical funnel plot (Figure 3).

Narrative synthesis of nutritional salivary biomarkers

This systematic review identified 34 nutritional salivary biomarkers in relation to nutritional status and/or dietary intake, as summarised in the league table (Supplementary File 5). The results for the most promising biomarkers including, glucose, vitamin D, TAC, calcium, fluoride and nitrate/nitrite are presented in this section. A summary of the results for the less promising salivary biomarkers is included in Supplementary File 6.

Glucose

In addition to the glucose meta-analysis, ten studies out of 17 reported on the correlation between salivary glucose and serum glucose; a positive correlation was found in six studies including, adults with T2D and controls [26,29,32] and in healthy men [89]. However, salivary and serum glucose only correlated amongst those with T2D, as salivary glucose was not detected in controls [50], whilst a correlation only existed between salivary glucose and fasting plasma glucose in those with T2D [33]. However, three studies found no significant correlation between salivary and serum glucose in adults with T2D [31,36] or in 19-72 year olds [78] and no significant correlation between salivary glucose and serum fasting plasma glucose in older adults with T2D and controls [30].

Vitamin D

Two studies investigated the relationship between salivary and serum 25-hydroxyvitamin D$_3$ [25(OH)D$_3$] in adults. One study found a significant positive linear relationship between salivary and serum 25(OH)D$_3$, as well as an increase in salivary 25(OH)D$_3$ levels after taking 10 days of 25(OH)D$_3$ supplementation [85]. Similarly, a positive correlation was found between salivary and...
serum 25(OH)D3 after adjusting for salivary flow rate in adults [102].

Total antioxidant capacity

An increasing trend in salivary TAC was found with an increased dietary antioxidant intake in adults [93] and a positive association and an inverse association between salivary TAC and simple carbohydrate and complex carbohydrate, respectively [74]. However, salivary TAC was not associated with micronutrient or macronutrient intake during pregnancy [61].

Calcium

Two studies found that post-menopausal women with a lower BMD had higher salivary calcium than post-menopausal women without bone mineral changes [88] or pre-menopausal or pregnant women [45]. Long-term supplementation of vitamin D combined with calcium, did not significantly affect salivary calcium in osteoporotic women [40].

Fluoride

Three studies investigated dietary fluoride in relation to salivary fluoride, with salivary fluoride levels significantly increasing after inclusion of a sodium fluoride supplement in children [52], a significant correlation in young children [86], but not repeated in adolescents [75].

Nitrate/Nitrite

Two studies reported salivary nitrate/nitrite in relation to diet. One study showed that adults had significantly higher salivary and plasma nitrate and nitrite levels after consuming a high nitrate diet, compared to a low-nitrate diet [97]. The other study found that salivary nitrate and nitrite levels returned to baseline levels seven days after cessation of the high-nitrate diet [103].

Discussion

This systematic review explored the potential of nutritional salivary biomarkers to aid nutritional status assessment and/or support traditional dietary assessment methods for patients. The results for the six most promising biomarkers, based on consistent evidence and biological plausibility, are discussed in this section. For nutritional status this included: glucose for T2D, vitamin D for vitamin D status and calcium related to BMD. For dietary intake this included; TAC, nitrate/nitrite
and fluoride. This evidence, even when considered promising, was based on a small number of studies of varying quality. Current literature, however, does not suggest a role for a large number of nutritional salivary biomarkers identified in the scoping exercise for this review, due to the inconsistent direction of significant associations across studies or the lack of evidence to inform a conclusion (see Supplementary Files 5 and 6).

**Salivary biomarkers and nutritional status**

One of the most promising biomarkers identified was salivary glucose in relation to T2D. The data generated in the meta-analysis, supports previous evidence which suggested that salivary glucose levels are significantly elevated in patients with T2D [112]. The positive correlation between salivary and serum glucose mirrors the increased serum glucose in T2D [26,29,32,33,50,89]. Increases in salivary glucose may reflect microvascular structural changes within the salivary glands which increase the glucose diffusion rate from the blood to oral cavity [112]. However, the meta-analysis was subject to publication bias as shown in Figure 3 and substantial heterogeneity ($I^2 = 94\%$) as the absolute values varied between studies. High heterogeneity may be due to the different age groups including, adults and children [26,27,36,37,28–35]. All studies in the meta-analysis analysed unstimulated saliva using colorimetric glucose kits, but one study was not included as glucose was only detected in adults with T2D [50]. The reason is unclear but perhaps levels were below the limit of detection in controls. Overall, the collection and analyses of glucose in unstimulated saliva is promising for T2D diagnoses and glucose monitoring. However, this was based on poor and fair quality studies and should be interpreted with caution. Higher quality studies are needed to confirm the use of salivary glucose to monitor and diagnose T2D.

Despite limited evidence, vitamin D (25(OH)D$_3$) in unstimulated saliva, positively correlated with serum 25(OH)D$_3$ in healthy adults [85] and after adjusting for salivary flow rate [102]. This corresponds to low serum 25(OH)D$_3$ in those with a vitamin D deficiency [113]. Therefore, the current analysis suggests that reduced salivary 25(OH)D$_3$ may also indicate this deficiency. This is important as measuring serum 25(OH)D$_3$ is expensive and universal screening is not supported
However, saliva was collected on three consecutive days which may restrict community use [102]. The results may not be representative due to small study samples sizes [85,102]. Larger studies are required to confirm the use of salivary 25(OH)D$_3$ for assessing vitamin D status.

Limited, but promising, evidence was reported for salivary calcium and BMD in post-menopausal women, as those with lower a BMD had higher unstimulated calcium levels compared to pre-menopausal or pregnant women [45] or post-menopausal women without bone mineral changes [88]. This suggests that salivary calcium may be a useful screening tool for bone mineral changes and has the potential for early diagnoses of osteoporosis [88]. Unfortunately, as the included studies did not assess serum calcium levels, the relationship between salivary and serum calcium is currently unclear. However, recent research found that adults in Sweden with elevated serum calcium at baseline developed osteoporosis more often than controls [114]. This finding corresponds with increased salivary calcium in those with a low BMD. Longitudinal studies with larger sample sizes are required, to confirm the use of salivary calcium in relation to BMD.

### Salivary biomarkers and dietary intake

Despite limited evidence, salivary TAC was promising, as a RCT showed an increasing trend in salivary and plasma TAC amongst intervention adults, who increased fruit and vegetable intake compared to controls [93]. Samples were analysed using the Trolox Equivalent Antioxidant Capacity (TEAC) assay [93]. Results suggested that both salivary and plasma TAC respond to an increased antioxidant intake similarly. However, no differences in salivary TAC [93] or nutrient intake during pregnancy [61] were detected using the ferric reducing antioxidant power (FRAP) assay. Therefore, the TEAC assay may be more sensitive than the FRAP assay in detecting salivary TAC changes. It is unclear which saliva collection is most appropriate, as this information was not included [93]. Cross-sectionally, a higher intake of simple carbohydrate was associated with higher unstimulated TAC in adults, using the Oxygen Radical Absorbance Capacity (ORAC) assay [74]. Results may differ if diet was recorded for longer, instead of 24 hours [61,74] or three days [93]. Further evidence is required to confirm the use of salivary TAC and the best assay.
Limited, yet promising evidence, suggested that salivary and plasma nitrate and nitrite respond in the same way to an increased dietary intake, as levels increased amongst those with a greater consumption of spinach and green leafy vegetables in adults and older adults compared to those on a low-nitrate diet [97]. Salivary nitrate and nitrite levels also returned to baseline levels seven days after cessation of the high-nitrate diet [103]. This increase in salivary nitrate and nitrite may be explained by dietary intake, along with nitrate produced from NO, both entering the enterosalivary nitrate-nitrite-NO-pathway, and absorbed from plasma into the salivary glands [97]. It was unclear how saliva was collected, but gas chromatography mass-spectrometry was used to analyse saliva samples and a food diary used to record diet [97,103]. Based on good quality evidence, salivary nitrate and nitrite was successful in validating dietary intake, but further studies are required.

The use of salivary fluoride to reflect fluoride intake was also promising, as unstimulated fluoride levels increased with higher fluoride intake in children living in higher fluoride areas, via food (moderate correlation) [86] or sodium fluoride supplementation over a 12 month period [52], compared to those living in lower fluoride areas. Similar results were achieved using a fluoride selective electrode [52] and acid diffusion [86]. Salivary and plasma fluoride levels may respond in the same way, as a strong positive correlation existed between plasma fluoride and total daily fluoride intake [86]. However, stimulated fluoride levels did not correlate with dietary fluoride intake in adolescents using a fluoride selective electrode [75]. Therefore, 24-hour dietary recalls may not be long enough to reflect salivary fluoride [75]. Further higher quality studies are needed to confirm the use of salivary fluoride in reflecting dietary fluoride intake.

Strengths and limitations

This is the first systematic review to investigate a comprehensive range of nutritional salivary biomarkers in relation to nutritional status and dietary intake. Broad inclusion criteria meant that all age groups, publication years, study design (with or without controls), and settings (any health condition), maximised the number of studies. Data extraction involved two independent reviewers, although only one reviewer conducted the risk of bias assessment. Six salivary biomarkers were
promising based on consistent significant evidence and biological plausibility. However, this
review was limited by the quality of the evidence, as the majority of studies lacked an assessment
of salivary assay performance, so results may be based upon unreliable methods. Comparisons
were also limited due to high variability in salivary collection and analysis methods. Many studies had
small sample sizes which may have lacked power to detect significant findings. A meta-analysis was
only possible for salivary glucose, but substantial heterogeneity and publication bias existed.

**Conclusions**

Saliva represents a biological matrix that is easily accessible, considered the least invasive of all
biological samples and its collection does not require specialised training. It can also be collected
by patients themselves, thus offering an alternative to invasive blood tests in an era of limited
access to healthcare facilities. At present, the evidence is generally limited, inconclusive and
inconsistent as the change in the direction of significant associations was not always consistent.
Despite this, some results were judged as promising, with the potential use of salivary glucose for
monitoring and diagnosing T2D, vitamin D for assessing vitamin D status, calcium levels related to
BMD, TAC for assessing dietary antioxidant intake, nitrate/nitrite for assessing dietary
nitrate/nitrite intake and fluoride for assessing dietary fluoride intake. In general, these promising
salivary biomarkers responded to nutritional state or dietary intake in the same way as their
corresponding serum/plasma biomarker. Therefore, using nutritional salivary biomarkers is still
largely uncertain and requires further research. At present they should not be used alone to assess
nutritional status or reflect dietary intake. Future studies should include salivary assay performance
to ensure reliable results and explore the methodology of commercially available serum kits for
detecting nutritional salivary biomarkers. More specific and sensitive methods may be required to
evaluate potential salivary biomarkers as indicators of nutritional status or dietary intake.

**References**


[34] P.A. Manjrekar, A. Hegde, Shirlaxmi, F. D’Souza, V. Kaveeshwar, A. Jose, S. Tasneem, R. Shenoy, Fructosamine in non-diabetic first degree relatives of type 2 diabetes patients: risk


Y. Ishioka, The sodium-potassium ratio in saliva of children with or without nutritional dystrophy; studies on the nutrition of children in Hirosaki area; 38th report, Tohoku J. Exp. Med. 64 (1956) 297–300.


