Reduced utility of serum IGF-1 levels in predicting retinopathy of prematurity reflects maternal ethnicity

M Ashwin Reddy,[1,2] Himanshu I Patel,[1,2] Shah M Karim,[1] Helen Lock,[1] Leslie Perry,[3] Catey Bunce,[2] Steve Kempley,[1,4] Ajay K Sinha[1,4]

[1]The Royal London Hospital, Barts Health NHS Trust, London, UK
[2]Moorfields Eye Hospital NHS Foundation Trust, London, UK
[3]Department of Clinical Biochemistry, Croydon University Hospital, London, UK
[4]Blizard Institute, Barts and the London School of Medicine and Dentistry, London, UK

Correspondence to
Maddy Ashwin Reddy, Department of Ophthalmology, Royal London Hospital, Whitechapel Road, London E1 1BB, UK; ashwin.reddy@bartshealth.nhs.uk

Received 29 May 2015
Revised 28 July 2015
Accepted 1 August 2015
Published Online First 24 August 2015

ABSTRACT

Aims To validate known risk factors and identify a threshold level for serum insulin-like growth factor 1 (IGF-1) in the development of severe retinopathy of prematurity (ROP) in an ethnically diverse population at a tertiary neonatal unit, 2011–2013.

Methods A prospective cohort masked study was conducted. Serum IGF-1 levels at 31, 32 and 33 weeks were measured and risk factor data collected including gestational age (GA), birth weight (BW), absolute weight gain (AWG) and maternal ethnicity. The eventual ROP outcome was divided into two groups: minimal ROP (Stages 0 and 1) and severe ROP (Stage 2 or worse including Type 1 ROP).

Results 36 patients were recruited: 14 had minimal ROP and 22 severe ROP. Significant differences between the groups were found in GA, BW, AWG and IGF-1 at 32 and 33 weeks. There was minimal rise in IGF-1 in Stage 2 patients and/or black patients (p=0.0013) between 32 and 33 weeks but no pragmatic threshold level of IGF-1 that could distinguish between minimal or severe ROP.

Conclusions There were significant differences in GA, BW, AWG and IGF-1 at 32 and 33 weeks between those babies with severe ROP and those with minimal ROP. However, there was no threshold level of IGF-1 at a time point between 31 and 33 weeks that can be used to exclude a large proportion of babies from screening. We also found ethnic differences in IGF-1 levels with infants born to black mothers having significantly lower IGF-1 levels at 32 and 33 weeks gestation. The determination of ROP risk using IGF-1 is a race-specific phenomenon.

Low insulin-like growth factor 1(IGF-1) serum levels and poor early or absolute weight gain (AWG) have recently been shown to be predictors for the development of retinopathy of prematurity (ROP)[1 2] in addition to birth weight (BW) and gestational age (GA). We have demonstrated that maternal ethnicity (ME) has a greater predictive power than AWG in our ethnically diverse population with black mothers having a protective effect on their infants' risk of ROP.[3] Complex algorithms such as WINROP have been used to identify which babies are at risk of treatment for ROP to avoid blindness with variations in sensitivity in different populations.[4–8] Their basis has been grounded in IGF-1 levels and AWG as a surrogate.

In view of the association between IGF-1 and ROP severity,[1] we aimed to define a threshold level at a particular time point that might exclude the large number of babies that are screened but do not require treatment.[4] The role of IGF-1 has not been assessed in a multiethnic population of neonates who were born and cared for in the same neonatal environment.

METHODS

This was a prospective masked cohort study conducted between 1 January 2011 and 1 February 2013 at a single tertiary neonatal unit in London, UK. Informed consent was provided from parents. Ethics committee approval was obtained. This research adhered to the tenets of the Declaration of Helsinki.

Recruitment

All at-risk infants (according to Royal College of Ophthalmology guidelines,[9] <32 weeks GA at birth and/or <1501 g birth weight) were eligible to be recruited in the study. Baseline data on ME, GA and BW were recorded. ME was classified according to white, South Asian and black[3] race. In addition, weight gain in the first 6 weeks of life (AWG) was collated as previously described.[3] The GA, BW, postmenstrual age (PMA) and weight at 6 weeks were used to calculated SD scores (SDS or z scores for weight at birth and 6 weeks) using LMSgrowth software which uses UK 1990 reference growth data.[10] The highest-stage ROP was recorded according to the International Classification of Retinopathy of Prematurity.[11] Any babies that reached prethreshold disease (Type 1 ROP) according to the Early Treatment of Retinopathy of Prematurity study received laser treatment.[12]

IGF-1 testing

The weeks between 31 and 33 PMA were chosen as the original study[1] demonstrated that serum IGF-1 levels for severe disease were at their lowest levels during this time period. The first blood sample for IGF-1 was taken at 31 weeks PMA. This was repeated at 32 weeks PMA and 33 weeks PMA. Three blood samples of 1 mL each were taken from infants. Routine blood samples were being taken from these infants on a weekly basis. Serum IGF-1 levels were analysed by an immunologist (HL) using an immunoenzymometric assay for the quantitative determination of IGF-1 in human serum (AC-27F1) test kits (Immunodiagnostics Systems). Before running study samples, the kit was evaluated against the analyser used routinely to measure IGF-1 by analysing samples that had been run on the standard analyser and external quality control samples. These results were comparable. Twenty-five microlitres of sample was run in duplicate. The assays were run manually but an automated plate washer was used to wash the plate three times between the conjugate and substrate

To cite: Reddy MA, Patel HI, Karim SM, *et al*. *Br J Ophthalmol* 2016;**100**:501–504.

incubation. Kit calibrators and two positive controls were included in each assay. A known positive sample was also included in each as an internal quality control. This was run as a patient sample at the end of each plate and the results were plotted on a Levey–Jennings chart to control for reproducibility.

Throughout the study, at-risk infants were screened by a paediatric ophthalmologist (MAR or HIP) as per standard protocol[13] (Retcam imaging using speculum after dilating with cyclopentolate 0.5% and phenylephrine 2.5%) every 1–2 weeks. The infants were classified according to ROP stage (below) and the screener was masked from the serum IGF-1 levels.

Data analysis

For ROP analysis, the maximum ROP for the worst eye after serial examinations was recorded for each infant and categorised as minimal ROP (no or Stage 1 ROP) or severe ROP (Stage 2/Stage 3 and/or Type 1 ROP). Spearman's rank correlation was used to assess evidence of association between ROP stage and continuous putative risk factors. Fisher's exact tests were used to assess evidence of association between ROP stage and categorical putative risk factors. Logistic regression was then used to assess associations between these factors and odds of developing severe ROP. IGF-1 levels were compared between different ethnic groups and at different time points using analysis of variance (ANOVA), with box plots of IGF-1 levels at different time points plotted by ethnic group and ROP severity. Pairwise comparisons were made using unpaired t tests with unequal variances. All statistical analyses were conducted using SPSS V.21. We cross-tabulated whether or not the baby had severe ROP against various levels of IGF-1 to see whether they reliably identified babies at risk.

RESULTS

Thirty-six infants were recruited. Infants were not recruited if there was a likelihood of transfer of the child from the research unit to another unit where blood testing would not be performed. Fifteen were born of white mothers, 15 of South Asian mothers and 6 of black mothers. The worst stage of ROP was Stage 0 or 1 (minimal ROP) in 14 infants, Stage 2 in 13 infants and Stage 3 or Type 1 ROP in 9 infants. Four of these nine babies required laser treatment.

Severity of ROP, demographics and IGF-1 levels

The development of severe ROP was negatively associated with GA ($r=-0.70$, $p<0.001$), BW ($r=-0.62$, $p<0.001$), AWG ($r=-0.48$, $p=0.003$) and IGF-1 levels at 32 weeks ($r=-0.46$, $p=0.005$) and 33 weeks ($r=-0.44$, $p=0.008$). Mean IGF-1 levels were significantly higher at 33 weeks compared with 31 and 32 weeks. There were statistically significant differences in GA, BW, AWG and IGF-1 levels at 32 and 33 weeks between babies with different stages of ROP as shown by ANOVA (table 1). Weight SDS scores at birth, 6 weeks of age and IGF-1 levels at 31 weeks were not statistically significantly different between babies of different ROP severity (table 1 and figure 1A). The difference in IGF-1 levels between 32 and 31 weeks (ANOVA $F=8.5$, $p<0.01$) as well as between 33 and 31 weeks (ANOVA $F=6.3$, $p<0.01$) was significantly different across the groups of severity of ROP (figure 1B). The mean rise in IGF-levels was higher in babies who developed minimal ROP (figure 1B).

ME, IGF-1 levels, AWG and outcome

IGF-1 levels in babies born to black mothers were statistically significantly lower at 32 and 33 weeks compared with babies born to non-black mothers (table 1 and figure 2A). Because similar values were seen in Asian and white babies, we combined these as non-black babies for pairwise comparisons. Mean (SD) IGF-1 levels in non-black babies were 17.2 (10.6) at 32 weeks and 22.3 (14.1) at 33 weeks, compared with values of 8.8 (3.1) and 9.4 (5.6) in black babies. ($p=0.0013$, $p=0.0013$). The rise in IGF-1 levels was higher in infants born to non-black mothers (figure 2B). All six babies born to black mothers did not require laser treatment. AWG in babies born to black mothers were lower compared with babies born to non-black mothers (table 1), although not statistically significantly so with our data (mean difference in AWG 178 g, $p=0.086$).

Prediction of severe ROP with addition of IGF-1 levels

Logistic regression was used to predict development of severe ROP (dependent variable) with GA, BW, AWG and IGF-1 levels as predictors. The odds of development of severe ROP was lower for every unit increase in IGF-1 levels between 33 and 31 weeks (OR=0.86, $p=0.01$). In the subgroup of infants with severe ROP, the development of proliferative ROP was higher for every unit increase in IGF-1 levels (OR=1.1, $p=0.2$).

Table 1 Patient demographics, IGF-1 levels at 31, 32 and 33 weeks corrected GA, severity of ROP and ME

	Number	GA (weeks)	BW (g)	SDS score at birth	AWG (g)	SDS score at 6 weeks	IGF-1 levels at 31 weeks (µmol/L)	IGF-1 levels at 32 weeks (µmol/L)	IGF-1 levels at 33 weeks (µmol/L)
All babies	36	26.4 (1.9)	846 (235)	−0.5 (1.1)	566 (232)	−1.6 (0.9)	13.6 (8.2)	15.8 (10.3)	20.1 (13.9)
ROP stages									
0–1	14	28.1 (1.2)	1026 (193)	−0.6 (1.3)	704 (184)	−1.4 (1.0)	14.3 (9.9)	21.6 (12.3)	27.5 (16.4)
2	13	25.9 (1.5)	781 (188)	−0.6 (1.0)	535 (245)	−1.7 (0.8)	12.6 (5.1)	10.6 (4.6)	13.7 (7.7)
3 or Type 1	9	24.5 (1.0)**	661 (163)**	−0.4 (0.9)	397 (153)**	−1.9 (0.9)	13.9 (9.9)	14.3 (8.7)*	17.6 (11.9)*
ME									
Asian	15	26.1 (2.0)	842 (274)	−0.5 (1.0)	553 (222)	−1.6 (1.0)	16.8 (8.8)	19.6 (12.7)	25.2 (17.0)
White	15	27.0 (1.8)	911 (205)	−0.5 (1.1)	639 (241)	−1.5 (0.8)	11.9 (7.2)	14.8 (7.7)	19.3 (9.8)
Black	6	25.7 (2.0)	694 (139)	−0.9 (1.3)	418 (182)	−2.3 (0.6)	9.7 (7.5)	8.8 (3.1)	9.4 (5.6)§
Non-black (Asian or white)	30	26.6 (1.9)	877 (240)	−0.5 (1.0)	596 (232)	−1.5 (0.9)*	14.3 (8.3)	17.2 (10.6)**	22.3 (14.1)**

Birth weight (BW), gestational age (GA), absolute weight gain in 6 weeks (AWG), maternal ethnicity (ME) and ROP stages (minimal ROP, Stage 0–1; ROP, Stage 2; ROP-proliferative stage (ie, Stage 3 or Type 1 ROP) of entire study cohort of babies. Data are shown as mean (SD). The values for ROP stages and ME were compared using analysis of variance (ANOVA). The values for non-black infants were compared with black infants using t test.
**p<0.01, *p<0.05, §p=0.05.
IGF-1, insulin-like growth factor 1; ROP, retinopathy of prematurity; SDS, SD scores.

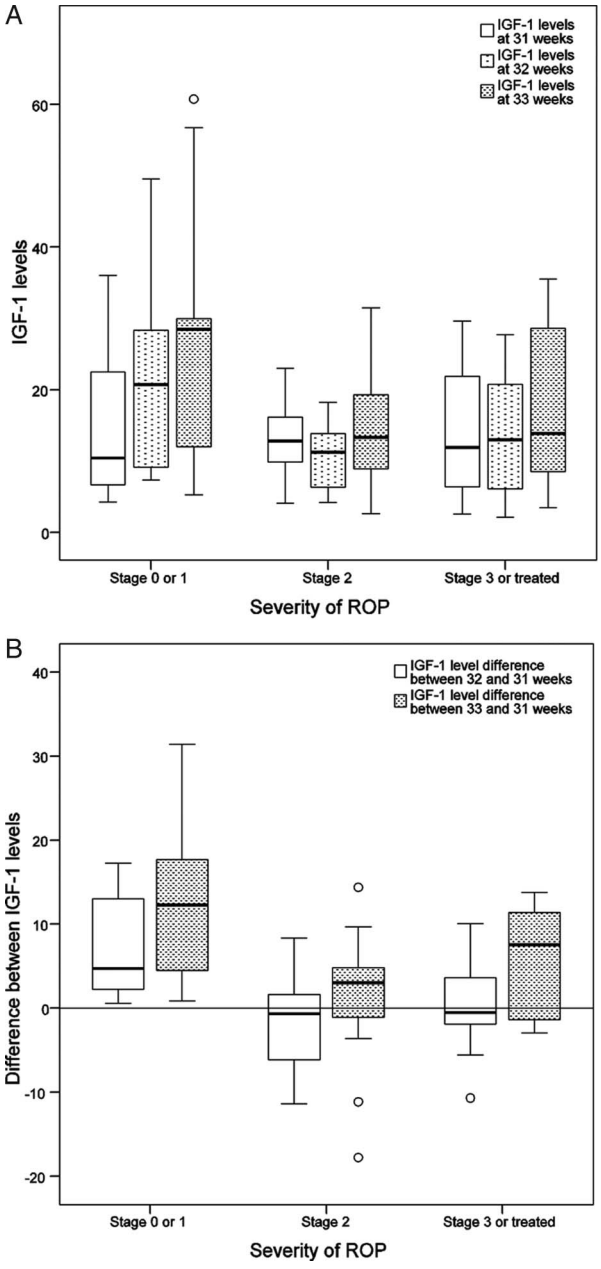

Figure 1 (A) IGF-1 levels (μmol/L) at 31, 32 and 33 weeks gestation in babies who developed different degrees of severity of ROP. (B) Differences in IGF-1 levels at 32–31 weeks and 33–31 weeks in babies who developed different degrees of severity of ROP. IGF-1, insulin-like growth factor 1; ROP, retinopathy of prematurity.

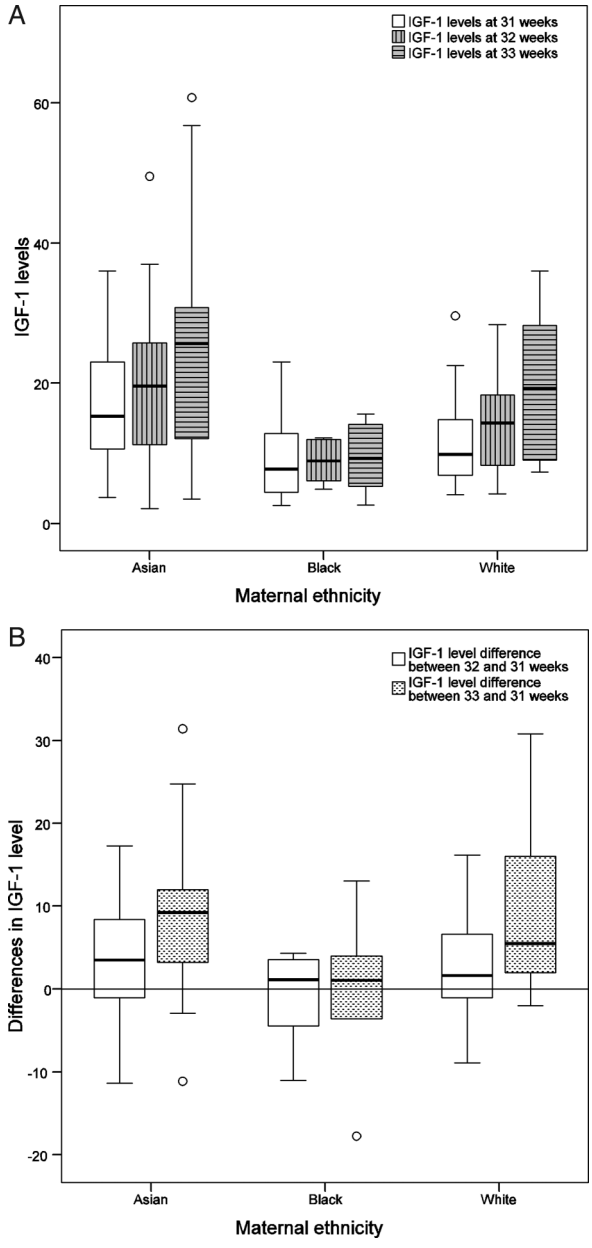

Figure 2 (A) IGF-1 levels (μmol/L) at 31, 32 and 33 weeks gestation in babies with different MEs. (B) The differences in IGF-1 levels at 32–31 weeks and 33–31 weeks in babies with different MEs. IGF-1, insulin-like growth factor 1; ME, maternal ethnicity.

Serum IGF-1 as an isolated predictor for severe ROP

Threshold levels of IGF-1 at the different time points were assessed to identify a single level that could exclude a large proportion of infants from conventional screening. Due to overlap between corresponding levels of IGF-1 in patients with minimal and severe ROP (figure 1A), no serum IGF-1 level at 31, 32 or 33 weeks was found that could be used as a pragmatic screening test for patients, that is, excluding a majority from conventional screening.

DISCUSSION

The development of proliferative (Stage 2/3/Type 1) ROP was associated with lower GA, BW, AWG and IGF-1 levels at 32 and 33 weeks gestation. We were keen to validate the original IGF-1 study[1] and also assess if a single threshold value of serum IGF-1 at a single time point was effective in determining whether babies should be screened or not. We have shown that IGF-1 levels rise over this period of time and that the mean differences between minimal ROP and severe ROP are significant at 32 and 33 weeks PMA. However, there is overlap in values and as a result, despite the small number in this study, we can conclude that there is no threshold level of IGF-1 at a time point between 31 and 33 weeks that can be used to exclude a large proportion of babies from screening.

Sample size was small in this study due to transfer of patients from the unit during 31 to 33 weeks (blood testing period) making many infants ineligible for the study. However, we still detected ethnic differences in IGF-1 levels with infants born to black mothers having significantly lower levels at 32 and

33 weeks gestation. The rise in IGF-1 levels between 31 and 33 weeks is lower in infants born to black mothers. Our previous work has shown a lower rate of ROP requiring treatment in infants of black mothers.[3] It has recently been shown that there is a racial difference between black and white adults for IGF-1 levels, with black controls having significantly lower levels of IGF-1 than whites.[14] To our knowledge this is the first study to confirm this finding in infants.

A surrogate for IGF-1 is AWG as there is a relationship between serum IGF-1 levels and AWG.[15] This is easier to measure and avoids blood samples being taken. We found that black babies had a trend for lower AWG compared with non-black babies which is in keeping with our IGF-1 results. This may explain why we had previously found that ME was a more important factor than AWG.[3]

Conventional screening will detect babies that require treatment. Algorithms that do not have 100% negative predictive value for the most severe form of the disease are best avoided. Although the WINROP algorithm has been proven to be effective in Sweden, there are several regions of the world where the sensitivity has dropped below 100%.[6 7 16] The basis of WINROP is the correlation between higher AWG (used as a surrogate for IGF-1) and reduced need for laser treatment.

A large multicentre study in North America used postnatal weight measurements, as a tool for the prediction of ROP in a racially diverse study population. Using the WINROP algorithm, the sensitivity achieved was 98.6%. Four of 149 babies who required treatment were missed and the implications for those infants are important. We used different categories of ROP severity from previous studies as we felt that any form of Stage 2 disease should have conventional screening to avoid the risk of infants being missed and then developing prethreshold disease. As a result we did not use the WINROP algorithm for this study.

In a Korean population, a sensitivity of 90% was achieved for prethreshold disease and recommendations were made for race/population-specific algorithms.[7] We echo these recommendations and provide a biological explanation why algorithms have struggled to achieve 100% sensitivity for the most severe form of disease in different populations.

We were surprised to find that babies of black mothers did not have an appropriate increase in IGF-1 levels over time as we have shown that there is a reduced risk of severe ROP in this group (in agreement with the majority of previous reports).[17–19] However, our findings are in keeping with racial differences in serum IGF-1 levels demonstrated in adults[14] and it is tempting to speculate that the protective effects of increasing IGF-1 levels and AWG are race-specific phenomena. There is an association between low initial levels of serum IGF-1 and/or AWG with severe ROP in certain ethnic groups. This may simply reflect the association between ROP and general illness, but IGF-1 is required for vascular development.[1] Hence, significantly lower levels of IGF-1 in black babies should result in more severe ROP. This was not found, suggesting that the relationship is more complex than was initially thought. Serum IGF-1 levels are not predictive in all populations. This should be confirmed in larger longitudinal studies.

Acknowledgements Brendan George's role as research nurse was very important for recruitment and venipuncture. Neonatal nurses Yolanda Solinap and Maria Crame were also instrumental in ensuring that the blood tests were performed.

We would like to thank Barts Health Charity for funding this project: Barts Charity Research Study 418/1197.

Contributors All authors made substantial contributions to the conception or design of the work or the acquisition, analysis or interpretation of data for the work; revised it critically for important intellectual content; gave final approval of the version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Funding Barts Charity (418/1197).

Competing interests None declared.

Ethics approval Local Ethics Committee, London.

Provenance and peer review Not commissioned; externally peer reviewed.

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